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Laboratory Experiment

A Greener Synthesis of Glycerol Phenylbutyrate ("Ravicti")

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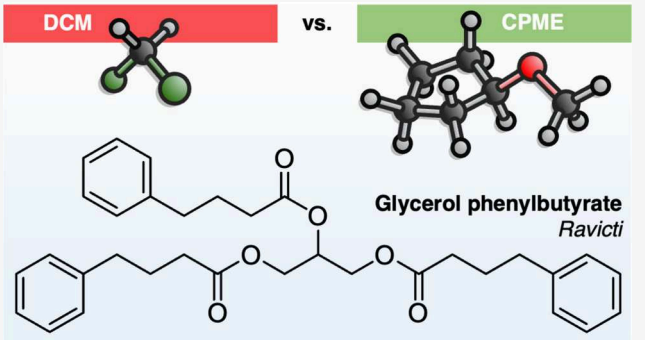
ABSTRACT: A laboratory experiment was developed to enable students to synthesize Ravicti, a drug used to treat Urea Cycle Disorders (UCDs). In response to legislation restricting the use of dichloromethane (DCM), otherwise known as methylene chloride, this synthesis uses cyclopentyl methyl ether (CPME) as a less hazardous, hydrophobic ether solvent. When implemented with students, they synthesized Ravicti in good yield and purity while discussing the adaptations to the traditional process together with the underlying reaction mechanisms. Students also determine relevant green metrics such as process mass intensity, which they use to quantify and explain the greenness of the conventional and adapted procedures.



KEYWORDS: *Upper-Division Undergraduate, Laboratory Instruction, Hands-On Learning/Manipulatives, Green Chemistry, Synthesis*

INTRODUCTION

It is critical that there is sustained effort to actively develop and integrate new resources into chemistry curricula to facilitate green chemistry instruction. Through this, the next generation of scientists, engineers and policymakers will be equipped with the ethos and tools to develop sustainable chemical manufacturing processes to minimize adverse effects on human health and environment. A holistic systems-based approach to instruction serves as an appropriate pedagogic framework to teach green chemistry; the application of the Twelve Principles of Green Chemistry, Life Cycle Assessment, and effective molecular design strategies are interdependent on one another and related systems.[1] Practical instruction is an integral component to chemistry education where significant work has been conducted to infuse green chemistry into laboratory courses and experiments.[2,3] To add to this portfolio of work, a laboratory experiment based on the synthesis of glycerol phenylbutyrate (1,2,3-propanetriyl tris(4-phenylbutanoate)), marketed as Ravicti, is described.

Ravicti is used to treat urea cycle disorders (UCDs) whereby individuals have genetic mutations in the enzymes involved in the urea cycle.[4] Such individuals cannot convert toxic ammonia (a metabolic waste product) into urea for excretion in urine. Instead, ammonia accumulates in the bloodstream which can cause neurological damage, seizures and death. Ravicti reduces blood ammonia levels, thereby treating the UCD. In addition to medicinal applications, Yazzie et al. report that glycerol phenylbutyrate synthesis is well-suited for undergraduate laboratory classes, due to its ease of production and use of low-cost reagents.[5] Interestingly, despite its low-cost synthesis, Ravicti was among the most expensive drugs sold in the U.S., costing patients approximately $700,000 annually.[6,7] Companies justify this by highlighting the rarity of UCDs, which affect only 1 in 35,000 newborns.[8,9] Thus, companies charge patients more to recuperate research and production costs from a small customer base.[10] However, some argue that the limited number of providers of Ravicti allow those companies to charge a high price point with minimal competition.[11] This may facilitate conversations in undergraduate classes about the ethics of overpricing orphan drugs like Ravicti by monopoly companies. Thus, glycerol phenylbutyrate synthesis, as shown in Scheme 1, is a beneficial reaction for undergraduates to study.

