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**Book Title:** Comprehensive Organic Chemistry Experiments for the Laboratory Classroom  
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## 4.1.1.9. Glycal Transformation into Surfactant 2-Deoxy Glycosides

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Number of sessions (duration of each session)	Hazard level	Difficulty level	Level of study
3 (4 h + 4 h + 5 h)	Moderate	High	Advanced
<b>Classes names</b> Enol ethers, alcohols, acetals, esters			
<b>Concepts involved</b> Acid-catalysed addition of an alcohol to an acetylated glycal (sugar enol ether with a double bond between C1 and C2) for the synthesis of an alkyl 2-deoxyhexopyranoside, purified by column chromatography; Zemplén deprotection by transesterification affords the unprotected target glycoside			
<b>Chemicals needed</b> 3,4,6-Tri-O-acetyl-1,5-anhydro-2-deoxy-D-arabino-hex-1-enitol ( <b>1</b> ), dodecanol, dichloromethane (previously dried over 4 Å molecular sieves), triphenylphosphane hydribromide, sodium hydrogen carbonate, anhydrous sodium sulfate, cyclohexane (CyHex), <i>n</i> -hexane, ethyl acetate, sodium methoxide, methanol (previously dried over 3 Å molecular sieves), Amberlite® IR120 (H <sup>+</sup> form), deuterated methanol, silica gel 60 Å (0.040–0.630 mm), 10% H <sub>2</sub> SO <sub>4</sub> solution in MeOH			
<b>Equipment and experimental techniques involved</b> Rotary evaporator, heating magnetic stirrer with integrated temperature control (alternatively, a heating magnetic stirrer with contact thermometer), separation funnel, reflux condenser; thin-layer chromatography aluminium sheets pre-coated with silica gel 60 and fluorescent indicator UV <sub>254</sub> , column chromatography equipment, melting point measurement apparatus, NMR spectrometer			
<b>Keywords</b> Anomeric effect, Brønsted catalysis, column chromatography, 2-deoxy-glycoside, Ferrier rearrangement, NMR, oxycarbenium ion, stereoselectivity			

Comprehensive Organic Chemistry Experiments for the Laboratory Classroom

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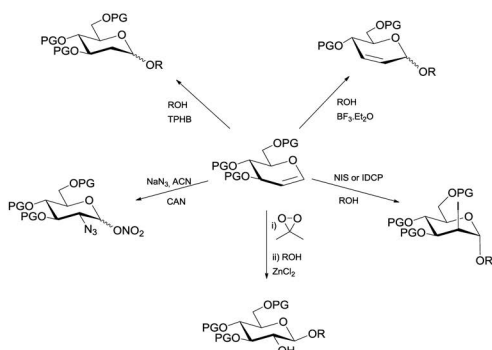
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## Background

Carbohydrates represent a unique family of polyfunctional molecules playing a role in tumour cell metastasis, infection, cell signalling, and fertilization, to name just a few examples of their importance in life processes. They have the potential to be chemically manipulated in a multitude of ways, being converted into compounds of interest for food, clothing, pharmaceutical, and agrochemical industries. Sugar-based surfactants are a versatile class of compounds, due to their low toxicity and biodegradation,<sup>1</sup> and their potential to interact with membranes, leading to several biological and medicinal applications.<sup>1,2</sup> The self-organization properties of these molecules depend on the nature of the sugar and the hydrophobic tail length, which therefore determine their application.<sup>3</sup> In particular, surfactant glycosides such as **5** and its analogues have proven to be biologically active, and their activity can be tuned by structural changes in the deoxygenation pattern and the anomeric configuration.<sup>2</sup> This protocol describes the stereoselective synthesis of 2-deoxy glycosides that affords, as major product, the bioactive anomeric configuration.

