Educationally Beneficial Enzymatic Organic Chemistry Laboratory Experiments

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Background

It may be that one of the greatest challenges within the teaching of Organic Chemistry (OChem) and other chemistry courses at the undergraduate level from an educational perspective is what to do with the lab section. While the lecture material is usually systematized, labs serve the purpose of trying to comprehensively introduce students to chemistry topics that might be of use and develop their proficiency in writing and performing such procedures. It is important to connect such chemistry topics to the prospective professions of the students to serve as many students as possible, which, in an OChem lab, leans quite heavily to the biological realm since most students are premedical in their degrees.

Enzymes are an obvious connection between the two fields, accomplishing reactions that are explicitly taught in OChem, and performing vital functions in the human body. Therefore, it seems wise to have students become acquainted with how enzymes can be used in catalyzing certain reactions. Additionally, at institutions like Liberty University, students in the biomedical sciences program have a biochemistry class without a lab section, so they cannot glean such experience there. In fact, at Liberty University in the 2023-2024 school year 87.5% of Biochemistry students were enrolled in Medical Biochemistry (no lab), with only 12.5% enrolled in Biochemistry I (with lab). It would be ideal then to have a lab in OChem in which the students use an enzyme to perform a reaction taught in OChem which forms or aids in the formation of a biologically significant molecule.

Moreover, as can be seen in Figure 2, the U.S. market for enzymes is very significant. Worldwide the market was estimated to be worth 60.48 billion USD in 2023. The U.S. market is growing considerably, meaning that there is demand for university laboratory material which addresses it.

Research Question

Besides the connection to the lecture material and extra concepts introduced, there are many other factors to be balanced when looking at prospective experiments like the cost of the reagents, safety/toxicity, length of the experiment, and level of difficulty. This research asks the question: Is there an enzymatic reaction experiment which practically could be imported into the OChem undergraduate class? It is desired that the experiments meets the qualifications listed above, with the hopeful goal of Liberty University employing such a procedure, making it immediately relevant.

Methods

Prospective experiments were taken from the Journal of Chemical Education and Journal of the American Chemical Society (Au). The various search terms and results are seen in Figure 1. Experiments were evaluated by a number of criteria. The length, cost, and toxicity of the experiments were evaluated to ensure they could be performed within the given time frame and budget with safety. Experiments were also evaluated based on the relevance of laboratory processes used such as thin layer chromatography and infrared spectroscopy, as well as the connection of the reaction mechanisms to those learned in the OChem lecture. The most applicable experiments were proposed.

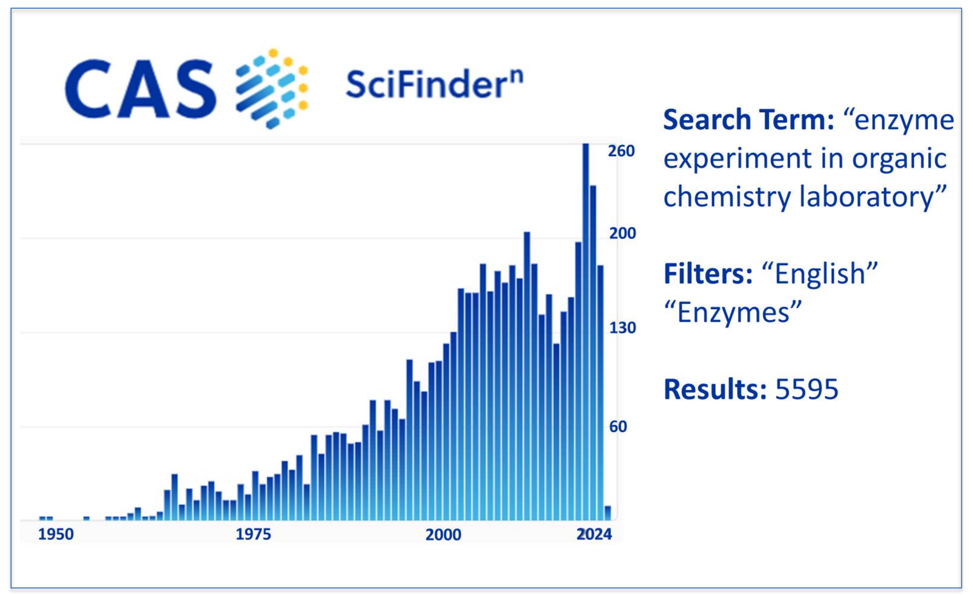

Figure 1. SciFinder literature search results.

