Biocatalyzed Regioselective Synthesis in Undergraduate Organic Laboratories: Multistep Synthesis of 2-Arachidonoylglycerol

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ABSTRACT: In order to introduce the concepts of biocatalysis and its utility in synthesis to organic chemistry students, a multistep synthesis of endogenous cannabinergic ligand 2-arachidonoylglycerol (2-AG) was tailored for use as a laboratory exercise. Over four weeks, students successfully produced 2-AG, purifying and characterizing products at each stage of the reaction series. This synthetic series also reinforces the importance of executing reactions under inert atmosphere, the strategic placement of protecting groups, and regioselectivity.


INTRODUCTION

Biocatalysis is a methodology used to facilitate traditional chemical transformations.1,2 Enzymes can catalyze numerous reactions while their inherent active-site chirality can impart regioselectivity, chemoselectivity, and enantioselectivity. Such preferences can be crucial in the synthesis of biologically active molecules.3,4 Moreover, biocatalysis can simplify synthetic schemes and increase yields by reducing the number of steps required to achieve the final product. This is of particular benefit in medicinal chemistry and the pharmaceutical industry.4,5 Since the field of biocatalysis is of increasing importance to current synthetic methodology, the laboratory experiment presented exposes students to this area of study in both a theoretical and practical manner.

Recent literature in the field of endocannabinoids has provided the impetus for adapting a novel synthetic strategy to a series of reactions applicable to the undergraduate organic chemistry laboratory. These publications explore the enzyme-mediated synthesis of the cannabinergic ligand, 2-arachidonoylglycerol (2-AG).6–7 2-AG8–10 is of particular interest as the endocannabinoid system is an important target of drug design and development.11–13

The endocannabinoid system includes signaling molecules9,14–16 for cannabinoid receptors (CB1 and CB2)17–20 as well as the enzymes that are responsible for their biosynthesis and degradation.21–24 2-AG is the predominant signaling molecule able to interact with the CB receptors in a fashion similar to Δ⁹-tetrahydrocannabinol (THC), a classical cannabinoid isolated from the Cannabis sativa plant.

Synthesis of 2-AG has been critical to the study of endocannabinoid system receptors. This experiment therefore provides a means to expose students to this area of study and facilitate discussions concerning the endocannabinoid field, which is otherwise lacking in undergraduate curricula. While 2-AG is a biologically active molecule, it has poor bioavailability, which eliminates concerns regarding its safety in the student laboratory. Furthermore, as 2-AG is rapidly metabolized in vivo, the danger for potential abuse is negligible.8–10

The outlined synthetic sequence (Scheme 1) exposes students to the challenges of multistep reactions and regioselectivity, while providing practical experience with a lipase-mediated synthesis. Several experiments related to the application of biocatalysts in organic synthesis have been published25–32 as have numerous articles outlining multistep syntheses.33–38 However, this experiment emphasizes the advantages of utilizing enzymatic steps as part of a multistep synthetic strategy to produce a biologically relevant molecule that may otherwise be too expensive to synthesize or may not be easily obtainable using classical methods. This synthesis demonstrates to students the strategic placement of protecting groups to form a desired product in minimal steps.

Received: March 26, 2016
Revised: September 4, 2016
Scheme 1. Lipase-Mediated Synthesis of 2-Arachidonoylglycerol

Table 1. Student Experimental Yields

<table>
<thead>
<tr>
<th>Step in Series</th>
<th>Yield Range (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1: Synthesis of 1,3-dibutyrylglycerol (4)</td>
<td>9−40, average = 24%</td>
</tr>
<tr>
<td>2: Synthesis of 2-arachidonoyl-1,3-dibutyrylglycerol (5)</td>
<td>13−63, average = 32%</td>
</tr>
<tr>
<td>3: Synthesis of 2-arachidonoylglycerol (1)</td>
<td>34−60, average = 48%</td>
</tr>
<tr>
<td>Overall yield of 2-AG (1)</td>
<td>2−8, average = 4</td>
</tr>
</tbody>
</table>

*Each yield was calculated from the proceeding molecule.*

**EXPERIMENTAL SECTION**

A synthetic sequence derived in part from recent publications was specifically developed for the undergraduate laboratory. The initial optimization of the multistep synthesis was performed, in part, by two undergraduate students as an independent study project. In total, the students completed the synthesis three times. The reaction series was then carried out by eight pairs of second-semester organic chemistry students, split into two sections, as part of their introductory organic chemistry laboratory course. The synthesis was completed over four lab periods, each 4 h in duration. Initially, 1,3-dibutyrylglycerol (4) (Scheme 1) is synthesized by selective esterification of glycerol (2), using vinyl butyrate (3) in the presence of Candida antarctica lipase B. The resulting butyryl groups act to protect these sites for the subsequent arachidonate ester placement at the free sn-2 position to form 2-arachidonoyl-1,3-dibutyrylglycerol (5). Subsequently, the butyryl groups are selectively removed in a lipase-mediated transesterification reaction to yield 2-arachidonoylglycerol (1). TLC and $^1$H NMR analysis are performed in order to determine the identity and purity of the products at each step in the synthesis.

The procedure is a general method that can be applied effectively to a range of fatty acids. Due to the high cost of some reagents, primarily arachidonic acid, the laboratory exercise can be prohibitive for certain budgets. In order to circumvent these concerns, a more cost-friendly acid such as palmitic or oleic acid may be substituted with minimal modification to the experimental details. The use of anhydrous solvents and inert reaction conditions (for all three reactions) ensures that acyl migration caused by exposure to ambient moisture is averted. Inert reaction conditions also prevent air oxidation of the unsaturated arachidonate group, thereby hindering the formation of oxygenated byproducts. Detailed procedures and product characterization data are provided in the Supporting Information.

