JOURNAL OF CHEMICAL EDUCATION

Laboratory Experiment

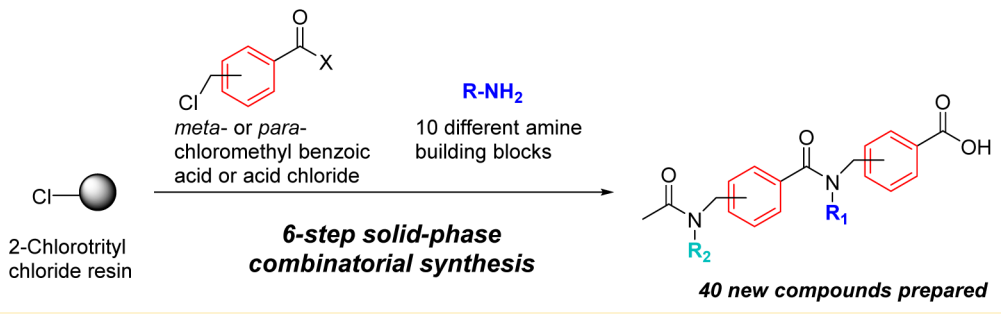
pubs.acs.org/jchemeduc

# Combinatorial Solid-Phase Synthesis of Aromatic Oligoamides: A Research-Based Laboratory Module for Undergraduate Organic Chemistry

Amelia A. Fuller*

Department of Chemistry & Biochemistry, Santa Clara University, 500 El Camino Real, Santa Clara, California 95053, United States

Supporting Information




**ABSTRACT:** A five-week, research-based experiment suitable for second-semester introductory organic laboratory students is described. Each student designs, prepares, and analyzes a combinatorial array of six aromatic oligoamides. Molecules are prepared on solid phase via a six-step synthetic sequence, and purities and identities are determined by analysis of LC−MS data. This experiment engages students in a research experience: none of the structurally diverse compounds prepared have been previously reported in the literature. After completion of this experiment, products are evaluated for their biological activity in the author's research laboratory. Although execution of biological experiments falls outside the learning objectives in this course, these applications motivate students. Through these hands-on experiences, students learn about organic chemistry reactions and modern techniques, and their research skills are enhanced.

**KEYWORDS:** *Second-Year Undergraduate, Laboratory Instruction, Organic Chemistry, Hands-On Learning/Manipulatives, Inquiry-Based/Discovery Learning, Amides, Combinatorial Chemistry*

Screening large numbers of diverse molecules is a common way to identify biologically active compounds,[1] and several modern technologies in organic chemistry have been developed to expedite the preparation and purification of such compound collections.[2−4] The efficient synthesis of combinatorial libraries on solid phase is a widely used and very important example of these. Herein, a research-focused laboratory experiment is described that introduces students to both combinatorial chemistry and multistep solid-phase synthesis. Students design and synthesize combinatorial libraries of previously unreported, structurally diverse molecules with potential biological relevance. Because a large number of students participate in laboratory courses, this is an ideal venue to prepare many diverse compound analogs. Few reports have detailed the incorporation of multistep solid-phase synthesis experiments in the undergraduate organic chemistry laboratory.[5−10] In particular, student synthesis of a combinatorial array is rare.[5−8] Most laboratory experiments that engage students in combinatorial chemistry entail one-step *solution* reactions to prepare known compounds.[11−17]

The products prepared in this experiment comprise the oligomeric meta- and para-substituted arylopeptoid (N-substituted aminomethyl benzamide) scaffold.[18,19] Arylopeptoids have attracted attention owing to their ease of synthesis on solid support,[19,20] interesting conformational properties,[20−22] and potential for biological activity.[23] Their synthesis on solid support entails iterating amide-bond forming reactions and displacement of benzylic halides with primary amines. Structural diversity has been explored by varying substitution of the aromatic ring and by varying the N-substituents,[19,20] making the synthetic sequence adaptable to combinatorial chemistry. The six-step synthesis of dimeric molecules in this experiment (Scheme 1) is technically and conceptually accessible for introductory organic chemistry students. Moreover, it employs critical reactions (substitution, acylation) traditionally covered in the introductory organic chemistry curriculum.