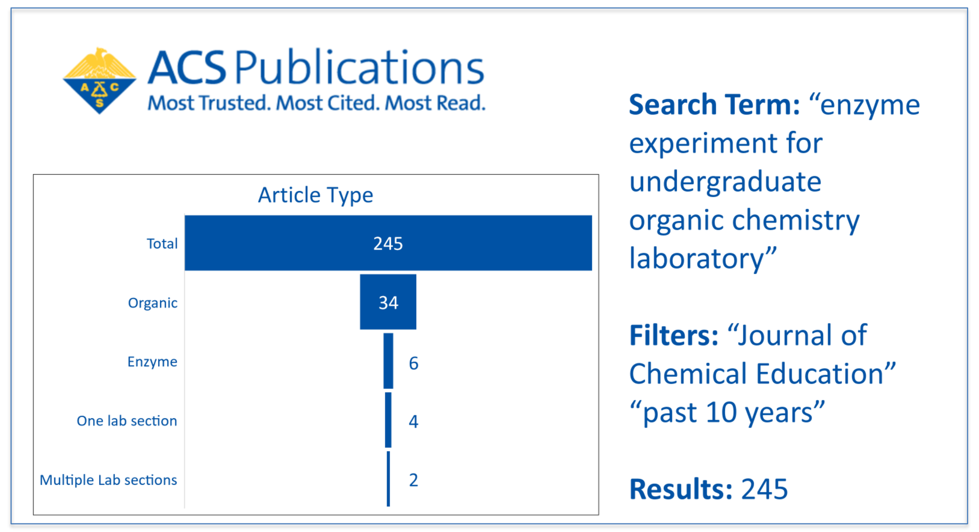

Figure 2. ACS Journal of Chemical Education literature search and filtering of results according to desired experiment parameters.

