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Book Title: Comprehensive Organic Chemistry Experiments for the Laboratory Classroom
Chapter: 48

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3.1.17. Cyclic Acetals for Regioselective Protection in Carbohydrate Synthesis: A Comparative Experiment

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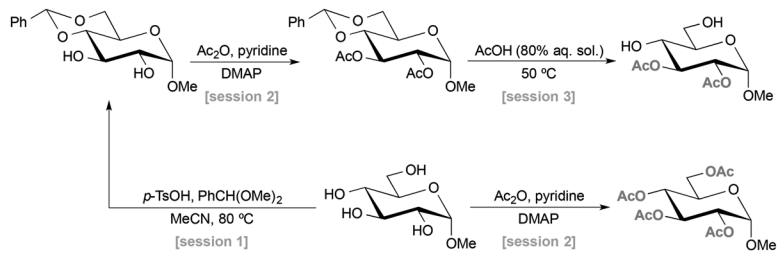

Number of sessions (duration of each session)	Hazard level	Difficulty level	Level of study
3 (4 h each)	Moderate	High	Intermediate

Classes names Alcohols, acetals, esters

Concepts involved This multi-step synthetic route is based on the selective protection of methyl α-D-glucopyranoside at positions 4 and 6 with a benzylidene acetal, acetylation of the remaining free hydroxy groups and selective benzylidene acetal hydrolysis. This protocol illustrates the importance of the benzylidene acetal and of its hydrolysis for sugar regioselective manipulation. Comparison of the target compound's NMR data with those of the peracetylated methyl α-D-glucopyranoside is the starting point for a valuable discussion on concepts such as multifunctionality, regioselectivity and the usefulness of acetal protecting groups in carbohydrate chemistry

Chemicals needed Methyl α-D-glucopyranoside, *p*-toluenesulfonic acid monohydrate, benzaldehyde dimethyl acetal, acetic anhydride, pyridine, 4-(dimethylamino)pyridine, hydrochloric acid (2 M aq. sol.), sulfuric acid, sodium chloride (sat. aq. sol.), sodium hydrogen carbonate (sat. aq. sol.), anhydrous magnesium sulfate, acetic acid (80% aq. sol.), petroleum ether (40–60 °C), dichloromethane, acetonitrile, toluene, methanol, ethyl acetate, deuterated chloroform, silica gel 60 Å (0.040–0.630 mm)

Comprehensive Organic Chemistry Experiments for the Laboratory Classroom
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Equipment and experimental techniques involved 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 precoated with silica gel 60 and fluorescent indicator UV_{254}, column chromatography equipment, melting point measurement apparatus, NMR spectrometer
Keywords Carbohydrate chemistry, column chromatography, cyclic acetal, NMR, regioselectivity

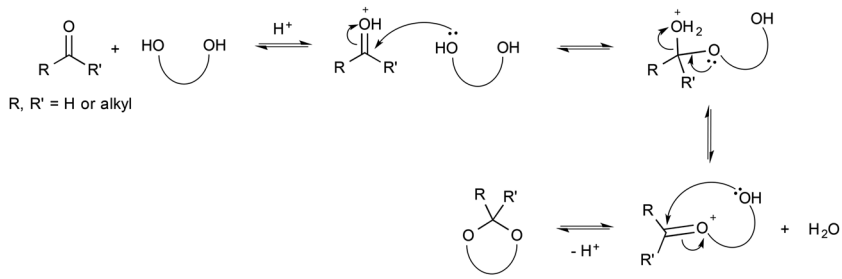
Background

The vast number of structural and functional roles of carbohydrates in living organisms makes them exceptional lead scaffolds for drug discovery. Indeed, it is possible to manipulate their natural interaction with biological targets and that is one of the main reasons why carbohydrates are broadly preferred as synthetic raw materials, and their stereochemistry and multifunctionality are crucial for the conception of selectively functionalized intermediates leading to a wide diversity of organic structures.[1–5]

