Nucleoside Chemistry and Flash Chromatography: An Integrated Approach to Teaching an Organic Chemistry Laboratory

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Abstract: We report here the development of an integrated approach to teaching an organic chemistry laboratory. The laboratory exercise focused on the syntheses of two modified nucleosides and used the syntheses as a vehicle to teach organic chemistry from the point of view of how science is done in the real world. The project not only taught advanced organic bench techniques, but also drew heavily from the basics of molecular biology. The laboratory exercise was taken from the work accomplished in an industrial research laboratory and introduced during the spring semester of 1998. Each three person group was provided with a file containing the relevant literature and given the task of completing the synthesis and purification in a three week period. The groups were encouraged to handle the science and chemistry in a manner similar to a research group in an industrial or academic setting. Each group presented their results in a written paper and an oral presentation.

Introduction

We have implemented a laboratory that incorporates nucleic acid chemistry into an organic chemistry laboratory. The laboratory is interdisciplinary in nature and focuses on the synthesis of two kinds of modified nucleosides and their purification by flash chromatography. Using the published information on nucleoside chemistry and the data available from an industrial research laboratory the students synthesize 5'-*O*-dimethoxytrityluridine and 3'-*O*-benzoyluridine [1–4].

These syntheses were chosen for the following reasons. First, modified nucleosides have been used extensively to design a new class of gene inhibitors called antisense compounds [5–7]. Modified nucleosides have also been used to treat AIDS, and the target molecules herein have been used in the chemical synthesis of DNA [8]. Secondly, the synthesis requires the students to learn many important bench techniques commonly used for the organic synthesis of biologically important molecules, including thin-layer chromatography (TLC), flash chromatography, Schlenk line techniques, and rotary evaporation. Additionally, the relevant intermediates and products have been extensively characterized in the literature and NMR and other important data were available [1–4]. Moreover, these experiments were taken from an actual research project directed by the professor elsewhere, so the reaction products and intermediates have been previously characterized by mass spectrometry and NMR [2]. Additionally, the products are well-characterized physically by TLC and melting-point analysis. This makes the synthesis well-suited for a small-school setting where no NMR or mass spectrometer are available. Finally, the major equipment required for the laboratory is a rotovap, melting point apparatus, Schlenk line, forced air to the hoods, and drying ovens.

Results

As a starting point for the project, 5'-*O*-dimethoxytrityluridine was synthesized (Figure 1A). Uridine was chosen because it does not require the use of protecting groups and does not present the purification problems sometimes associated with the more complex purines [1, 4]. Moreover, tritylation at the 5'-hydroxyl was preferential due to the large size of the trityl group [2, 3]. Uridine (**1**) was combined with a slight excess of dimethoxytritylchloride (**2**) in pyridine to form 5'-*O*-dimethoxytrityluridine (**3**, DMTU). The reaction progress was easily followed by TLC and the product purity in the reaction mixture was estimated to be greater than 70%. The experimental yields were further quantified by weighing the residue after purification by flash chromatography (see below). Purity after chromatography was estimated by TLC at better than 98%; product yields, however, were about 50%, depending on the student group. One of the convenient features of the TLC was that the products could be visualized by using a hand-held UV lamp, and by exposing the plate to acid vapors, which turned the tritylated spots orange. It was thus very easy for students to identify DMTU. Furthermore, the ΔR_f for DMTU and uridine was 0.24, which made this compound an excellent candidate for purification by flash chromatography.

Once the students had successfully synthesized DMTU, the next step was to make 3'-*O*-benzoyluridine (3BU, **5,** see Figure 2). This reaction was complex and required more skill from the students and therefore was completed after the synthesis of DMTU. Uridine (**1**) was first converted to its dibutylstannylene derivative (**4**) to activate the 2' and 3' positions of the nucleoside [1, 4]. The first reaction was quantitative; yields were consistently better than 95%, as determined by weighing the resulting residue. The residue was pure, based on its melting point of 232–234 ºC and the fact that its recrystallization from ethanol did not change the melting point. These results were identical to those published [4]. Once the reaction was complete (see below), the benzoyl chloride was added directly and the reaction was allowed to proceed. The reaction progress was again easily followed by TLC and only one major product, **5,** was formed. The ΔR_f was 0.18 for 3BU, which facilitated purification of this compound

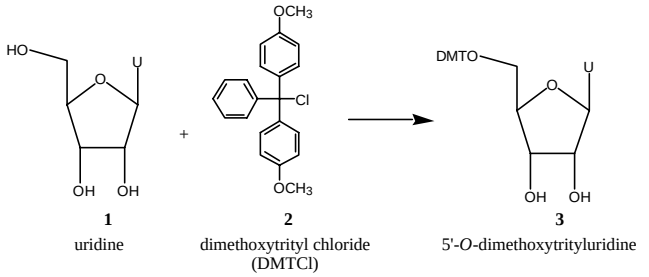

Figure 1. Synthesis of 5'-*o*-dimethoxytrityluridine, **3.**

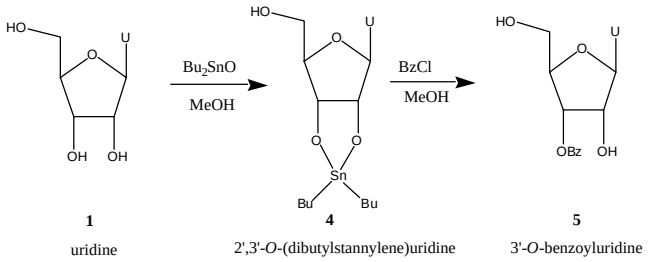

Figure 2. Synthesis of 3'-*o*-benzoyluridine, **5**. (BU_2SNO is dibutyltin oxide, BZCL is benzoyl chloride).

by flash chromatography. Although we have found that this compound can be purified directly using flash chromatography, the product yield and purity can be further analyzed by first partitioning the evaporated residue from the above between ether and water. The aqueous portion was concentrated by rotary evaporation and allowed to crystallize. Recrystallization from aqueous ethanol resulted in a product with a melting point of 211–215 ºC, which was consistent with published results [4].

