Combinatorial Synthesis and Discovery of an Antibiotic Compound

An Experiment Suitable for High School and Undergraduate Laboratories

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This paper describes an experimental exercise designed to introduce students to combinatorial chemistry as it is applied in drug discovery. Care has been taken to ensure that students perform all aspects of the discovery process: synthesis of libraries, screening for the desired activity, and deconvolution of the library to identify the active individual compound. The laboratory was designed for high school biology and chemistry classes as part of the Science Partnership Scholars program at TSRI. However, it is readily adaptable to an undergraduate laboratory course by expansion to include a discussion of reaction mechanism, alternate methods of library synthesis and deconvolution, and structure–activity relationships.

Background and Rationale

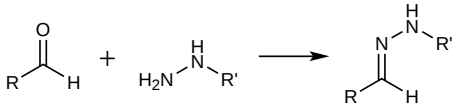
In the past 10–15 years, combinatorial chemistry has dramatically changed the paradigm for the discovery of novel compounds with desired properties (*1*). Although it has found application in materials science (*2*), catalysis (*3*), and molecular recognition (*4*), its greatest influence has been on drug discovery (*1*). In both academic and industrial laboratories, it has been used to generate huge libraries of novel compounds containing leads that inhibit enzymes, block receptors, bind DNA, and disrupt disease pathways. The method is a wonderful example of the fruitful interaction of chemical synthesis and biology and amply demonstrates the utility of interdisciplinary approaches to complex problems.

The distinguishing characteristics of combinatorial synthesis are its use of mixtures and its exploitation of the exponential relationship between the number of starting-material components and the number of products formed in a reaction. It is this relationship that is responsible for the astounding number of compounds in many libraries, sometimes in excess of 2,000,000 (*5*). The outstanding benefit of the method is its ability to identify active compounds in a mixture by performing fewer reactions and fewer tests for the desired property than if the compounds were synthesized and tested individually.

We sought to express these concepts in an experimental exercise in which students simultaneously prepare libraries of compounds, screen them for antibiotic activity, and deconvolute their libraries to identify the active individual compound. This approach, which emphasizes the use of mixtures, complements a previous report in this *Journal* for the demonstration of parallel synthesis (*6*).

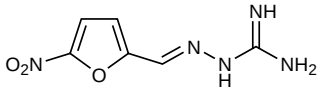
Combinatorial Synthesis of Hydrazones

We selected a two-component coupling reaction for library synthesis. This is the simplest reaction type upon which combinatorial libraries have been based, and it has been applied widely. We chose hydrazone formation as the basis of our library synthesis (eq 1) because it demonstrates the appropriate characteristics for combinatorial use: it is high yielding; there is little or no workup; there is no necessary purification. In addition, the reaction has practical benefits for use in an exercise: it proceeds well in water; no reagents are required; it is very fast; it is accompanied by a color change. All reactions in this exercise take place in solution phase. While much combinatorial work is performed on solid support, it complicates the experimental procedures and is unnecessary for demonstrating the combinatorial method.



(1)

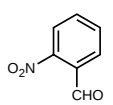
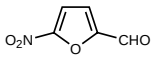
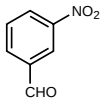
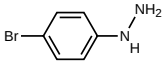
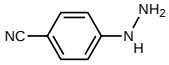
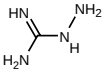
In the experiment, three aldehydes (A1, A2, A3) and three hydrazines (B1, B2, B3) are reacted in water to form six mixtures (M1–M6) of three hydrazones each. The starting-material aldehydes and hydrazines were selected to produce eight inert compounds and a single hit, the water-soluble nitrofuran antibiotic guanofuracin (structure **1**) (*7*). The six libraries are then screened for antibiotic activity against *Escherichia coli* using the cup agar diffusion method (*8*). The results of the antibiotic screens, visible after a 12–24-h incubation period, enable the deconvolution of the mixtures.



guanofuracin (**1**)

Tables 1 and 2 serve as visual aids in determining the contents of each mixture and in deconvoluting them. The part above and to the left of the lines in Table 1 represents the starting materials for the reactions carried out; the rest represents all the compounds synthesized. The same 3 × 3 matrix also describes all six of the mixtures produced (Table 2): mixtures M1–M3 are represented in the columns of the table,

Table 1. Aldehyde and Hydrazine Reactants and All Possible Hydrazone Products

	 A1	 A2	 A3
 B1	A1–B1	A2–B1	A3–B1
 B2	A1–B2	A2–B2	A3–B2
 B3	A1–B3	A2–B3	A3–B3

and mixtures M4–M6 are represented in the rows. When the column and row corresponding to the two mixtures that show antibiotic activity are shaded (as in Table 2), the intersection corresponds to the active compound (A2–B3).

The key insight emphasized in the data analysis is that nine hydrazones are synthesized and screened for antibiotic activity by carrying out only six reactions and six antibiotic screens. In the discussion of results, the concept and design of the experiment are extrapolated to larger systems (e.g., a 10×10 matrix containing 20 mixtures of 10 compounds each) and multiple hits. The advantages and disadvantages of the simple deconvolution method used are addressed, and comparisons are made among it and other deconvolution strategies.

Hazards

No significant hazards are associated with this experiment.

Conclusions

This experiment was favorably received by the high school chemistry and biology teachers who participated in the TSRI Science Partnership Scholars program. Many have adopted it for use in their classrooms, where it has proven successful and stimulating. Since a thorough understanding of the chemical reactions occurring is not necessary to appreciate the combinatorial methods demonstrated, teachers felt comfortable using the experiment with biology students who had not yet taken high school chemistry. The synthesis of libraries and setup of antibiotic screens requires about one hour, so the experiment fits well into high school schedules. In addition, the ready availability and safety of all required microbiology and chemistry materials proved particularly attractive to the teachers.

Table 2. Deconvolution of Hydrazone Libraries

	M1 ↓	M2 ↓	M3 ↓
M4 →	A1–B1	A2–B1	A3–B1
M5 →	A1–B2	A2–B2	A3–B2
M6 →	A1–B3	A2–B3	A3–B3

If antibiotic activity is observed in mixtures M2 and M6, the active antibiotic compound is A2–B3.

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[w]Supplemental Material

Further background material, detailed procedures for this experiment, and notes for the instructor are available in this issue of *JCE Online*.

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