This experiment introduces students to a research environment in which they apply reactions described in the literature to prepare combinatorial arrays of *new* molecules. Herein,

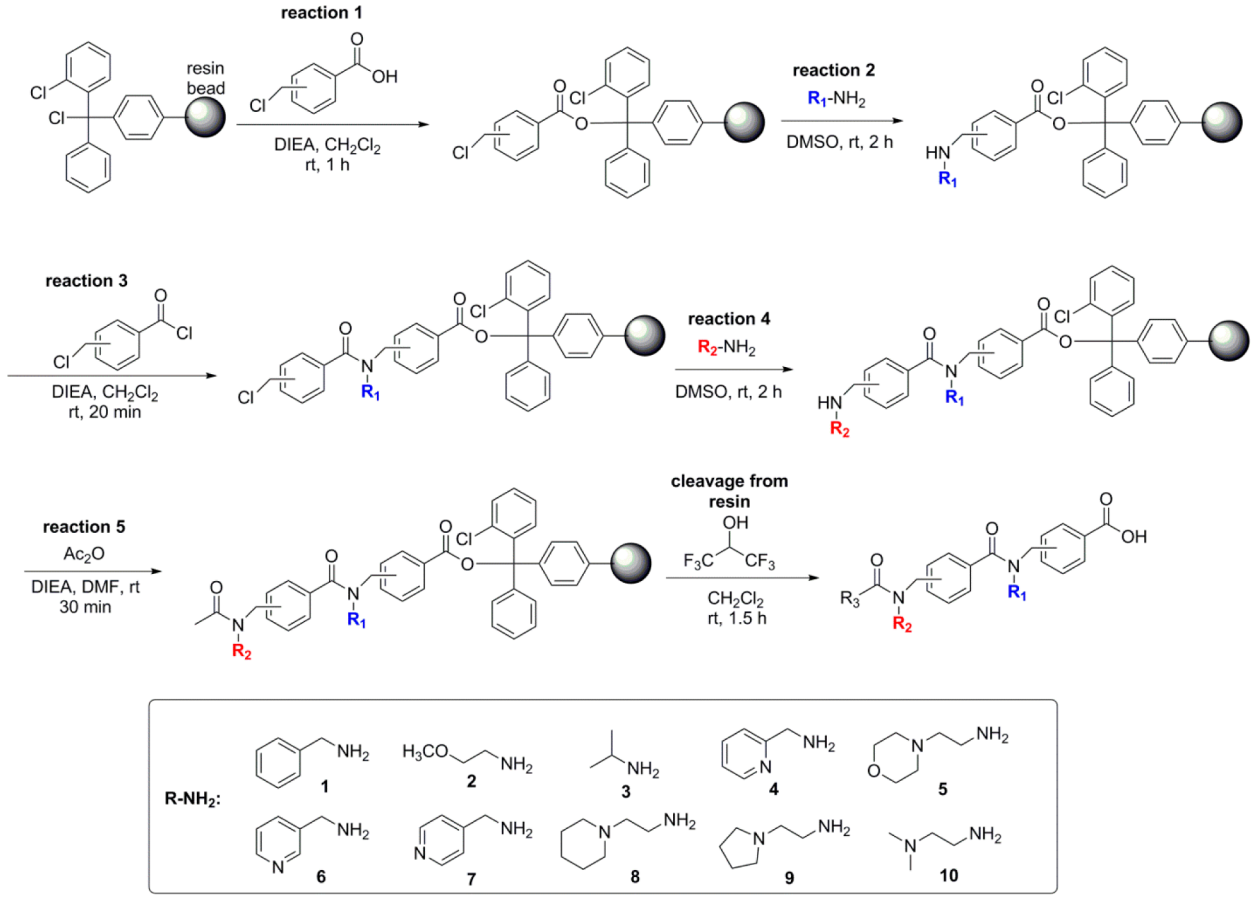
**Received:** August 14, 2015
**Revised:** January 20, 2016





DOI: 10.1021/acs.jchemed.5b00671
J. Chem. Educ. XXXX, XXX, XXX−XXX

**Scheme 1. Synthesis of Oligoamide Targets on Solid Phase.**[a]




[a]DIEA = *N*,*N*-diisopropylethylamine, DMSO = dimethyl sulfoxide, DMF = *N*,*N*-dimethylformamide, $Ac_2O$ = acetic anhydride.