Current issues with published educational protocols of the synthesis of Ravicti include the use of dichloromethane (DCM) as a solvent.[5] This is highly problematic for deployment with undergraduate students (and more broadly) because since April 2024, the United States Environmental Protection Agency issued an intervention to significantly restrict the use of dichloromethane to reduce the risk to human health.[12,13] It was determined that DCM poses an unacceptable risk of cancer,[14] acute neurotoxicity, and chronic liver toxicity due to exposure arising from many of its uses.[15] Given this, there is an imperative for educators to find alternative solvents to DCM for implementation in teaching laboratories.[16] As such, in the case of the synthesis of Ravicti, alternative pathways have been explored such as through the

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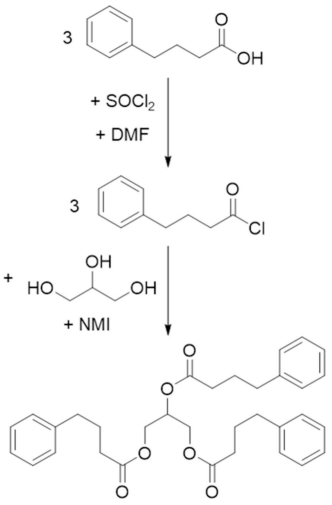
Scheme 1. Two-Step Synthesis of Glycerol Phenylbutyrate (Ravicti)[a]



[a]DMF is *N*,*N*-dimethylformamide (catalytic amount). NMI is *N*-methylimidazole.

adoption of carbodiimides as coupling agents to avoid the use of thionyl chloride and DCM but using heptane as a solvent.[17] There remains, however, significant issues from a green chemistry perspective of using toxic and wasteful coupling agents for undergraduate instruction.[18]

The laboratory experiment reported in this work serves as a greener alternative to the published educational literature, making use of alternative and greener solvents to DCM. It is suitable for implementation in an upper-division undergraduate teaching lab and can be used to introduce aspects of green chemistry while providing further experience of synthetic preparatory and characterization techniques. The learning objectives associated with this laboratory experiment are to

1. Safely and effectively use laboratory techniques such as rotary evaporation and column chromatography in the synthesis of glycerol phenylbutyrate.
2. Propose mechanisms for both steps of glycerol phenylbutyrate synthesis.
3. Evaluate the "greenness" of a reaction using quantitative green metrics (atom economy, reaction mass efficiency and process mass intensity) and appreciate their individual limitations.
4. Critically assess adjustments made to the method in terms of broader environmental and health impacts, using the 12 principles of green chemistry.

This experiment can be applied for students studying chemistry modules and is designed for upper division/third year students over 1 or 2 days. The latter can be enacted (if only half-day lab sessions of 3−4 h are available) by performing the second reaction with the acid chloride and glycerol on the second day. The second reaction could be performed either a few days or up to 1 week later. It is possible that the second reaction could be conducted with a longer interval between sessions though this was not tested. Additionally, if time is restricted, the experiment could be stopped ahead of performing chromatography such that students finish the day with the crude glycerol phenylbutyrate in around 4−5 h. In this work, following attaining ethical approval from the Institutional Review Board (reference: 2026_06_01), the laboratory experiment was implemented with a group of 10 upper division chemistry students that had completed Organic Chemistry II laboratory classes over a period of 2 half-days with an interval of 1 week. The success of the reaction was evaluated using the green chemistry metrics of Atom Economy, Reaction Mass Efficiency and Process Mass Intensity (PMI) where students compared how green the synthesis they performed was to the same reaction using dichloromethane.

INSTRUMENTATION

^{1}H nuclear magnetic resonance (NMR) spectra were obtained using a Jeol ECS-400 NMR spectrometer (400 MHz ^{1}H) at ambient temperature in chloroform-d. Spectra were referenced to the solvent signal. Infrared (IR) spectra were obtained using a PerkinElmer FTIR/FTNIR Spectrum 400 between 400 cm^{-1} and 4000 cm^{-1}.

Gas chromatography (GC) was performed using an Agilent Technologies 7890B GC, with a flame ionization detector (GC-FID), fitted with a Rxi-5HT capillary column (30 m, 250 mm × 0.25 mm nominal, max temperature 400 °C). Hydrogen was used as the carrier gas at a flow rate of 2 mL/min with a split ratio of 30:1 and a 1 μL injection. The initial oven temperature was 50 °C and was increased instantly at a rate of 30 °C/min to 300 °C and held at this temperature for 5 min, with a total run time of 13.3 min. Injection temperature was 290 °C and the detector temperature was 320 °C.