**HAZARDS**

Standard safety precautions should be practiced at all times. Students must wear safety goggles and gloves. Any skin or eye contact, inhalation, or ingestion should be avoided with all chemicals. Extra care must be taken when working with solvents used in reactions, purification, and spectral analysis such as dichloromethane, ethanol, $n$-hexane, ethyl acetate, and chloroform. While all organic solvents are flammable, dichloromethane and chloroform are suspected carcinogens, and $n$-hexane is a known neurotoxin. The reagent vinyl butyrate is flammable and capable of acute toxicity. $N,N$-dimethylaminopyridine (DMAP) is acutely toxic, and $N$-dimethylaminopropyl-$N'$-ethylcarbodiimide (EDCI) is both acutely toxic and corrosive. The hazards of the synthesized products are presently unknown, requiring students to use caution and treat them as hazardous.

**RESULTS AND DISCUSSION**

The synthetic sequence was incorporated into an introductory organic chemistry laboratory course. However, the exercise may also be appropriate for an advanced organic laboratory course with increased expectations and emphasis regarding conceptual understanding, yield maximization, and spectral interpretation.

Students were able to setup and execute reactions without undue difficulty, using the majority of the time allotted in the lab period. None of the students previously performed a true multistep synthesis. Accordingly, they learned to be aware of, and strive for, yield maximization to ensure requisite product quantities were achieved for subsequent reactions. The students’ yields (Table 1) at each step were noticeably, and expectedly, lower than those reported in the literature. In part, the low yields can be attributed to the less than optimal reaction times that were required in order to adhere to the proposed four-week timeline. Additionally, yields would have been higher if multiple chromatographic steps were implemented. However, due to time constraints, each product was only chromatographed once.

The pedagogical goals of this laboratory activity are 3-fold. First, students are expected to successfully navigate and
complete a multistep organic synthesis. Second, these experiments will cultivate students’ ability to purify crude compounds and assess the degree of purity by TLC. Third, students will gain practical experience analyzing and characterizing organic compounds using $^1$H NMR spectroscopy. The pedagogical goals of this laboratory activity were assessed by the following means and deemed satisfactorily met. Each student pair successfully synthesized the requisite quantity of each product in the multistep synthesis, ultimately enabling the synthesis of 2-AG. Students were able to purify crude compound and assess the degree of purity by TLC (Supporting Information). After column chromatography, students determined which fractions contained pure product (i.e., the presence of a single spot correlating to product $R_f$ value) and which fractions would benefit from further purification (i.e., the presence of multiple spots). Students were also able to use TLC to determine the progress of each reaction by observing the disappearance of starting material and the appearance of product after analyzing $R_f$ values. 1,3-dibutyrylglycerol (4) is characterized by an $R_f$ value of 0.45 (30:70 ethyl acetate/hexane). Once the arachidonate ester group is introduced in step 2, a product spot with a higher $R_f$ value (0.7, 20:80 ethyl acetate/hexane) should be observed. Upon deprotection of the primary hydroxyl groups in step 3, a spot corresponding to an $R_f$ value of 0.35 (50:50 ethyl acetate/hexane) should be observed in order to confirm product formation. Lastly, using $^1$H NMR analysis, students characterized their products by assessing the chemical shift, multiplicity, and integration of each signal of their spectra (Supporting Information).

To confirm the synthesis of 1,3-dibutrylglycerol (4), students assessed whether peaks corresponding to the ester-related protons were present around 4.1 ppm (two doublet of doublets, and a multiplet). Upfield peaks relating to the butyryl groups should also be observed: a triplet at 2.34 ppm, a sextet at 1.67 ppm, and a triplet at 0.96 ppm. Additionally, students were looking for the lack of vinyl proton peaks between 5 and 6 ppm, which would indicate residual vinyl butyrate starting material. For the spectrum of 2-arachidonoyl-1,3-dibutyrylglycerol (5), the appearance of peaks resulting from the addition of the arachidonate group should be observed: a multiplet ranging from 5.32 to 5.46 ppm, a multiplet around 2.8 ppm, a multiplet at 2.3 ppm, a quartet at 2.12 ppm, a quartet at 2.06 ppm, a multiplet at 1.74 ppm, a multiplet around 1.3 ppm, and a triplet at 0.9 ppm. The esterification also results in an observable shift in the sn-2 proton from 4.1 to 5.3 ppm. Finally, in order to confirm the synthesis of 2-arachidonoylglycerol (1), students should look for the slightly upfield shift of the sn-2 proton peak from 5.3 to 4.94 ppm. Additionally, the peaks relating to the protons of the butyryl groups should now be absent. Some students who lacked a strong background in $^1$H NMR analysis did find product confirmation more challenging. However, six of the eight pairs of students were able to successfully analyze the representative spectra.

**CONCLUSION**

An experimental procedure for the synthesis of 2-AG (1) was adapted from current literature as a second-semester undergraduate organic chemistry laboratory experiment. *Candida antarctica* lipase B was utilized in order to control the regioselectivity of the reactions. Use of the enzyme ensured that protecting groups were exclusively placed and subsequently removed from primary hydroxyl groups. Without the advantage of regioselectivity, the synthesis of 2-AG would be much more laborious, requiring additional steps and the use of multiple protecting groups. This laboratory exercise reinforced the utility of biocatalysis in medicinal chemistry, executing reactions under inert atmosphere, chromatography, and the challenges of performing a multistep synthesis, preparing students for future studies in science.

**REFERENCES**


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**Notes**

The authors declare no competing financial interest.

**ACKNOWLEDGMENTS**

We are appreciative of S. Pavlopoulos and C. Todd for their superlative guidance and support. Additionally, we would like to thank the students of Manhattanville College’s CHM 2006 lab class for their effort and enthusiasm.