As a final objective the compounds described above were purified by flash chromatography [9]. TLC was used to determine the conditions for the purification of DMTU and 3BU. The evaporated residue from each reaction was loaded onto its own column containing a 12-cm bed of silica gel and fractionated with 5% methanol in 99:1 dichloromethane:triethylamine (see below). Uridine is fully retained on the column under these conditions, while DMTU and 3BU were selectively eluted. Fractions containing the desired products were combined and concentrated in a preweighed round-bottom flask. Experimental yields were determined by weighing the residue; purity was evaluated by TLC with comparison to the appropriate standards.

Experimental Procedures

Materials. All reagents and solvents were purchased from either Sigma or Aldrich Chemical Co.

5'-O-Dimethoxytrityluridine (3). Anhydrous pyridine (50 mL) was transferred via cannula into a dry 250-mL round-bottom flask [3]. Uridine (2.0 mmol, 0.488 g) and dimethoxytritylchloride (2.2 mmol, 0.75 g) were added to the flask along with 1 mL of triethylamine and stirred for 1 hour at room temperature. The reaction progress was followed by TLC and product purity in the reaction mixture was greater than 70%. However, because the pyridine does not easily evaporate from the plate, it is recommended that the plates first be eluted with diethyl ether and then with 5% methanol/dichloromethane. Under these TLC conditions a single product was obtained (R_f 0.29). After the reaction was complete, the solution was evaporated to a gum using a rotary evaporator. The experimental yields were determined by weighing the residue obtained after flash chromatography. Purity of the purified residue was better than 98% based on TLC and the yields were 50% or better, depending on the student group.

2',3'-O-(Dibutylstannylene)uridine (4). Uridine (2 mmol, 0.488 g) and dibutyltin oxide (2 mmol, 0.500 g) were carefully added to 100 mL of anhydrous methanol that had been transferred into a 250-mL round-bottom flask using a cannula [4]. The mixture was refluxed employing a Friedrich condenser until the solution turned clear (30 min). The heat was removed and the solution was allowed to cool to room temperature. If the solution did not become clear then the reaction had not progressed to completion and the students did not proceed to the next step described below. This reaction is quantitative; uridine is essentially completely converted to the stannylene derivative [4]. This was confirmed by evaporating a small quantity and determining the melting point, which was 232–234 ºC. Moreover, the melting point of residue recrystallized from ethanol did not change.

3'-O-Benzoyluridine (5). Triethylamine (10 mmol, 1.4 mL) was added directly to the solution of **4** described above [4]. Benzoyl chloride (10 mmol, 1.2 mL) was added to the stirring mixture at room temperature. The reaction was allowed to proceed for 10 minutes and then was evaporated to dryness in vacuo. The reaction products were analyzed by TLC, eluting with 5% methanol/dichloromethane. A single reaction product was obtained with an R_f of 0.24. The purity of the product in the reaction mixture, based on TLC analysis, was estimated to be greater than 80%. The experimental yield, determined by weighing the purified residue after flash chromatography, was about 62%, depending on the student group. However, the product was further characterized by partitioning the residue (before flash chromatography) between ether and water (100 mL). The aqueous phase was concentrated to 30 mL and crystallized in an ice–water bath. The residue was recrystallized from ethanol and the residue gave a melting point of 213–214 ºC.

Flash Chromatography. The above products were separated and purified by flash chromatography in a 55 × 2.5-cm column with silica gel (grade 60, 230–400 mesh; Aldrich) equilibrated in 99:1 dichloromethane:triethylamine [9]. The products were loaded onto a 12-cm bed of silica gel and washed with 2 column volumes of 99:1 dichloromethane:triethylamine. The products of interest were eluted using 5% methanol in 99:1 dichloromethane:triethylamine. Fractions were monitored by TLC as described above. The fractions of interest were combined, evaporated to dryness, and stored under nitrogen for further analysis by TLC. Under these conditions uridine was fully retained on the column; DMTU and 3BU were readily eluted. Fractions containing the appropriate products were combined and concentrated to a gum in a preweighed round-bottom flask.

Safety and Other Tips. The students wore safety glasses and gloves for all the procedures described above. All reactions and experimental manipulations were carried out in the fume hoods. Glassware and silica gel were stored at 110 ºC prior to all reactions and chromatography. Although the reactions described herein were not extremely sensitive to moisture, anhydrous techniques were used for all experimental manipulations. It should be noted that some of the reagents and solvents described in this laboratory are toxic and must be handled with caution. Both dibutyltin oxide and benzoyl chloride are toxic; student use was monitored and carefully controlled. Benzoyl chloride was transferred using a syringe; dibutyltin oxide was weighed in the hood.

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