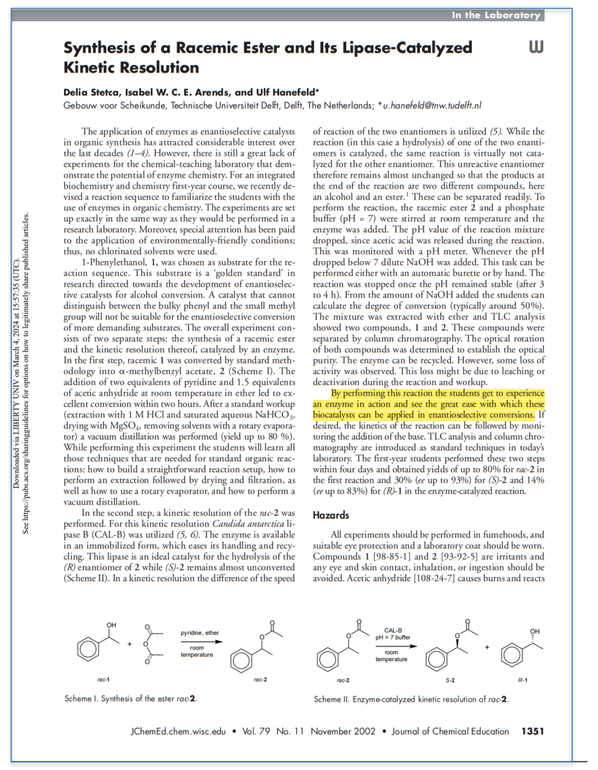

Figure 3. First page of Racemic ester experiment article

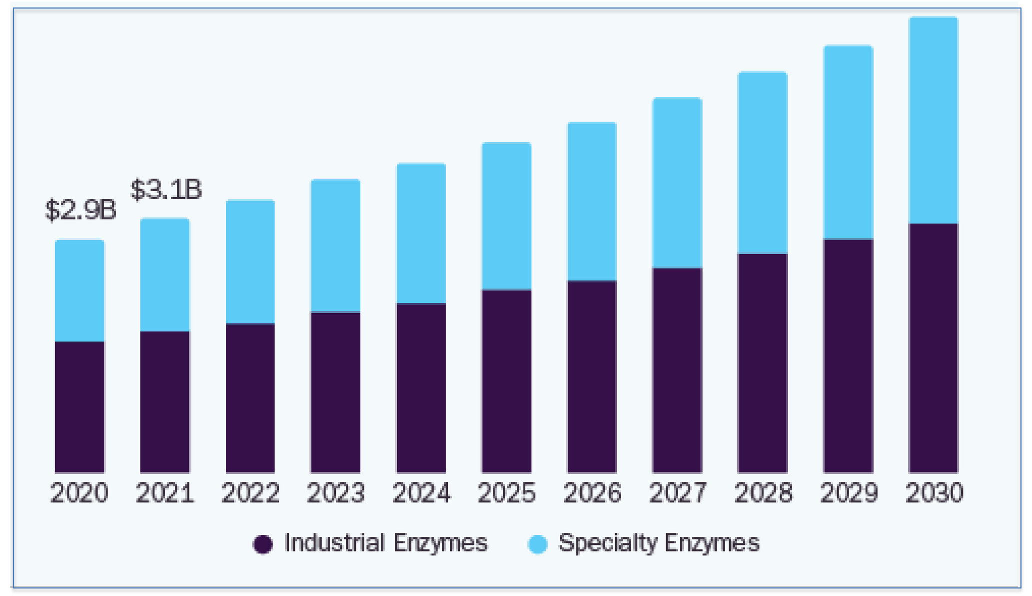

Figure 4. U.S. Enzyme Market Data. The projected compound annual growth rate (CAGR) of the market is predicted as 6.7%. [4]

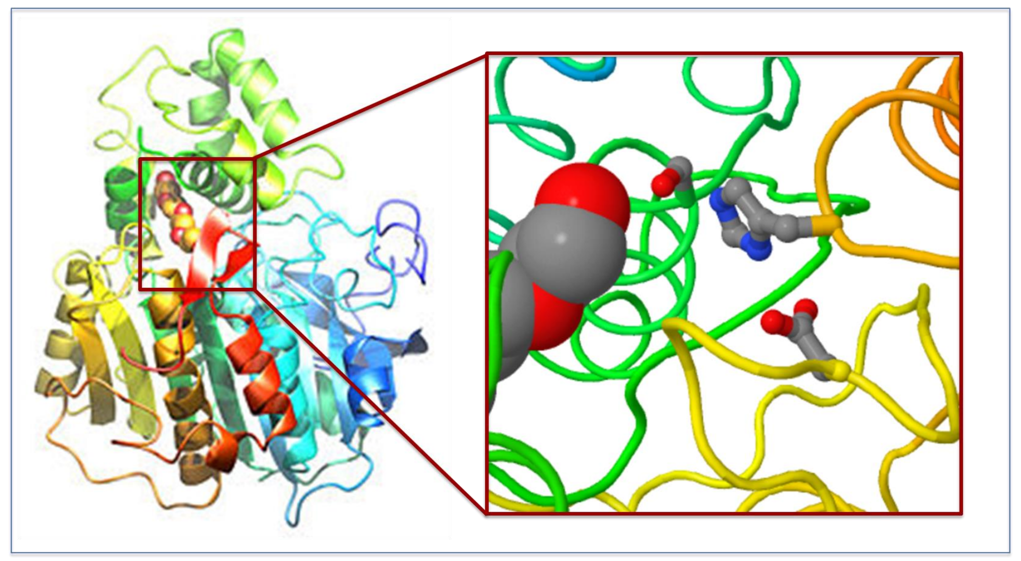

Figure 5. Enzyme structures of Lipase B full structure (left) and catalytic triad active site (right). [5]