**Table 1. Timeline for Laboratory Lectures and Student Laboratory Tasks**

| Week | Tasks |
|---|---|
| 1 | • Laboratory lecture: introduction to combinatorial chemistry and experimental design, introduction to use of the Bill-Board equipment |
| | • Students design arylopeptoid product array, complete calculations for reactions 1−4 (to be completed outside of lab), execute reaction 1 |
| 2 | • Students prepare solutions for reactions 2 and 4, execute reactions 2 and 3 |
| 3 | • Students execute reactions 4 and 5 |
| 4 | • Laboratory lecture: introduction to LC−MS techniques and instrumentation |
| | • Students cleave reaction products from resin, prepare samples for LC−MS |
| | • Students observe LC−MS instrument demonstration |
| 5 | • Laboratory lecture: analysis of LC−MS data |
| | • Students analyze LC−MS data, store samples |

procedures are established for students to execute six parallel reactions on solid support. Students vary the amines used in reactions 2 and 4; the reactivity of some of the amines chosen for this laboratory has not yet been reported. Consequently, reaction outcomes are unknown. The synthesis of diverse molecules engages students in a larger research endeavor; their products are screened for inhibition of bacterial growth in the author's research laboratory. Although biological evaluations are not executed in this experiment, the connection between organic synthesis and potential "real-world" pharmaceutical applications captures student interest and enthusiasm. Additionally, the advantages of research-focused curricula to promote student learning have been well documented.[24−26]

Implementing this experiment was motivated by addressing two main learning goals. First, it contextualizes and, thus, enhances students' understanding of important concepts, including mechanistic details of organic transformations and the advantages of solid-phase synthesis. Second, student participation in these experiments strengthens research-applicable skills, including analysis of unknown experimental outcomes, proper laboratory notebook documentation, and working and communicating as a research team.

## EXPERIMENTAL SUMMARY

### Overview and Timeline

These procedures have been performed twice in a second-year undergraduate organic chemistry laboratory course designated for chemistry and biochemistry majors, and results from the most recent offering of the course (in 2015) are detailed here. Enrollment for the course was 14 and 16 students supervised by