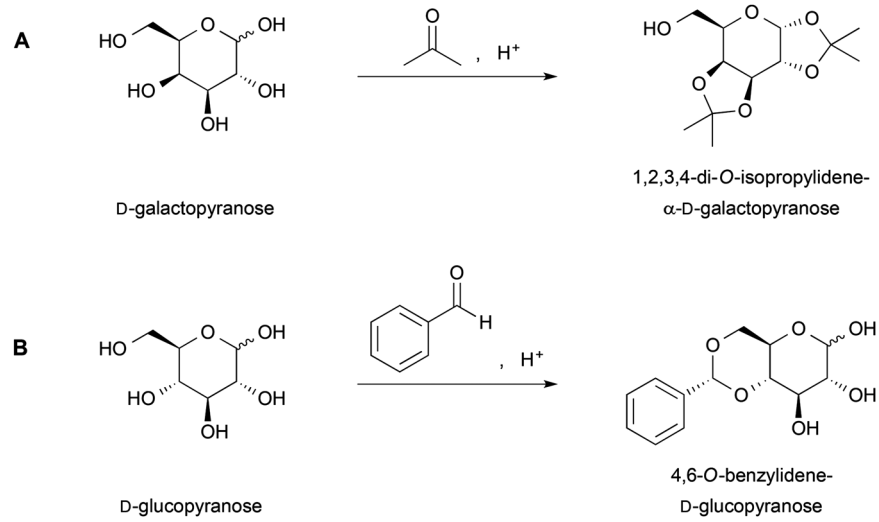
The use of protecting groups to allow regioselective reactions of hydroxy groups is, therefore, particularly important in synthetic carbohydrate chemistry. While benzyl ethers and esters, such as acetates or benzoates, are commonly used for the indiscriminate protection of primary, secondary or anomeric hydroxy groups, stereochemically hindered trityl or silyl ethers are usually preferred for the selective protection of the primary hydroxy group.[2]

When it comes to regioselective and simultaneous protection of more than one hydroxy group in the sugar, building a cyclic acetal is the correct choice. Its formation results from the acid-catalysed reaction of two suitably oriented hydroxy groups with aldehydes or ketones (Scheme 3.1.17.1). While the former class of compounds preferentially forms six-membered ring acetals involving the primary hydroxy group, ketones form five-membered ring acetals to avoid the unfavourable alkyl group in an axial position.[3]

Isopropylidene and benzylidene groups are commonly used for carbohydrate acetal protection. Whereas the five-membered isopropylidene is formed by reaction of acetone with *cis*-arranged vicinal hydroxy groups (Scheme 3.1.17.2A), the six-membered benzylidene acetal is typically used for regioselective 4,6-*O*-protection of carbohydrates, affording products with free hydroxy groups at positions 1, 2 and 3 (Scheme 3.1.17.2B),[3] which become available for further transformations. Although benzylidene acetal formation employs benzaldehyde as the carbonyl reagent, transacetalation with benzaldehyde dimethyl acetal has proven just as effective and requires much less harsh reaction conditions.[4]



Scheme 3.1.17.1 Mechanism for the acid-catalysed reaction of aldehydes or ketones with diols to give cyclic acetals.[3]



Scheme 3.1.17.2 D-Galactopyranose and D-glucopyranose protection with (A) isopropylidene group and (B) benzylidene group.

Another major advantage of cyclic acetal protection is the possibility of selective acetal removal. For example, the commercially available methyl α-D-glucopyranoside is itself an acetal and, therefore, labile to acid hydrolysis. However, when protected with benzylidene or isopropylidene groups, the use of mild acidic conditions permits isopropylidene or benzylidene acetal deprotection, keeping the integrity of the anomeric centre, which requires more acidic conditions to be hydrolysed.[5]

This short project is intended to enrich the education of students attending an organic chemistry course by applying synthetic methodologies to monosaccharides as multifunctional and stereochemically complex small molecules. Students will explore and understand the usefulness of cyclic acetals, namely the benzylidene acetal, in the context of a multi-step synthetic route. Throughout the proposed set of experiments, students are challenged to apply different purification techniques commonly used in the organic chemistry laboratory. By comparing the ^{1}H NMR spectra of acetylated methyl α-D-glucopyranoside with and without previous benzylidene protection, they will be able to confirm the regioselective manipulation of the starting material and the selective acetal hydrolysis.

Additional Safety

Manipulation of compounds 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. *p*-Toluenesulfonic acid and hydrochloric acid are corrosive acids and may cause severe skin and eye burns (H314). Long-term exposure to pyridine can cause sterility in males (H360). Benzaldehyde dimethyl acetal and acetic anhydride are harmful in contact with skin and eyes (H312, H318). Chloroform is classified as a carcinogen (H351) and therefore it is not used for extraction purposes, being replaced by dichloromethane, which is much less toxic (H305, H315). Most of the solvents used are flammable, and thus should be kept away from heat, sparks or flames (H226).