Glycosides are cyclic acetals involving the anomeric centre and their synthesis requires reaction of an activated glycosyl donor with an alcohol. Moreover, the glycosyl donor must be conveniently protected to avoid autoglycosylation. Noteworthy is also the anomeric effect, which results from the presence of electronegative substituents (X) at the anomeric carbon of a pyranose ring, which prefer to adopt an axial orientation rather than an equatorial one. This electronic effect occurs because there is a lone pair of electrons of the endocyclic oxygen anti-periplanar to the C-X bond; therefore, a partial donation of this lone pair to the anti-bonding ( $\sigma^*$ ) orbital of the C-X bond takes place, stabilizing the axial position of X.<sup>4</sup>

Amongst the structural diversity of glycosyl donors, 1,5-anhydro-2-deoxy-1-enitols (Figure 4.1.1.9.1), commonly referred to as glycals, have been recognized as versatile building blocks for the preparation of stereochemically rich molecules, the structural diversity and multifunctionality of which are an important asset for several applications and carbohydrate transformations. Their reactivity can be tuned towards the synthesis of 2-deoxy or 2,3-unsaturated sugars, depending upon the catalyst used. While  $\text{Ph}_3\text{P}\cdot\text{HBr}$  is known to catalyse sugar 2-deoxygenation,<sup>1-3</sup> other Brønsted and Lewis acids as well as heterogeneous acid zeolite catalysis afford mainly 2,3-dideoxy-2,3-unsaturated glycosides.<sup>5</sup> Moreover, glycals can be converted into 1,2-*trans* glycosides *via* epoxidation followed by treatment with an alcohol, or can react with nucleophiles after direct activation by *N*-iodosuccinimide (NIS) or iodonium di-*sym*-collidine perchlorate (IDCP), *via* nucleophilic opening of the intermediate cyclic iodonium ion. They can also be converted into 2-amino-2-deoxy sugars by reaction with sodium azide in the presence of ceric ammonium nitrate (CAN), followed by reduction (Figure 4.1.1.9.1).<sup>4,6</sup>



### Did you know...?

Glycals were given the suffix 'al' by Emil Fischer, who thought he had synthesized an aldehyde. Nowadays, the use of the terms glucal, rhamnal,... is not recommended by IUPAC, since these enol ethers do not exhibit the configuration of glucose, or rhamnose, inferred by the name.

**Figure 4.1.1.9.1** Usefulness of 1,5-anhydro-2-deoxy-1-enitols as building blocks in carbohydrate chemistry (PG: protecting group).

In this experiment, the synthesis of surfactant 2-deoxy glycosides *via* reaction of 3,4,6-tri-*O*-acetyl-1,5-anhydro-2-deoxy-*D*-*arabino*-hex-1-enitol (tri-*O*-acetyl-*D*-glucal, not recommended name) with dodecanol in the presence of triphenylphosphane hydrobromide (TPHB) is illustrated. The acid-catalysed addition of an alcohol to an acetylated glycal usually occurs with concomitant Ferrier rearrangement, giving the 2,3-unsaturated derivative as major reaction product. However, TPHB, first described by Bolitt *et al.*<sup>7</sup> to prepare 2-deoxy glycosides from glycals, circumvents Ferrier rearrangement. TPHB catalysed glycosylation is carried out under simple experimental conditions, affording sugar-based surfactants type 5 in a stereoselective manner and permitting an easy access to biologically important molecules.

## Additional Safety

All experiments should be carried out in a fume hood, with proper safety equipment, such as protective clothing, gloves, and safety goggles. Students are advised to consult GHS hazard and precautionary phrases for all reagents. Dodecanol and TPHB are harmful when in contact with skin and eyes (H314, H315, H319). Sodium methoxide is a flammable solid (H251), reacts violently with water (EUH014) and causes burns by ingestion or inhalation (H314). Therefore, it should be carefully handled.

## Experimental Procedure

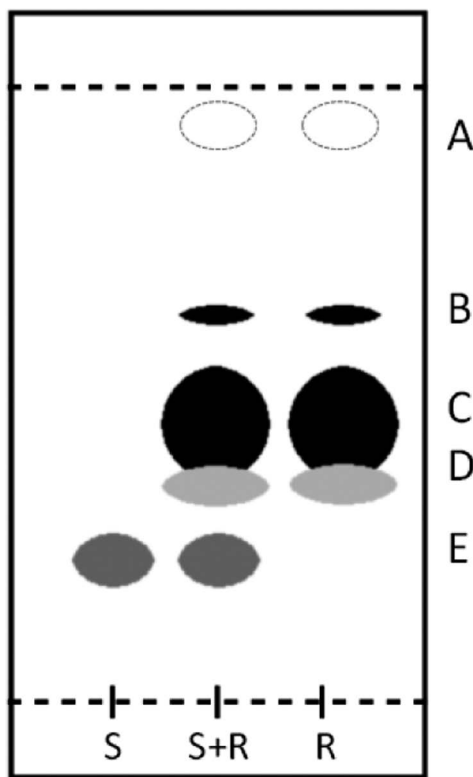
### Laboratory Session 1: Glycosylation Reaction (4 h)