DOI: 10.1021/acs.jchemed.5b00671
J. Chem. Educ. XXXX, XXX, XXX−XXX

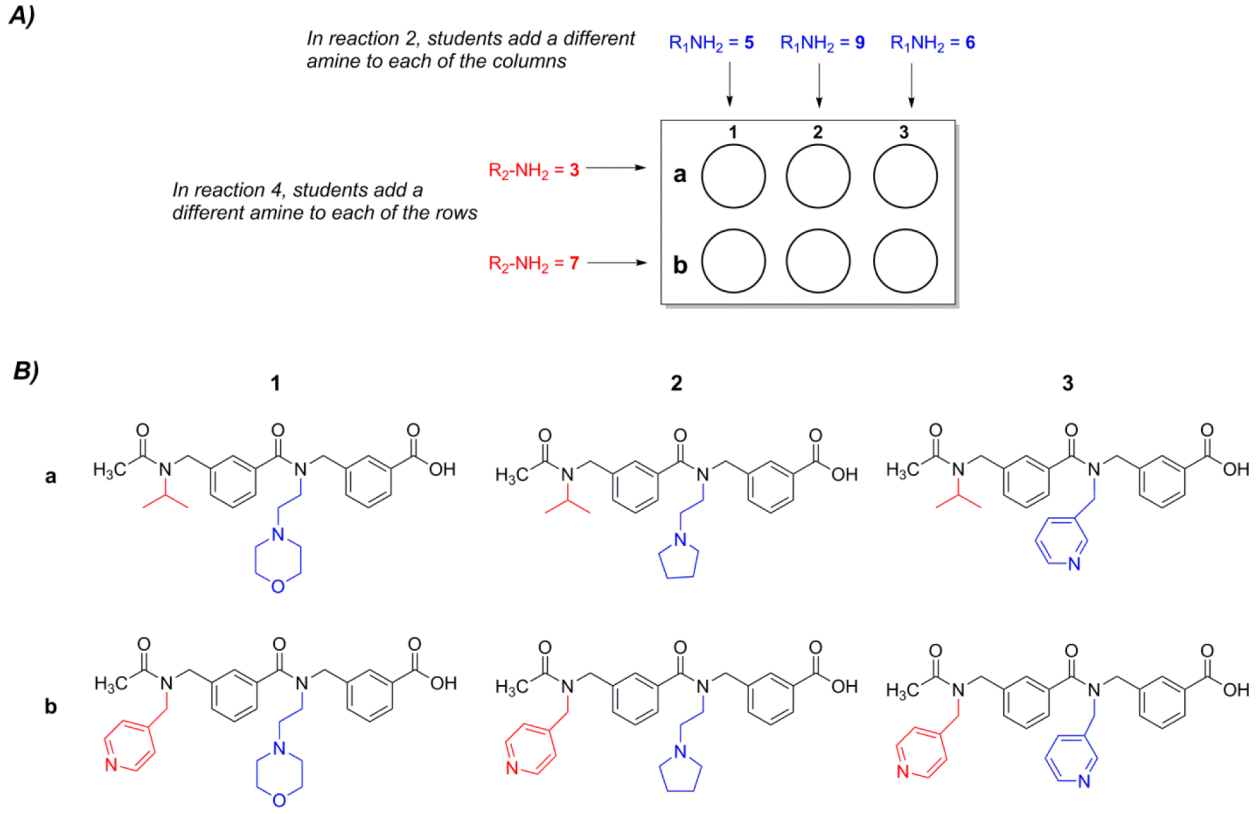



**Figure 1.** Using the Bill-Board to design a combinatorial array of products. (A) Combinatorial schematic, including identities of amines used for the synthesis of the example product array shown. (B) Example product array that follows this design.

an instructor and one undergraduate student teaching assistant. Five, 4 h lab periods were allocated for students to design and execute the experiment, then analyze their experimental outcomes (Table 1).

## Materials

Parallel synthesis of six compounds in a spatially addressable manner is enabled by the commercially available Bill-Board apparatus.[5−8] The Bill-Board apparatus comprises three modular parts. A reaction plate arranges six reaction vessels in a plastic grid. A plastic drain tray fitted below the reaction plate collects rinsed solutions from reaction vessels. After the resin cleavage reaction, the drain tray is replaced with a vial tray for collection of the crude product solutions into glass vials. With the Bill-Board, students use an "air-push" apparatus (a modified plastic pipet) to pressurize reaction vessels to accelerate drainage.

Implementation of this experiment necessitates an initial investment in the Bill-Board equipment as well as a diversity of reagents. In this experiment, each student is provided a Bill-Board apparatus and an "air-push" apparatus. Institutions may economize by pairing students on each Bill-Board and/or by reducing the number of combinatorial reagents available.

## Experimental Design and Synthesis

Students design an array of six unique arylopeptoid products from the available reagents (Scheme 1). Working in pairs, students collaborate to choose reagents and generate a ChemDraw file with chemical structures of their products. Students choose either meta- or para-substituted rings for both rings in all compounds of the array. Students then choose at least three different amines (**1**−**10**) for use in reactions 2 and 4 (Figure 1): three unique amines are used for reaction 2, one in each of the three columns of the Bill-Board. For reaction 4, two different amines are used; these can replicate the amines used in reaction 2. Each student prepares all reaction solutions for reactions 1−4. Solutions for reaction 5, for the resin-cleavage reaction, and for the chloranil test are provided.