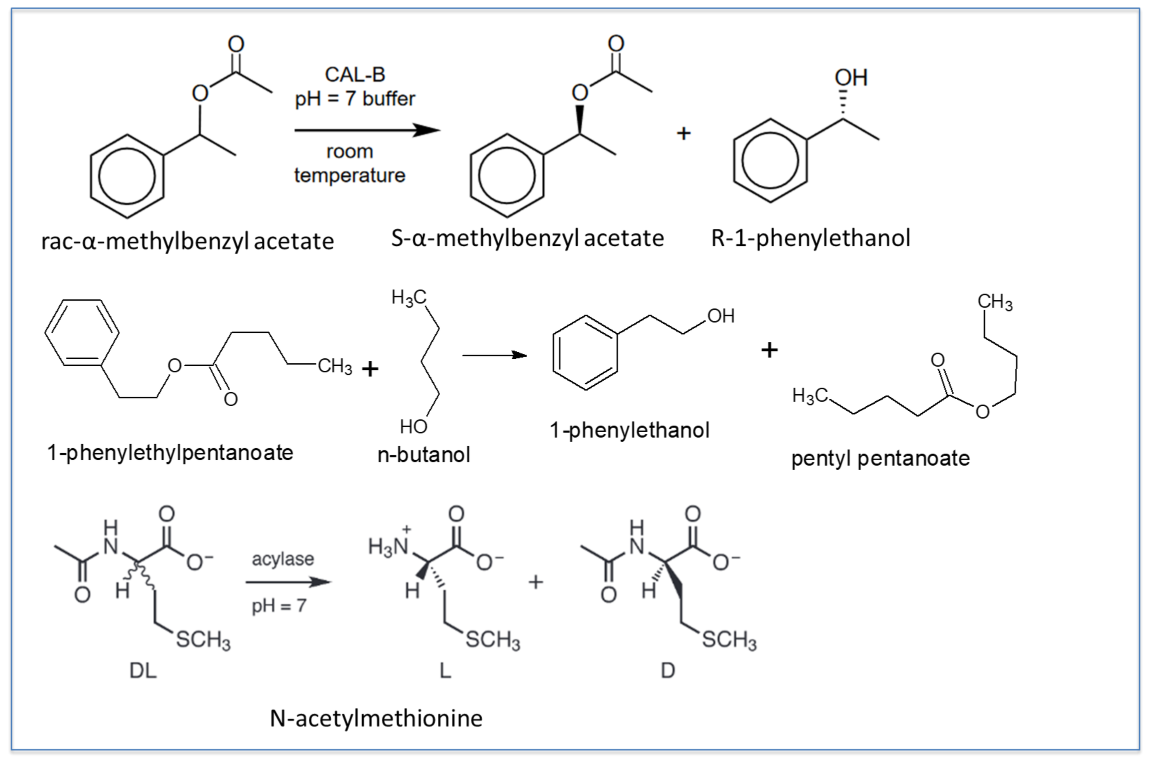

Figure 6. Reaction mechanisms for experiments with reagents of α-methylbenzyl acetate (top), 1-phenylethylpentanoate (middle), and N-acetylmethionine (bottom). [1-3]

