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Carbohydrate Experiments in the Organic Laboratory: A Robust Synthesis and Modification of Thioglycosides

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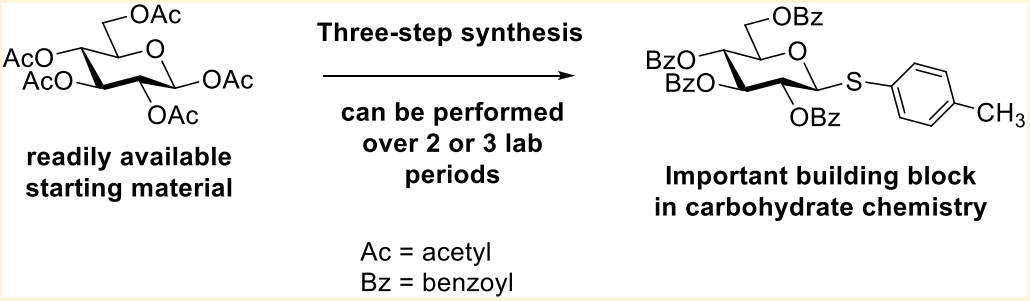
Supporting Information



ABSTRACT: A robust laboratory protocol for the three-step synthesis of *p*-tolyl 2,3,4,6-tetra-*O*-benzoyl-1-thio-β-D-glucopyranoside from commercial glucose pentaacetate is reported. The previously reported procedures have been modified to accommodate laboratories and students without proper training or equipment needed to conduct air-sensitive chemistry. The educational focus is on the various considerations and reactions that are applied in protecting group strategies. The three-step synthesis is designed to be performed over two 5-hour lab sessions but can be expanded to three sessions. Purification of all compounds is performed through crystallization. The reaction products are characterized using NMR spectroscopy, thin-layer chromatography, melting point, IR spectroscopy, mass spectrometry, and optical rotation. This newly developed protocol was performed by undergraduate students working individually in a 3000-level organic chemistry laboratory. The students generated products with high purity in acceptable yields (42−88%).

KEYWORDS: *Laboratory Instruction, Upper-Division Undergraduate, Organic Chemistry, Analogies/Transfer, Hands-On Learning/Manipulatives, NMR Spectroscopy, Bioorganic Chemistry, Carbohydrates, Stereochemistry, Synthesis*

The study of carbohydrates is one of the most rapidly growing fields in the scientific community today.[1] Previously only considered as an energy source, advancements in technology[2] and the development of sensitive analytical techniques have helped elucidate other important biological roles of carbohydrates. This improved understanding has led to their increased application as therapeutic and diagnostic agents.[3] Due to the great complexity of the mammalian glycome and the high level of diversity in microbial glycans, isolation of complex carbohydrates from natural sources in pure form is quite difficult and often low-yielding. Hence, chemical synthesis has come to the forefront as a mode to obtain glycans of interest for biological studies. Synthesis also allows for facile derivatization of a target compound, aiding in drug discovery and design.[4] Forming complex oligosaccharides from simple monosaccharide building blocks requires a carefully planned synthetic route involving various protecting and leaving group strategies.

The concept of protecting group strategies is widely applied in the synthetic field. Despite its applicability in both academic and industrial research, students' exposure to it at the undergraduate level is limited. Although typically mentioned in standard organic lecture classes, protecting group strategies are seldom applied in sophomore teaching laboratories. Because of their polyhydroxylated nature, carbohydrates make good models for demonstrating the variety and practicality of leaving and protecting groups. The synthetic demand for pure carbohydrate compounds has given rise to elegant protecting group strategies en route to synthetic targets.[5,6] These strategies are designed to take advantage of reactivity or conformational differences between free hydroxyl groups and, in doing so, selectively change the substituents. These structural modifications are done either to alter the compound's reactivity or to allow for selective functionalization in subsequent reactions.