1. In a fume hood, heat an oil bath to 40 °C on a heating magnetic stirrer with integrated temperature control (alternatively, a heating magnetic stirrer with contact thermometer can be used).
2. In a 25 mL round-bottomed flask equipped with a magnetic stir bar, prepare a solution of 3,4,6-tri-*O*-acetyl-1,5-anhydro-2-deoxy-*D*-*arabino*-hex-1-enitol (1, 0.500 g, 1.84 mmol) in dry dichloromethane (2 mL). Add dodecanol (0.43 mL, 1.93 mmol, 1.05 equiv.) and a solution of  $\text{Ph}_3\text{P}\cdot\text{HBr}$  (0.063 g, 0.184 mmol, 0.1 equiv.) in the same solvent (1 mL). Adapt a reflux condenser and stir the reaction mixture under reflux for 2 h 30 min. Check reaction completion by thin-layer chromatography eluted with CyHex/EtOAc (3 : 1), staining with a 10%  $\text{H}_2\text{SO}_4$  solution in MeOH, followed by heating at 120 °C (Figure 4.1.1.9.2).
3. Cool the reaction mixture to room temperature and dilute it with dichloromethane (10 mL). Wash the solution with a saturated  $\text{NaHCO}_3$  solution ( $2 \times 15$  mL) and then water (15 mL). After separation of both phases, dry the organic phase with anhydrous  $\text{MgSO}_4$ , remove the desiccant by filtration, and evaporate the solvent under reduced pressure.
4. Keep the residue (yellowish oil) at 0 °C until the next session.

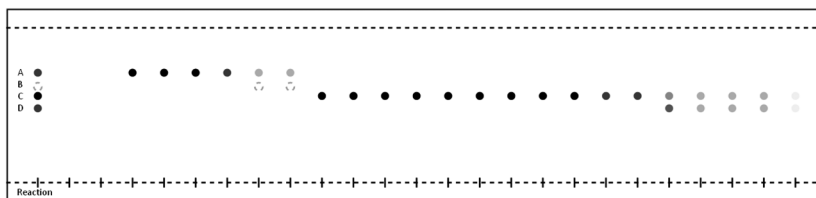
### Laboratory Session 2: Product Purification (5 h)

1. To isolate the products from the reaction mixture, assemble a chromatography column:
  - Prepare the eluent CyHex/EtOAc (15 : 1) (1500 mL).
  - Fill a chromatography column (3.5 cm diameter  $\times$  12 cm height) with silica gel (30 g) in CyHex/EtOAc (15 : 1).
  - Dissolve the reaction residue in eluent (*ca.* 3 mL), adding one or two drops of dichloromethane to assure complete dissolution, and apply it to the column chromatography.
  - After discharging *ca.* 80 mL of dead volume, collect fractions in a set of 10 mL vials.

2. Follow the course of the column chromatography by eluting TLC plates with EtOAc/CyHex (1:3). Stain eluted TLC plates by dipping them in a 10%  $\text{H}_2\text{SO}_4$  solution in MeOH, followed by heating at 120 °C (Figure 4.1.1.9.3).
3. Combine the fractions containing the pure dodecyl 4,6-di-*O*-acetyl-2,3-dideoxy- $\alpha$ -D-*erythro*-hex-2-enopyranoside (**4**,  $R_f = 0.71$ , EtOAc/CyHex (1:3)), in a 250 mL labelled and tared round-bottom flask and evaporate the solvent in the rotary evaporator.
4. Combine the fractions containing the pure dodecyl 3,4,6-tri-*O*-acetyl-2-deoxy- $\alpha$ -D-*arabino*-hexopyranoside (**2**,  $R_f = 0.58$ , EtOAc/CyHex (1:3)) in a 500 mL labelled and tared round-bottom flask and evaporate the solvent in the rotary evaporator.



**Figure 4.1.1.9.2** Schematic TLC of the reaction (R) and of glycal (starting material, S). A:  $\text{PPh}_3$  (only UV-visible); B: compound **4**; C: compound **2**; D: compound **3**; E: glycal **1**.