Both members of the pair prepare the same six compounds, but each individual prepares his/her own solutions and executes his/her own six reactions on a unique Bill-Board. Reaction progress is monitored by the colorimetric chloranil test: the prepared test solutions (see Supporting Information) are added to a few beads removed from the reaction vessel. A blue or green bead color indicates a secondary amine. Reactions determined to be incomplete are repeated. Products are cleaved from resin and collected into labeled vials.

To reinforce student understanding of the chemical transformations and simultaneously to utilize the long reaction times, student pairs present handouts prepared using ChemDraw software that detail mechanisms of these reactions and of the chloranil test.

## Student Analyses of Experimental Outcomes

Each student prepares one sample per reaction for analysis by liquid chromatography−mass spectrometry (LC−MS). Data are collected by the instructor or a laboratory assistant, then distributed to students. A lab lecture and handout introduces students to the conceptual background of this instrumental technique and guides them through data analysis. Students assess the success and reproducibility of their reactions and compare relative purities and polarities of the products.



DOI: 10.1021/acs.jchemed.5b00671
J. Chem. Educ. XXXX, XXX, XXX−XXX

Table 2. Selected Survey Response Data from 19 Students' Self-Reported Learning Gains

| Question | Average[a] | Standard Deviation |
|---|---|---|
| As a result of your work in this class, what gains did you make in your understanding of the following: experimental organic chemistry reactions on solid support? | 4.5 | 0.8 |
| As a result of your work in this class, what gains did you make in the following skills: analyze and interpret experimental data? | 4.4 | 0.7 |
| As a result of your work in this class, what gains did you make in the following skills: working with others | 4.4 | 0.7 |
| As a result of your work in this class, what gains did you make in the following: enthusiasm for chemistry? | 4.6 | 0.6 |
| As a result of your work in this class, what gains did you make in the following: confidence that you can be a good independent researcher in faculty-supervised research? | 4.3 | 0.7 |
| How much did the following aspects of the class help your learning: the instructional approach taken in this class (i.e., focus on research topics)? | 4.4 | 0.6 |
| How much did the following aspects of the class help your learning: class reaction mechanism presentations? | 4.4 | 0.8 |

[a]Scale: 1 = no gains/help, 2 = a little gain/help, 3 = moderate gain/help, 4 = good gain/help, 5 = great gain/help. From 19 students, 2013 and 2015 laboratory courses.

## HAZARDS

Because all chemicals used in these experiments are hazardous, students should have prior laboratory experience and safety training. Work should be carried out exclusively in fume hoods with sashes pulled to the lowest possible position, and students and instructors should wear gloves, lab coats, pants, shoes, and goggles at all times. Proper syringe technique should be demonstrated and used. 2-Chlorotrityl chloride resin is an irritant. Dichloromethane is a health hazard and a skin and eye irritant. DMSO is flammable. DMF, methanol, and acetaldehyde are flammable and health hazards; DMF is a potential reproductive toxin and methanol has acute toxicity. Acetic anhydride is flammable, corrosive, and has acute toxicity. 4-(Chloromethyl)benzoic acid is corrosive and an inhalation health hazard. 3-(Chloromethyl)benzoic acid, 3-(chloromethyl)benzoyl chloride, and 4-(chloromethyl)benzoyl chloride are corrosives and irritants. All amines are corrosive, and many are flammable. Isopropylamine and *N*,*N*-diisopropylethylamine also have acute toxicity. Chloranil is an irritant and toxic to the environment. 1,1,1,3,3,3-Hexafluoro-2-propanol is corrosive and an irritant. Products are new compounds; they should be assumed to have biological activity, and direct contact should be strictly avoided.

## RESULTS AND DISCUSSION

To emulate a research laboratory, students prepared reaction solutions using directions comparable to those in the literature. For example, for reaction 2, students were instructed to add 20 equiv of amine as a 2 M solution in DMSO. A worksheet to guide them through these challenging calculations was developed (Supporting Information). Calculations were checked by an instructor prior to the start of the experiments.