In recent years a growing number of carbohydrate-based teaching lab experiments have been described.[7] These experiments range from relatively simple[8,9] to rather complex syntheses.[10−13] Unfortunately, these protocols typically require anhydrous conditions,[9,11−13] column chromatography,[11−13] long reaction times,[9] and/or form problematic side products,[10,11] ultimately limiting their utility to advanced students only. Presented herein is a description of a robust three-step

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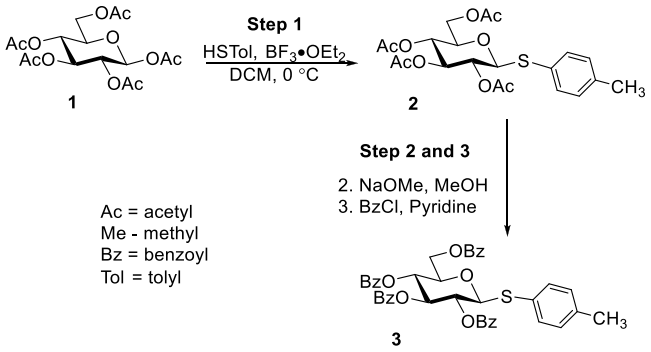
synthesis of *p*-tolyl 2,3,4,6-tetra-*O*-benzoyl-1-thio-β-D-glucopyranoside (**3**) (Scheme 1) that can be performed over two or

Scheme 1. Synthetic Route for the Preparation of Compound 3



three laboratory periods. Modifications of the previously reported protocols for the synthesis of this compound[14−16] allow these reactions to be performed without the need for anhydrous conditions or column chromatography, broadening the application of these reactions to teaching laboratories at all levels. Furthermore, common aspects of carbohydrate chemistry, including functionalization at the anomeric center and protecting group manipulation, are also covered, providing a useful connection to this important area of organic synthesis.

Compound **3** is a benzoylated tolyl thioglucoside that has appeared numerous times in the literature.[15,17] Benzoates are commonly used as protecting groups in synthesis because they are more stable than acetates toward many common reaction conditions, specifically in glycosylation. Compound **3** has been broadly used in carbohydrate chemistry as a glycosyl donor to introduce β-glycosidic linkages. The synthesis of this substrate can be followed using a variety of spectroscopic methods that confirm the structural identity of each compound. ^{1}H and ^{13}C NMR spectroscopy are two of the most common methods for structural identification in chemical synthesis.[18] Preparation of this compound provides multiple educational opportunities for the students from both a conceptual and lab technique perspective. This reaction sequence contains many potential discussion points and pedagogical aspects, such as chemoselectivity, stereoselectivity, and kinetic versus thermodynamic control.

■ EXPERIMENT DESCRIPTION

The following is a general experimental overview covering the techniques, educational aspects, and characterization methods for each reaction. More detailed protocols designed for student use can be found in the student handout in the Supporting Information.

Step 1: Introduction of the *p*-STolyl Anomeric Group to Pentaacetylglucose 1

The objective of the first experiment is to successfully introduce the *p*-tolylthio group (STol) at the anomeric position of commercially available 1,2,3,4,6-penta-*O*-acetyl-β-D-glucopyranose (**1**) (Scheme 1). The reaction proceeds under acidic conditions with boron trifluoride diethyl etherate (BF_3·Et_2O), which catalyzes the reaction. Although nearly all of the acetyl protecting groups will coordinate with the Lewis acid BF_3·Et_2O, only the anomeric (C-1) acetate will depart, ultimately being replaced by the *p*-thiocresol nucleophile. This is known as chemoselectivity, and it is due to the acetal functionality at the anomeric carbon (see the Supporting Information for more detail). The synthesis begins with the preparation of a solution of β-D-glucose pentaacetate **1** in bulk dichloromethane (DCM). An ice bath is used to cool the solution, and the remaining reagents, *p*-thiocresol (HSTol) and BF_3·Et_2O, are added. It is noteworthy that this reaction proceeds efficiently without the use of anhydrous conditions, dry DCM, and an inert argon atmosphere, which are commonly employed when reactions of this type are performed. The reaction progress is followed by thin-layer chromatography (TLC) and visualized by charring with 20% sulfuric acid in methanol solution, although the product can be observed using a UV lamp. Upon completion of the reaction and a subsequent aqueous workup, the product is purified by crystallization. Three different crystallization methods varying in times and yields (see the Supporting Information) have been developed for this experiment. The final product, *p*-tolyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranoside (**2**), shown in Scheme 1, is then used in the following session as the starting material.