EXPERIMENTAL SECTION

All chemicals were used as received unless otherwise stated. In one embodiment, 1.34 g of 4-phenylbutyric acid in 1.3 mL of cyclopentyl methyl ether (CPME) is added 0.04 g of *N*,*N*-dimethylformamide dropwise, followed by dropwise addition of 0.76 mL of thionyl chloride in 0.7 mL of CPME over 5−10 min. The reaction mixture is stirred for 1 h at room temperature. Upon completion, the solution is concentrated *in vacuo* to yield 4-phenylbutanoyl chloride. To the isolated acyl chloride, 1.6 mL of CPME is added and the mixture cooled to 0 °C using an ice bath. A solution made of 0.15 g of glycerol from a fresh bottle and 0.68 mL of *N*-methylimidazole is prepared separately and added to the mixture over 1−2 min. A further 4.6 mL of CPME is added to dilute the resulting slurry and stirred for 1.5 h. Upon completion, the mixture is diluted with an additional 10 mL of CPME and extracted with 20 mL of water, 20 mL of 7% sodium bicarbonate, and a further 20 mL of water. The organic phase is dried and concentrated *in vacuo* to yield crude glycerol phenylbutyrate as a pale-yellow oil. The crude product is purified by column chromatography (15−25% ethyl acetate:*n*-heptane on silica) to yield glycerol phenylbutyrate as a colorless oil. Overall yields varied between students with an average of 65%.

^{1}H NMR (400 MHz, chloroform-d) δ ppm: 1.90 (6H, m), 2.31 (6H, m), 2.61 (6H, m), 4.12 (2H, dd, J = 12.0, 6.0 Hz), 4.28 (2H, dd, J = 12.0, 4.0 Hz), 5.25 (1H, m), 7.15−7.30 (15H, m).

IR ν_{max} (cm^{-1}) 3062.73, 3026.92 (aryl C−H stretch), 2947.93 (alkyl C−H stretch), 2861.57, 1736.36 (C = O stretch), 1603.35, 1496.72 (aryl C−C stretch), 1453.82 (alkyl C−H bend), 1416.32, 1373.11, 1333.37, 1305.43, 1238.52, 1156.08, 1134.16 (C−O stretch), 1098.77, 1081.99, 1030.63, 1003.89, 962.49, 910.52, 846.06, 810.97, 744.48, 698.23 (aryl C−H bend), 564.81, 491.57.

HAZARDS

All steps should be carried out in a fume hood. There are several chemicals with associated hazards that are highlighted in the risk assessment as part of the student lab script and technician guide in the Supporting Information. Particular care

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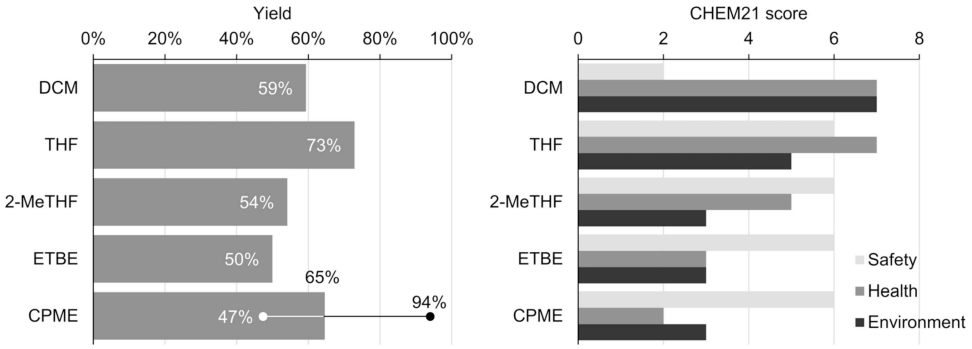

Figure 1. Left: Yields of glycerol phenylbutyrate in different solvents. Range in CPME represents lowest to highest yield obtained by students. Right: Scores from the CHEM solvent selection guide. Low scores are preferable.

and attention need to be taken when handling *N*,*N*-dimethylformamide (may damage the unborn child, H360), thionyl chloride (toxic if inhaled, H331), *N*-methylimidazole (toxic in contact with skin, H311). Flammable solvents are also used, and silica gel poses a chronic toxicity hazard if inhaled. Practitioners should also be aware of the evolution of gases in the first step of the reaction (sulfur dioxide and hydrogen chloride, both corrosive and toxic if inhaled, H331). Given the above, approval from the school safety officer should be sought when conducting this experiment with upper division undergraduate students.