**Figure 4.1.1.9.3** Illustrative scheme of the column chromatography followed by TLC. A: dodecyl 4,6-di-*O*-acetyl-2,3-dideoxy- $\alpha$ -D-*erythro*-hex-2-enopyranoside (**4**); B: dodecanol; C: dodecyl 3,4,6-tri-*O*-acetyl-2-deoxy- $\alpha$ -D-*arabino*-hexopyranoside (**2**); D: dodecyl 3,4,6-tri-*O*-acetyl-2-deoxy- $\beta$ -D-*arabino*-hexopyranoside (**3**).

- Compound 2 will also be eluted from the column contaminated with the corresponding  $\beta$ -anomer, 3,4,6-tri-*O*-acetyl-2-deoxy- $\beta$ -D-*arabino*-hexopyranoside (3,  $R_f$  = 0.47; EtOAc/CyHex (1:3)). Combine the fractions with the  $\alpha/\beta$  mixture in a 500 mL labelled and tared round-bottom flask and evaporate the solvent in the rotary evaporator.
- Weigh the flasks with compounds 2, 2/3, and 4.
- Prepare two NMR samples, one containing the isolated compound 2 (30 mg) and the other containing the mixture of compounds 2 and 3, by dissolving them (30 mg) in  $CDCl_3$  (0.5 mL each). Transfer the solutions to two NMR tubes. Run a  $^1H$  NMR experiment on both samples.

### Laboratory Session 3: Deacetylation Reaction (4 h)

- Prepare a solution of sodium methoxide in dry methanol (1 M, 1 mL).
- In a 25 mL round-bottomed flask equipped with a magnetic stir bar, prepare a solution of compound 2 (0.150 g, 0.374 mmol) in methanol (2 mL) and add the previously prepared sodium methoxide solution (0.2 mL). Stir the reaction mixture at room temperature for 1 h 30 min.
- Neutralize the reaction mixture with Amberlite (IR-120,  $H^+$  form). Filter the resin off and evaporate the solvent.
- Recrystallize the residue from EtOAc/*n*-hexane (3:1) as follows: dissolve the residue in EtOAc (3 mL) and heat under reflux until complete dissolution. Add hexane (1 mL) and allow the solid to precipitate in an ice bath for 30 min. Filter the solid (dodecyl 2-deoxy- $\alpha$ -D-*arabino*-hexopyranoside, 5) under reduced pressure and take it to the high vacuum line for 30 min.
- Measure the melting point (mp 113.9–115.5 °C).
- Dissolve compound 5 (30 mg) in MeOD (0.5 mL) and transfer the solution to an NMR tube. Run  $^1H$  NMR,  $^{13}C$  NMR, COSY, and HMQC experiments.

### Waste Disposal

The neutralized aqueous solution may be diluted and discarded in the appropriate waste collectors. Dichloromethane, cyclohexane, *n*-hexane, ethyl acetate, and methanol should be collected in the appropriate waste collector recipients (chlorinated or non-chlorinated organic solvents, accordingly).

### Results Interpretation and Additional Questions

- Calculate the isolated yield for 3,4,6-tri-*O*-acetyl-2-deoxy- $\alpha$ -D-*arabino*-hexopyranoside (2) and the dodecyl 4,6-di-*O*-acetyl-2,3-dideoxy- $\alpha$ -D-*erythro*-hex-2-enopyranoside (4). Calculate the 2-deoxy glycoside yield obtained in the  $\alpha/\beta$  mixture and give its  $\alpha/\beta$  proportion, as determined by the anomeric signal integration of both anomers in the  $^1H$  NMR spectrum of the mixture.
- Justify mechanistically why the glycosylation reaction affords both  $\alpha$  and  $\beta$  anomers and comment on their relative proportion.
- Compare the melting point obtained for compound 5 with that given in the literature and justify any differences you may find.
- Using the mono and 2D NMR spectra acquired, assign both  $^1H$  and  $^{13}C$  NMR signals for dodecyl 2-deoxy- $\alpha$ -D-*arabino*-hexopyranoside. How do you recognize the anomeric configuration in the  $^1H$  NMR spectrum?
- If the starting material is a benzyl protected glycal, do you expect the Ferrier rearrangement to occur? Justify your answer based on the allylic rearrangement mechanism.

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