Step 2: Removal of the Acetyl Protecting Groups on Compound 2 (Deprotection)

The final benzoylated product **3** is obtained through two synthetic steps performed during the second lab period. The first step involves deacetylation employing NaOMe in MeOH (Zemplén saponification conditions).[19] The reaction completion is confirmed through TLC by comparison of the starting material and product. The far more polar tetraol product has a lower R_f value than the starting material. Due to their similar polarities, partially deacetylated intermediates also have low R_f values, it is important to allow the reaction to proceed until there is only one signal on the TLC plate (solvent system suggestions are given in the Supporting Information). This short reaction, which typically requires 30 min, is quenched by neutralization with acidic ion-exchange resin. The latter is then filtered off and rinsed, and the combined filtrate is evaporated. After the first evaporation, the resulting oily residue is dissolved in DCM and evaporated again to afford a white solid. This second evaporation step is important for the removal of residual methanol, which can hinder the next reaction. The product can be used in the subsequent reaction directly; if desired, some material can be saved for characterization.

Step 3: Acylation of the Tetraol-β-STol Glucoside via the Installation of Benzoyl Groups (Protection)

Benzoylation can be performed during the same or the following lab period. The synthesis begins with the preparation of a solution of the tetraol *p*-tolyl thioglycoside starting material in reagent-grade pyridine. This solution is chilled in an ice bath before the addition of benzoyl chloride (BzCl). Progress of the reaction is monitored by TLC, and since pyridine has a high boiling point (115 °C), the TLC plate must be dried on the house vacuum for 5−10 min prior to development. This demonstrates to the students the importance of altering standard techniques in order to accommodate different reaction conditions. Following an acidic aqueous workup and evaporation, the oil residue is readily crystallized from hot ethanol.

Although the previously discussed experiments are designed for two 5-hour laboratory sessions, they can be expanded to three sessions depending on the needs of the class. This is

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accomplished by stopping after the deacetylation reaction. Similarly, these protocols may be performed significantly faster by classes with a smaller number of students. The focus of these reactions is on protecting group manipulation, which in this case is the conversion of the relatively labile acetyl groups to the more desirable benzoyl groups. To perform this modification, the acetyl groups are first removed in a deprotection step under saponification conditions. The newly unmasked free hydroxyl groups can then be protected in the benzoylation reaction, forming the targeted product **3**. In these reactions, the deprotection and protection steps are both esterification reactions. This demonstrates how the same type of reactions can be utilized in different ways for protecting group manipulations. These concepts, along with others, are discussed in greater detail in the Supporting Information.

HAZARDS

$BF_3{\cdot}Et_2O$ should be handled with care. This strong acid is often commercially sold with a Teflon seal, which requires the material to be withdrawn with a syringe and needle of adequate length. To avoid vacuum buildup in the bottle during the syringe withdrawal, the seal should be vented to an air source, such as a balloon. *p*-Thiocresol should not be weighed out hastily as residual compound can cause an unpleasant odor to linger. If a sulfuric acid in methanol TLC solution is to be made, it requires the use and dilution of concentrated sulfuric acid. In this case, the acid should be slowly poured into a volume of methanol. Once the acid has been added, the mixture must be allowed to cool to room temperature. During the workup of the third reaction (**2** → **3**), the sample is washed multiple times with 1 N aqueous HCl. Students should be made aware of accumulating pressure due to the presence of pyridine. In most cases the solvents employed in these reactions and for analysis are flammable, toxic, or carcinogenic. See the Supporting Information for full hazardous risks of all chemicals used.