Experimental Procedure

Laboratory Session 1 (4 h): Preparation of Methyl 4,6-*O*-Benzylidene-α-D-glucopyranoside

1. In a fume hood, heat an oil bath on a heating magnetic stirrer with integrated temperature control or, alternatively, on a heating magnetic stirrer with contact thermometer, to maintain the reaction temperature at 82 °C.
2. In a 25 mL round-bottomed flask equipped with a magnetic stir bar, prepare a solution of methyl α-D-glucopyranoside (1 g, 5.15 mmol) in acetonitrile (5 mL) and add *p*-toluenesulfonic acid monohydrate (0.02 g, 0.11 mmol, 0.02 equiv.) and benzaldehyde dimethyl acetal (1.55 mL,10.30 mmol, 2 equiv.). Clamp the flask, equipped with a reflux condenser, in the preheated oil bath and let the reaction mixture stir at 82 °C for 2 h.
3. Cool the reaction mixture to room temperature, add dichloromethane (50 mL) and transfer the solution into a separation funnel. Wash the organic phase with an aqueous saturated solution of sodium hydrogen carbonate (50 mL) and then an aqueous saturated solution of sodium chloride (brine, 50 mL).
4. Dry the organic phase with anhydrous magnesium sulfate ($MgSO_4$), filter and evaporate the solvent under reduced pressure.
5. Re-suspend the dry residue in dichloromethane (use the smallest necessary volume for complete dissolution under heat), add petroleum ether (30 mL) and keep the flask on an ice bath.
6. Filter the formed crystals under reduced pressure and transfer them to a tared crystallizing dish with spout and conserve them in a desiccator in vacuum until the next session.

Laboratory Session 2 (4 h): Acetylation Reactions

Beginning of the laboratory session – Weigh the crystallizing dish containing the compound prepared in the previous session, calculate the reaction yield and determine the melting point of the obtained white solid.

Part A – Acetylation of Methyl 4,6-O-Benzylidene-α-D-glucopyranoside

1. Add the earlier synthesized methyl 4,6-*O*-benzylidene-α-D-glucopyranoside (0.9 g, 3.19 mmol, 1 equiv.), 4-(dimethylamino)pyridine (DMAP) (0.03 g, 0.25 mmol, 0.1 equiv.) and pyridine (5 mL) to a 25 mL round-bottomed flask equipped with a magnetic stir bar. Stir at room temperature until complete dissolution (approx. 5 min).
2. Add acetic anhydride (1.21 mL, 12.8 mmol, 4 equiv.) and stir for 30 min at room temperature.
3. Add dichloromethane (10 mL) and transfer the solution to a separation funnel. Neutralize with a 2 M aqueous HCl solution (15 mL) and extract the aqueous phase with dichloromethane (15 mL). Combine the organic phases and repeat the procedure by washing with a 2 M aqueous HCl solution (15 mL) followed by extraction with dichloromethane (15 mL).
4. Combine all the organic phases and wash them with a saturated solution of sodium hydrogen carbonate (50 mL) and then with brine (50 mL).
5. Dry the combined organic phases with anhydrous $MgSO_4$, filter under reduced pressure, pour the filtrate to a previously tared flask and evaporate the solvent with a rotary evaporator until dryness.
6. Keep the flask in a desiccator, under vacuum, until the next session.

Part B – Acetylation of Methyl α-D-Glucopyranoside

1. Add methyl α-D-glucopyranoside (0.5 g, 2.57 mmol, 1 equiv.), DMAP (0.03 g, 0.25 mmol, 0.1 equiv.) and pyridine (5 mL) to a 25 mL round-bottomed flask equipped with a magnetic stir bar. Stir at room temperature until complete dissolution (5 min).
2. Add acetic anhydride (1.95 mL, 20.6 mmol, 8 equiv.) to the solution and stir for 30 min at room temperature.
3. Add dichloromethane (10 mL) and transfer the solution to a separation funnel. Repeat the neutralization process and carry out the extraction as described for part A.
4. Keep the flask in a desiccator, under vacuum, until the next session.

Laboratory Session 3 (4 h): Benzylidene Acetal Hydrolysis and ^{1}H NMR Data Acquisition

Beginning of the laboratory session – Weigh the flasks containing the compounds prepared in session 2, calculate reaction yields and determine the melting point of the solid compound.