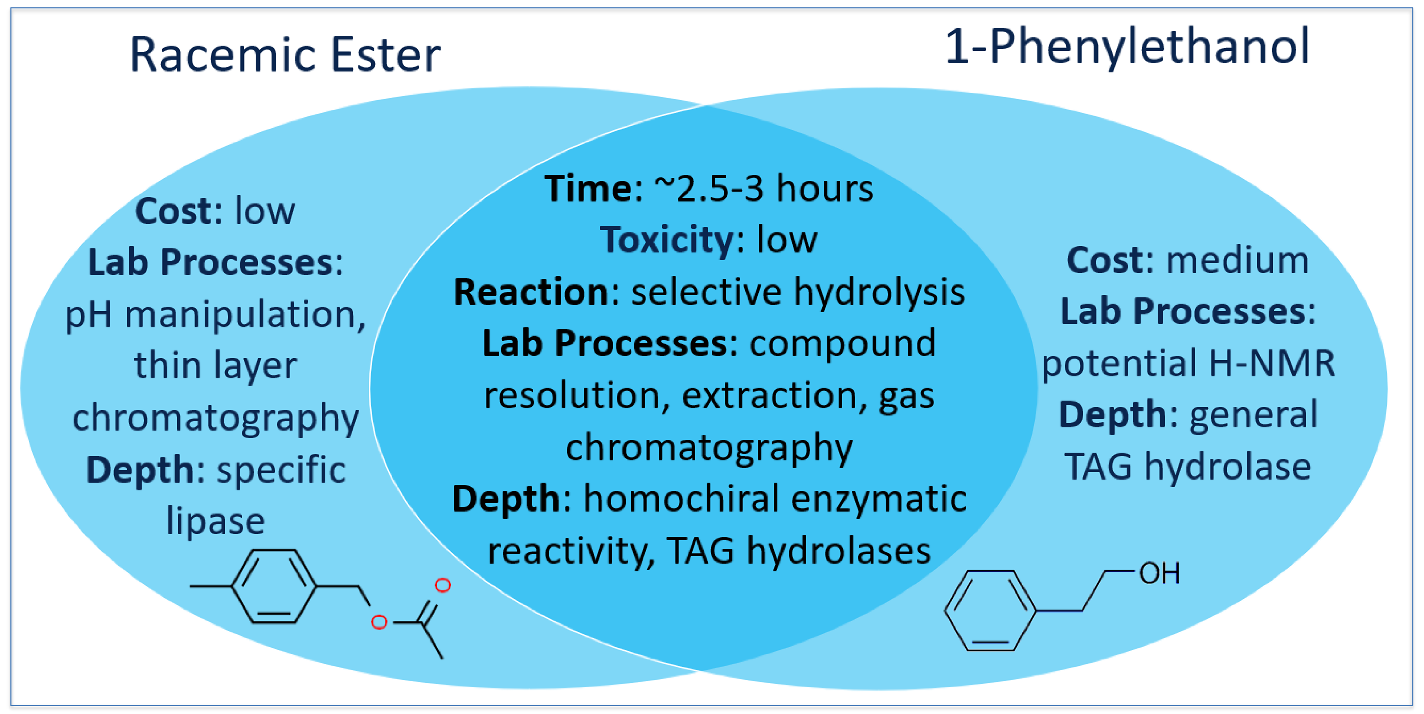

Figure 7. Venn diagram comparison of racemic ester and 1-phenylethanol experiments. [1,3]

Compound	Toxicity	Price
α-Methylbenzyl acetate	irritation to skin, eyes, and respiratory tract, may be harmful if swallowed or inhaled	$86.10 / 100 mL
CAL-B (Lipase B from *Candida antarctica*)	avoid skin contact or inhalation	$158 / 10 g
KH_2PO_4	LD 2,500 mg/kg	$110 / 500 g
NaOH	Causes severe skin burns and eye damage, may be corrosive to metals	$81 / 500 g
Ether	Extremely flammable liquid and vapor	$627 / 4 L

Table 1. Racemic ester chemical analysis data. The article did not provide set amounts of components to be utilized, so amount data was not included. While the experiment consists of multiple parts, the first of which is the synthesis of α-methylbenzyl acetate, only the second experiment was of interest so racemic α-methylbenzyl acetate was purchased and only the materials of the steps of interest were included. [3,6]

Compound	Amount	Toxicity	Price
Novozym 435	2 g / student	irritation to skin, eyes, and respiratory tract, may be harmful if swallowed or inhaled	$600/kg
n-Butanol	.820 g / student	irritation to skin, eyes, and respiratory tract, may be harmful if swallowed or inhaled	$155 / 4 L
Ether	35 mL / student	Extremely flammable liquid and vapor	$627 / 4 L
Pentanoic acid	10.5 g / student	Causes severe skin burns and eye damage	$77.10 / L
(1-Bromoethyl)benzene	13.95 g / student	irritation to skin, eyes, and respiratory tract, may be harmful if swallowed or inhaled	$184 / 100 g
Aliquat 336	3.03 g / per student	Causes severe skin burns and eye damage	$93.90 / 250 mL
KOH	66 g	Causes severe skin burns and eye damage, may be corrosive to metals	$30.40 / 25 g
Ethanol	225 mL	Highly flammable liquid and vapor, causes serious eye irritation	$163 / 1 L