In the course of the most recent laboratory iteration, 42 syntheses were undertaken. Because two pairs' libraries coincidentally included two identical compounds, the synthesis of 40 unique molecules was undertaken. None has been reported in the literature previously, so reaction outcomes were unknown prior to this laboratory experiment. As is common in chemistry research, not all syntheses were equally successful (Supporting Information). The majority of syntheses (25, 60%) reproducibly furnished the desired product in at least 50% crude purity as evaluated by integration of the UV chromatogram. Another three compounds were prepared in more than 50% crude purity by one of the two partners. Fifteen compounds were prepared reproducibly in at least 80% crude purity. These results validated the robustness of these reactions and their suitability to the skill set of introductory organic chemistry students.

Analysis of the class data revealed some reactivity trends. Both meta- and para-substituted reagents are suitable for these reactions; both were present in the 15 purest compounds. All 10 amines available were used, but the 15 products prepared reproducibly with over 80% crude purity comprise only amines **1**−**6**; these represented the most reliable, efficient reagents for this synthesis. At least one of the 10 compounds prepared reproducibly in 50−80% crude purity contains one or more of the remaining amines (**7**−**10**). These amines were thus also competent reagents for these syntheses. Lower purities may be attributable to inexperience of the students or to product or reagent instability.

Results from the biological evaluation of student-prepared compounds in the author's research laboratory were communicated to students by e-mail after completion of the course. To date, no compounds that inhibit bacterial growth have been identified.

Assessment of student learning was carried out by weekly evaluation of laboratory notebooks and mechanism presentations. Because all students synthesized different and new molecules, detailed and organized record keeping was emphasized and carefully checked. Mechanism presentations contributed to their conceptual understanding of reactions in this laboratory, as suggested by student survey responses (vide infra).

Student learning in this laboratory was also assessed by analyzing survey responses for Student Assessment of their Learning Gains[27] (full instrument included in the Supporting Information). Students reported substantial gains in understanding of solid-phase chemistry, interpretation of data, and working with others (Table 2). Additionally, students reported gains in their enthusiasm for chemistry and confidence as researchers, and were uniformly strongly supportive of the research-based project and the mechanism presentations to help their learning.

## SUMMARY

A new laboratory experiment was developed that engages second-year undergraduate students in the combinatorial synthesis of novel, diverse molecules with potential bioactivity. This research-focused experiment represented an engaging way for students to prepare diverse molecules for application in the author's research program. Simultaneously, students were trained in modern techniques while augmenting their understanding of concepts typically taught in an organic chemistry sequence. Moreover, students learned essential research skills, including notebook keeping, experimental design, and analyzing new experimental outcomes.



DOI: 10.1021/acs.jchemed.5b00671
J. Chem. Educ. XXXX, XXX, XXX−XXX

## ■ ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available on the ACS Publications website at DOI: 10.1021/acs.jchemed.5b00671.

Student handouts with detailed procedures and example LC/MS data for these experiments (PDF, DOCX)
Instructor materials (PDF, DOCX)
Meta template (CDX)
Para template (CDX)
Post-lab student survey information (XLSX)

## ■ AUTHOR INFORMATION


### Corresponding Author

*E-mail: aafuller@scu.edu.


### Notes

The authors declare no competing financial interest.

## ■ ACKNOWLEDGMENTS

Financial support for this work has been provided by the Department of Chemistry and Biochemistry at Santa Clara University and by the National Science Foundation under CAREER Grant CHE1056520. I am also grateful to Owen Gooding for his assistance with LC−MS instrumentation, to John Hyde for assistance with lab preparations, to Erik Schaumann and Bridget Ralston, the undergraduate student TAs who have helped me in this class, and to the enthusiastic students who enrolled in the lab.

## ■ REFERENCES


(1) Sundberg, S. A. High-Throughput and Ultra-High-Throughput Screening: Solution- and Cell-Based Approaches. *Curr. Opin. Biotechnol.* **2000**, *11* (1), 47−53.