RESULTS AND DISCUSSION

The final products of all reactions can be characterized using various analytical techniques. Practically, students are expected to confirm compounds **2** and **3** by NMR spectroscopy. NMR analysis of **2** in $CDCl_3$ shows the appearance of the aromatic and methyl peaks of the *S*-tolyl group at 7.35, 7.1, and 2.32 ppm, respectively. The β-orientation of the STol group can be confirmed by calculating the $J_{1,2}$ value to be 10.6 Hz. Additionally, a ^{13}C NMR spectrum can confirm the presence of the STol functional group through the appearance of the corresponding aromatic carbon signals (127.5−133.9 ppm) and the upfield shift of the anomeric carbon (86.2 ppm).[14] In a similar manner, product **3** is dissolved in $CDCl_3$ and characterized by ^1H NMR spectroscopy, specifically the appearance of additional peaks in the aromatic region, corresponding to the benzoyl groups (6.94−8.07 ppm).[14]

Prior to the start of the multistep synthesis, students are given the structure of the starting material **1** and the final product **3** and asked to search the literature for possible synthetic routes. The students meet with the instructor to discuss their findings. Although ultimately the same optimized procedure (listed in the Supporting Information) will be followed, this exercise gives students practical experience looking through the literature to find viable protocols. The document describing this exercise can be found in the Supporting Information. The developed synthesis was performed by an undergraduate student in a research lab setting under the supervision of a graduate student. Additionally, this protocol was reproduced in 3000-level undergraduate organic teaching lab classes of seven and 18 students working individually. The experiments were performed once by each student over the course of three lab sessions as described above. Upon completion, the students write a formal report. This report is assessed on the basis of their discussion of various topics such as the compound uses, building block synthesis in carbohydrates, mechanisms, and the overall process of multistep syntheses. In addition, students fully characterize and comment on each compound using NMR spectroscopy, melting point data, TLC (R_f values), IR spectroscopy, and optical rotation. In all cases students adequately performed both the prelab assignment and postlab report. The students enrolled in the 3000-level course were able to obtain yield averages of 64 and 59% and ranges of 42−87% and 24−85% for experiments 1 and 2, respectively.

Despite the robust and reproducible nature of the described protocol, some limitations or potential problematic steps were found by the undergraduate students. The major problems occur in the purification steps of the first and third reactions. In the case of the first reaction, STol introduction, some students noticed a pink color in their samples after crystallization. This color could be removed with subsequent washing with hexanes in most cases. However, two students were unable to remove this impurity, and the following week their samples had decomposed. It is possible that this impurity is due to partial oxidation of *p*-thiocresol to *p*-toluenesulfonic acid. This can be easily removed through additional washes in the separatory funnel. In the case of the third reaction, benzoylation, some students were unable to adequately remove pyridine during the workup. This is believed to have led to reduced yields upon crystallization.

The modified protocol for the synthesis of *p*-tolyl 2,3,4,6-tetra-*O*-benzoyl-1-thio-β-D-glucopyranoside (**3**) (benzoylated β-STol glucoside) from commercially available peracetylated β-D-glucose (**1**) has been developed (see the Supporting Information for specifics). This synthesis is designed as a multistep reaction (two 5-hour sessions) for the second-year organic teaching lab. This multistep synthesis requires students to be familiar with basic lab techniques (TLC, crystallization, analytical techniques) and common lab equipment (rotary evaporator, heat gun, house vacuum, basic glassware). Additionally, this protocol avoids more advanced techniques and/or additional equipment such as the utilization of dry solvents, inert atmosphere, and column chromatography purification.

Although all of the lab techniques performed are fairly basic, the multistep nature of this protocol requires students to be adequately skilled, specifically with crystallization, as it is applied twice. Considering this, the implication of this multistep lab would best occur toward the end of the course. This is more of a concern for second-year students rather than more advanced undergraduate students.

Students are provided with an opportunity to learn or improve their understanding of many basic principles and techniques of organic synthesis. An important aspect of laboratory growth occurs when students conduct the TLC analysis of the benzoylation step. Because the reaction is conducted in pyridine, the TLC plate must be dried prior to

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the development. This technique demonstrates the importance of understanding the reaction conditions.

The two conceptual lessons are focused around protecting and leaving group chemistry (protection/deprotection and chemoselectivity). These concepts can be applied to functionalization of many other compounds. More advanced topics, including neighboring group participation and kinetic versus thermodynamic control of the reaction, are discussed in the Supporting Information.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available on the ACS Publications website at DOI: 10.1021/acs.jchemed.9b00446.

Instructor notes, reaction notes, complete characterization and spectral data, and NMR spectra (PDF, DOCX)

Student prelab assignment (PDF, DOCX)

Report grading rubric (PDF, DOCX)

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The authors declare no competing financial interest.

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