Table 2. 1-phenylethanol experiment chemical analysis data. [1,6]

Results and Conclusions

The literature results (Figures 1-2) show that there are a plethora of experiments that could be chosen from when proposing an enzymatic OChem experiment. Among the three analyzed experiments, it was concluded that the racemic ester experiment [3] was the most profitable to be implemented. The experiment using N-acetylmethionine [2] was not ultimately profitable because of the high cost of D_2O for an H NMR step and calculations via Michaelis-Menten kinetics and Lineweaver-Burke plots which OChem students are not exposed to.

The racemic ester experiment and 1-phenylethanol experiment were very similar as seen in Figure 7, which has data obtained from Tables 1 and 2 [1,3]. Both experiments can be done within the given timeframe, have low toxicities, showcase a selective hydrolysis reaction of racemic mixtures, and even use similar laboratory processes. The way separation is evaluated can likely be chosen to fit the needs of the course, whether GC, TLC, or H-NMR. The 1-phenylethanol experiment employs general precision in laboratory techniques and would be best if Liberty University purchased and was desiring to utilize an H-NMR instrument. Nonetheless, the experiment requires more preparation since the 1-phenylethylpentanoate reagent seen in Figure 6 would be synthesized beforehand from potassium valerate and (1-bromoethyl)benzene [1].

The racemic ester experiment, as seen in Table 1, uses only five compounds, three of which are already commonly used in the OChem lab, and which are relatively inexpensive [3]. It has flexibility for manipulation of amount of reaction performed, ensures students practice proper lab safety while not being overly hazardous, and is environmentally friendly in that no chlorinated solvents are used.

This experiment also introduces students to enzyme chemistry in a way connected to biochemistry and biology classes the students have taken. The reaction site of Lipase B and its mechanism utilizes a lid with a catalytic triad which upon conformational change catalyzes the reaction (Figure 5). This mechanism exhibits elegant beauty by using a simple concept to great effect to catalyze reactions that would never occur under ordinary conditions. This same reaction and mechanism is used elsewhere to catalyze the hydrolysis of triglycerides to form glycerol and fatty acids. This major aspect of fatty acid metabolism would be of particular interest to biomedical students. Therefore, both experiments are proposed for implementation, but the racemic ester experiment shows the most promise.

Future Work

It is desired that the Liberty University Department of Biology and Chemistry considers using the racemic ester experiment in its Organic Chemistry laboratory section due to its multifaceted usefulness. More research should be done as to the logistics of implementation such as amount of reaction performed, which evaluative processes to employ, and place of implementation into the laboratory curriculum. A control experiment in which racemic α-methylbenzyl acetate has its (R) enantiomer hydrolyzed via *Candida antarctica* lipase B should be performed to confirm the functionality of the racemic ester experiment.

References

[1] Drouin, J., Costante, J., & Guibe-Jampel, E. (1997). A Thermostable Microbial Enzyme for Fast Preparative Organic Chemistry: The Preparation of (R-(+)-1-Phenylethanol from (+/)-1-Phenylethyl Pentanoate and n-Butanol. Journal of Chemical Education, 74(8), 992. https://doi.org/10.1021/ed074p992

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[3] Stetca, D., Arends, I. W. C. E., & Hanefeld, U. (2002). Synthesis of a Racemic Ester and Its Lipase-Catalyzed Kinetic Resolution. Journal of Chemical Education, 79(11), 1351. https://doi.org/10.1021/ed079p1351

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[5] https://proteopedia.org/wiki/index.php/Lipase_from_Candida_antarctica_in_closed_state

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[7] https://oakwoodchemical.com