DEVELOPMENT OF THE GREENER PROTOCOL

The procedure was adapted from that reported by Yazzie et al. in the following ways.[5] The scale of the reaction was reduced significantly to minimize waste and risk. The excess of 4-phenylbutyric acid over glycerol was increased slightly from 4 to 4.5 equiv to improve conversion. Using 5 and 6 equiv of 4-phenylbutyric acid did not increase the yield further. It was also observed that the formation of the acid chloride was more reliable when the reaction time was shortened from 90 to 60 min to negate incipient hydrolysis from trace moisture. The use of a freshly opened bottle of glycerol greatly improved the reliability of results which we attributed to the presence of water.

A solvent substitution was implemented to eliminate the use of DCM from both steps of the reaction. Yields were recorded in DCM for reference and compared to tetrahydrofuran (THF), 2-methyltetrahydrofuran (2-MeTHF), ethyl *t*-butyl ether (ETBE), and CPME. The first step, production of an acid chloride, reliably reached full conversion within 60 min, of which >90% was isolated. After the second step, the monoester of glycerol phenylbutyrate was a commonly observed impurity in all solvents. The average yield in DCM was 59% across both steps (Figure 1). THF resulted in a greater yield of 73% but isolated products were of lower purity, and the aqueous work up required the addition of a hydrophobic solvent (e.g., DCM). 2-MeTHF and ETBE were less effective than DCM, but CPME reliably produced yields of 65% (but in some cases as low as 47%).

The greenness of the solvents was evaluated with the CHEM21 solvent selection guide,[19] with safety, health, and environment scores recalculated for this assessment (Figure 1). Ethers were chosen because they are inert under the reaction conditions, although they are prone to form peroxides and must be stabilized and monitored. Conversely, DCM is regarded as safe in terms of flammability and stability. The purpose of substituting DCM is its suspected carcinogenicity and its role in workplace fatalities even after short-term exposure.[13] This has resulted in strict USA regulation making the use of DCM in the laboratory challenging to justify.[12] DCM is also very volatile which leads to fugitive emissions and consequently air pollution. Thus, DCM scores poorly in the CHEM21 solvent selection guide health and environment categories. Like DCM, THF is also a suspected carcinogen (H351) and so not a green substitute solvent. 2-MeTHF, ETBE, and CPME are somewhat opposite to DCM in the sense they are flammable and thus pose a safety risk, but have less severe health and environment hazards. CPME is the overall least concerning solvent overall and so was adopted as the solvent for both stages of the reaction.

CPME was also used as the extraction solvent for the aqueous workup. In a further optimization study, the number of aqueous washes was reduced from 5 to 3 with no change to purity. The necessity of column chromatography to purify the product is debatable. We observed a range of purities postworkup and so it is generally recommended to perform column chromatography. The *n*-hexane in a *n*-hexane-ethyl acetate solvent system was replaced with *n*-heptane to slightly lessen the health hazard. However, note that both solvent substitutions, in favor of CPME and *n*-heptane, introduce higher boiling point solvents, which will require more energy and time to remove using a rotary evaporator.

The final procedure was evaluated using Process Mass Intensity (PMI, the ratio between mass used and product mass). For comparison, data from Yazzie et al.[5] and a patented procedure[20] using the same reagents are included in Figure 2. Chromatography is the biggest contributor to waste, which is not used in the patented procedure. The majority of the waste is solvent. The PMI values are also affected by the scale of the reaction. In order to be able to effectively stir and separate the compounds, relatively more organic solvent and water is needed as the scale decreases. In our hands, this has meant an increase in PMI (most notably from the workup) despite the absolute quantity of waste being lower by virtue of working at almost an order of magnitude smaller scale. The PMI is sensitive to the yield, which will vary between students, because PMI is mass input divided by product mass. The PMI range was 393 g/g (65% yield) to 540 g/g (47% yield). At maximum efficiency (100% yield) the PMI would be approximately 250 g/g with two-thirds of the waste attributable to chromatography and a further quarter of the waste from the workup. Therefore, our modifications to the procedure have increased the relative mass of waste compared to the product in order to reduce the absolute quantity of