Benzylidene Acetal Hydrolysis

1. In a fume hood, heat an oil bath on a heating magnetic stirrer with integrated temperature control or, alternatively, on a heating magnetic stirrer with contact thermometer to maintain the reaction temperature at 50 °C.
2. Add methyl 2,3-di-*O*-acetyl-4,6-*O*-benzylidene-α-D-glucopyranoside (0.2 g, 0.55 mmol) and an aqueous solution of acetic acid (3 mL, 80% v/v) to a 25 mL round-bottomed flask with a magnetic stir bar. Stir until complete dissolution (*ca.* 5 min). Clamp the flask, equipped with a reflux condenser, in the preheated oil bath and let the reaction mixture stir at 50 °C for 2 h.
3. Meanwhile, fill a chromatography column (3 cm diameter × 10 cm height) with a slurry of silica gel in petroleum ether/ethyl acetate (1 : 1).
4. After cooling the reaction mixture to room temperature, add toluene (10 mL) and evaporate until dryness.
5. Dissolve the reaction residue in dichloromethane (2 mL), and add one or two drops of methanol to assure complete dissolution.
6. Apply the sample to the chromatography column.
7. Elute with petroleum ether/ethyl acetate (1 : 1) (150 mL) and collect 20 mL fractions. Control by TLC with the eluent petroleum ether/ethyl acetate (1 : 1) until all benzaldehyde has come out of the column (see Supplementary material). Staining should be performed by dipping the plate in a 10% methanolic solution of H_2SO_4 followed by heating at 120 °C.
8. Elute with ethyl acetate (100 mL) and discharge the eluted solvent that does not contain benzaldehyde or product as confirmed by TLC.
9. Elute with a mixture of ethyl acetate and methanol (10 : 1, 200 mL) and collect the fractions, confirming by TLC (same eluent as above) the beginning and the end of methyl 2,3-di-*O*-acetyl-α-D-glucopyranoside elution.
10. Pour the collected fractions containing the product into a 250 mL round-bottomed tared flask and evaporate the solvent with a rotary evaporator until dryness.
11. Determine the mass of the residue, which can be immediately used to run compound ^{1}H NMR spectra. Calculate the crude yield (yield prior to drying the residue in a vacuum desiccator).

Sample Preparation for ^{1}H NMR Spectra Acquisition

1. Dissolve the compound (15 mg) in $CDCl_3$ (500 μL) and transfer the solution to an NMR tube.
2. Dissolve methyl 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranoside (15 mg), obtained in laboratory session 2, in $CDCl_3$ (500 μL) and transfer the solution to an NMR tube.
3. Record the ^{1}H NMR spectra of the two samples prepared.

Waste Disposal

The neutralized aqueous solutions may be diluted and discarded in the waste collector. Used organic solvents should be collected in appropriate waste collector recipients (chlorinated and non-chlorinated, accordingly).

Results Interpretation and Additional Questions

1. In the process of benzylidene acetal formation, which hydroxy group is expected to react first? Describe the reaction mechanism starting from benzaldehyde dimethyl acetal.
2. Why does benzaldehyde (or its corresponding dimethyl acetal) selectively react with positions 4 and 6 of monosaccharides to form six-membered ring acetals?
3. Why is it advantageous to use DMAP in the acetylation reactions instead of using pyridine alone? Describe the mechanism by which DMAP-catalysed acylation occurs.
4. Analyse the acquired ^{1}H NMR spectra:
   a. Confirm the presence of two or four acetate groups in the synthesized products.
   b. Compare the chemical shifts of H2, H3, H4 and H6 signals of both derivatives emphasizing their importance for the assignment of the acetyl functionalization pattern.
   c Regarding the spectrum of methyl 2,3-di-*O*-acetyl-α-D-glucopyranoside, assign the signal corresponding to the protons of the methoxy group at the anomeric centre, thus confirming the occurrence of selective acetal hydrolysis at positions 4 and 6.
5. Compare the melting point of methyl 4,6-*O*-benzylidene-α-D-glucopyranoside with that reported in the literature and suggest plausible reasons for any existing differences. Comment on the purity of this synthetic intermediate taking into account the applied purification method.
6. Give the overall yield for the three-step synthesis. Comment on the global efficiency of the synthetic route.

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