(2) Plunkett, M. J.; Ellman, J. A. Combinatorial Chemistry and New Drugs. *Sci. Am.* **1997**, *276* (4), 68−73.

(3) DeWitt, S. H.; Kiely, J. S.; Stankovic, C. J.; Schroeder, M. C.; Cody, D. M. R.; Pavia, M. R. Diversomers": An Approach to Nonpeptide, Nonoligomeric Chemical Diversity. *Proc. Natl. Acad. Sci. U. S. A.* **1993**, *90* (15), 6909−6913.

(4) Kirschning, A.; Monenschein, H.; Wittenberg, R. Functionalized Polymers—Emerging Chemical Tools for Solution-Phase Chemistry and Automated Parallel Synthesis. *Angew. Chem., Int. Ed.* **2001**, *40* (4), 650−679.

(5) Scott, W. L.; Denton, R. E.; Marrs, K. A.; Durrant, J. D.; Samaritoni, J. G.; Abraham, M. M.; Brown, S. P.; Carnahan, J. M.; Fischer, L. G.; Glos, C. E.; Sempsrott, P. J.; O'Donnell, M. J. Distributed Drug Discovery: Advancing Chemical Education through Contextualized Combinatorial Solid-Phase Organic Laboratories. *J. Chem. Educ.* **2015**, *92* (5), 819−826.

(6) Scott, W. L.; O'Donnell, M. J. Distributed Drug Discovery, Part 1: Linking Academics and Combinatorial Chemistry To Find Drugs for Developing World Diseases. *J. Comb. Chem.* **2009**, *11* (1), 3−13.

(7) Scott, W. L.; Alsina, J.; Audu, C. O.; Babaev, E.; Cook, L.; Dage, J. L.; Goodwin, L. A.; Martynow, J. G.; Matosiuk, D.; Royo, M.; Smith, J. G.; Strong, A. T.; Wickizer, K.; Woerly, E. M.; Zhou, Z.; O'Donnell, M. J. Distributed Drug Discovery, Part 2: Global Rehearsal of Alkylating Agents for the Synthesis of Resin-Bound Unnatural Amino Acids and Virtual $D^3$ Catalog Construction. *J. Comb. Chem.* **2009**, *11* (1), 14−33.

(8) Scott, W. L.; Audu, C. O.; Dage, J. L.; Goodwin, L. A.; Martynow, J. G.; Platt, L. K.; Smith, J. G.; Strong, A. T.; Wickizer, K.; Woerly, E. M.; Zhou, Z.; O'Donnell, M. J. Distributed Drug Discovery, Part 3: Using $D^3$ Methodology to Synthesize Analogs of an Anti-Melanoma Compound. *J. Comb. Chem.* **2009**, *11* (1), 34−43.

(9) Pohl, N. L. B.; Kirshenbaum, K.; Yoo, B.; Schulz, N.; Zea, C. J.; Streff, J. M.; Schwarz, K. L. Student-Driven Design of Peptide Mimetics: Microwave-Assisted Synthesis of Peptoid Oligomers. *J. Chem. Educ.* **2011**, *88* (7), 999−1001.

(10) Utku, Y.; Rohatgi, A.; Yoo, B.; Kirshenbaum, K.; Zuckermann, R. N.; Pohl, N. L. Rapid Multistep Synthesis of a Bioactive Peptidomimetic Oligomer for the Undergraduate Laboratory. *J. Chem. Educ.* **2010**, *87* (6), 637−639.

(11) Nichols, C. J. Automated Combinatorial Chemistry in the Organic Chemistry Majors Laboratory. *J. Chem. Educ.* **2010**, *87* (1), 87−90.

(12) Wade, P. A.; Rutkowsky, S. A.; King, D. B. A Simple Combinatorial Experiment Based on Fischer Esterification. *J. Chem. Educ.* **2006**, *83* (6), 927−928.

(13) Gung, B. W.; Taylor, R. T. Parallel Combinatorial Synthesis of Azo Dyes. *J. Chem. Educ.* **2004**, *81* (11), 1630−1632.