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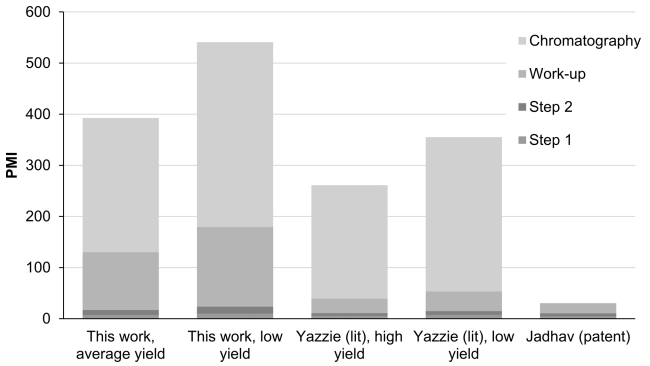

Figure 2. Process Mass Intensity (PMI) of the synthesis of glycerol phenylbutyrate in this work (meaning the average and lowest yield of students as given in Figure 1) and other reported instances in the literature. The yield range from this work is 47−65%, and in the literature 64−87%,[5] and 94%.[20] Chromatography conditions assumed the same across all applicable entries.

waste (from 1.9 to 0.2 kg per student based on scaling down the same procedure) and also eliminate the use of DCM.

IMPLEMENTATION WITH STUDENTS

To facilitate the consolidation and application of organic chemistry knowledge in the context of the laboratory experiments, through postlaboratory questions, students are tasked with determining the curly arrow mechanisms for both steps of the synthesis to include the catalytic formation of the acyl chloride and esterification of glycerol. Students are then asked to determine and compare relevant metrics (atom economy, reaction mass efficiency and process mass intensity) for their reaction and the equivalent synthesis using DCM. Finally, with this information students must evaluate the enhancements made from the previous synthetic route to the synthesis conducted as part of the laboratory experiment, making relevant links to the 12 principles of green chemistry. Student answers to these questions together with the purity and yield of the product were adopted as the assessment tool for this laboratory experiment.

The laboratory experiment was implemented with a group of upper division undergraduate chemistry students taking an organic chemistry lab class. The synthesis was repeatable with consistent yields and purities reported. The experiment was conducted over two half-day sessions where the second reaction between the acid chloride and glycerol was performed on the second day.

Students indicated that the pharmaceutical relevance of synthesizing Ravicti was appealing, while providing a glimpse into what a career within the pharmaceutical sector may consist of. The students fully characterized their products and answered the postlaboratory questions well. Most students successfully outlined the mechanisms for the formation of the acyl chloride and glycerol esterification. Students determined associated green metrics accurately and were able to make sensible comparisons between the synthetic protocols, drawing links to the 12 Principles of Green Chemistry. Through this, students were able to meet the learning objectives of the laboratory experiment well.

CONCLUSION

The synthesis of Ravicti has been adapted to make use of alternative and greener solvents to DCM whereby CPME was found to be the optimal solvent in both steps of the synthesis. This enables the synthesis of Ravicti to be achieved in undergraduate laboratories in response to changing legislation in relation to the permissible use of DCM. Further to students synthesizing a product used in the pharmaceutical sector, students are able to determine green chemistry metrics and use these as a comparator with previously published protocols to evaluate greenness. Furthermore, while green enhancements have been made in this new protocol, there are factors that limit how green the process is, offering the opportunity to highlight the compromises involved in enacting green chemistry through due consideration of the entire reaction system.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available at https://pubs.acs.org/doi/10.1021/acs.jchemed.5c01672.

Student lab script (PDF)
Instructor guide (PDF)
Technician guide (PDF)
Ravicti metrics (PDF)

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Notes

The authors declare no competing financial interest.

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