(14) Duarte, R.; Nielsen, J. T.; Dragojlovic, V. Synthesis of Chemiluminescent Esters: A Combinatorial Synthesis Experiment for Organic Chemistry Students. *J. Chem. Educ.* **2004**, *81* (7), 1010−1015.

(15) Wolkenberg, S. E.; Su, A. I. Combinatorial Synthesis and Discovery of an Antibiotic Compound. *J. Chem. Educ.* **2001**, *78* (6), 784−785.

(16) Miles, W. H.; Gelato, K. A.; Pompizzi, K. M.; Scarbinsky, A. M.; Albrecht, B. K.; Reynolds, E. R. An Indexed Combinatorial Library: The Synthesis and Testing of Insect Repellents. *J. Chem. Educ.* **2001**, *78* (4), 540−542.

(17) Birney, D. M.; Starnes, S. D. Parallel Combinatorial Esterification. *J. Chem. Educ.* **1999**, *76* (11), 1560−1561.

(18) Combs, D. J.; Lokey, R. S. Extended peptoids: A New Class of Oligomers Based on Aromatic Building Blocks. *Tetrahedron Lett.* **2007**, *48* (15), 2679−2682.

(19) Hjelmgaard, T.; Faure, S.; Staerk, D.; Taillefumier, C.; Nielsen, J. Efficient and Versatile COMU-Mediated Solid-Phase Submonomer Synthesis of Arylopeptoids (Oligomeric *N*-Substituted Aminomethyl Benzamides). *Org. Biomol. Chem.* **2011**, *9* (19), 6832−6843.

(20) Hjelmgaard, T.; Faure, S.; De Santis, E.; Staerk, D.; Alexander, B. D.; Edwards, A. A.; Taillefumier, C.; Nielsen, J. Improved Solid-Phase Synthesis and Study of Arylopeptoids with Conformation-Directing Side Chains. *Tetrahedron* **2012**, *68* (23), 4444−4454.

(21) Hjelmgaard, T.; Plesner, M.; Dissing, M. M.; Andersen, J. M.; Frydenvang, K.; Nielsen, J. Advances Toward Aromatic Oligoamide Foldamers: Synthesis and X-Ray Structures of Dimeric Arylopeptoids with Conformation-Directing Side Chains. *Eur. J. Org. Chem.* **2014**, *2014* (19), 3971−3975.

(22) Hjelmgaard, T.; Roy, O.; Nauton, L.; El-Ghozzi, M.; Avignant, D.; Didierjean, C.; Taillefumier, C.; Faure, S. Macrocyclic Arylopeptoids—A Novel Type of Cyclic *N*-Alkylated Aromatic Oligoamides Forming Nanotubular Assemblies. *Chem. Commun.* **2014**, *50* (27), 3564−3567.

(23) Worm-Leonhard, K.; Hjelmgaard, T.; Petersen, R. K.; Kristiansen, K.; Nielsen, J. Discovery of New PPAR$\gamma$ Agonists Based on Arylopeptoids. *Bioorg. Med. Chem. Lett.* **2013**, *23* (14), 4162−4165.

(24) Seymour, E.; Hunter, A.-B.; Laursen, S. L.; Deantoni, T. Establishing the Benefits of Research Experiences for Undergraduates in the Sciences: First Findings from a Three-Year Study. *Sci. Educ.* **2004**, *88* (4), 493−534.

(25) Russell, S. H.; Hancock, M. P.; McCullough, J. The pipeline - Benefits of Undergraduate Research Experiences. *Science* **2007**, *316* (5824), 548−549.

(26) Weaver, G. C.; Russell, C. B.; Wink, D. J. Inquiry-Based and Research-Based Laboratory Pedagogies in Undergraduate Science. *Nat. Chem. Biol.* **2008**, *4* (10), 577−580.

(27) Student Assessment of Their Learning Gains. www.salgsite.org, accessed December 4, 2015.




DOI: 10.1021/acs.jchemed.5b00671
*J. Chem. Educ.* XXXX, XXX, XXX−XXX