D-GLUCURONO-6,3-LACTONE AS AN INTERESTING COMPOUND IN THE SYNTHETIC CHEMISTRY OF CARBOHYDRATES RELATED TARGETS

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Isopropylidene acetals and ketals are frequent protecting groups in the chemistry of carbohydrates, particularly in organic synthesis. The yield of these derivatives must be high. Besides already published syntheses, we report a new way of synthesizing these derivatives in high yields and efficient deprotection. Acetyl and benzoyl groups are frequent protecting groups, and their use in carbohydrate chemistry, particularly on the derivative of D-glucurono-6,3-lactone was shown. Reactions of 1,2,5-tri-*O*-acetyl-D-glucurono-6,3-lactone with BF_3 and $(CH_3CH_2)_3SiH$, $LiBH_4$, respectively and I_2, were also reported.

Keywords: isopropylidene; acetyl; Lewis acid

Introduction

When two OH groups are in the 1,2- or 1,3- positions their protection very often takes the form of acetals, ketals or orthoesters. This is particularly important in the synthesis of carbohydrates. This type of protection is very frequently used in the chemistry of steroids, glycerides, nucleosides and cyclitols [1].

The most widespread groups of this type are isopropylidene and benzylidene, although it is not rare to have acetals in the form of formaldehyde and acetaldehyde.

The usual route for their synthesis is to treat glycol with a large excess of aldehyde or ketone in the presence of an acid catalyst. When acetone or benzaldehyde are used, they usually also serve as solvents. Gaseous hydrochloride or hydrochloric acid, sulfuric and *p*-toluolsulfonic acid, and zinc-chloride as Lewis acid are used as catalysts. Assuming that the acetylation reaction is in equilibrium and that every time a water molecule is released, the thermodynamic equilibrium shifts in favor of reactants, there is a problem in removing water so that the reaction can be completed. Very rarely is it possible to get acetals or ketals from vicinal dialdehydes and glycols, considering that this mechanism is of the S_N2 type. Which acetal types or ketals are chosen in the synthesis depends on the ease of the hydrolysis of the obtained derivatives, which is usually necessary for the further course of synthesis [2,3].

Cyclic isopropylidene ketals are widely used in the chemistry of steroids, carbohydrates, and nucleosides and the reason for this is the accessibility of acetone, which can be used as a solvent to obtain the isopropylidene ketal. The second reason for this wide use is the possible selective hydrolysis of primary-secondary in the presence of disecondary isopropylidene ketal [2].

The reaction with the appropriate anhydride or acid chloride in pyridine at 0-20 ºC gives the desired product in most cases [4]. In the case of tertiary alcohols, acylation is very slow, so it must be sped up with a catalytic amount of 4-dimethylaminopyridine [4]. Scandium triflate was proven to be a better catalyst for acylation [5].

Acetate can be cleaved under mildly basic conditions [4]. In the carbohydrate's chemistry, acetates can be cleaved selectively in the presence of benzoates and *p*-bromobenzoates using *p*-toluenesulfonic acid monohydrate (1 equivalent per acetate) in DCM-methanol mixture at 4 °C in 7 h or room temperature in 24 h [6].

Although there is a huge available literature on protecting groups, still there is a necessity to increase the yield in the protection step. Sometimes, the deprotection can be difficult as in the case of 5-*O*-acetyl-1,2-*O*-isopropylidene-α-D-glucofuranurono-6,3-lactone, where under various conditions high degree destruction and only relatively poor yields of 1,2-*O*-isopropylidene-α-D-glucofuranurono-6,3-lactone can be achieved [7]. Here, we tried to increase the yields in the protection step using different reagents and/or conditions, but also to investigate the products of the reactions of the obtained protected derivatives as they are potentially important intermediates in the synthesis of many bioactive compounds.

Materials and methods

Apparatus

The NMR analyses were performed on a Bruker AC–250 instrument with standard Bruker software. All analyses were carried out using regular 5 mm NMR tubes.

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Reagents

All chemicals used for syntheses were of the analytical reagent grade. Solutions were prepared for NMR analyses in $CDCl_3$ (Merck, Germany), purity of 99.8%. The chemical shifts are referred to as tetramethylsilane (TMS, δ_H= 0.00 ppm) in $CDCl_3$.

Synthesis of 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose (**1**)

α-D-glucose (20 g, 0.1066 mol) was powdered in the mortar and transferred into the three-necked round bottom flask. Then 250 mL of freshly purified and dried acetone was added and cooled in the ice bath. Slowly, with constant stirring 16 mL of concentrated sulfuric acid was added dropwise, taking care the temperature does not exceed 10 ºC. The reaction was kept for 5 h at room temperature with constant stirring. Afterward, the mixture was cooled in the ice bath; the neutralization was done using a saturated solution of $NaHCO_3$ in methanol. The obtained salts were filtered off and washed and the acetone filtrate was evaporated using a vacuum evaporator. The pale-yellow oil was obtained. The product was recrystallized from acetone. 16.2 g of the product were obtained, m.p. 110 ºC, with a yield of 56%. The melting point was in accordance with the literature data [8].

The product was purified on the column of silica gel using benzene:methanol=2:1 as an eluent.

Synthesis of 1,2-*O*-isopropylidene-α-D-glucofuranose (**2**)

1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose (**1**) (5 g, 0.0192 mol) was placed in a round bottom flask (100 mL), then it was added 10 mL 40% acetic acid and the hydrolysis of the 5,6-*O*-isopropylidene group on water bath at 70 ºC for 55 min was performed. After that time one spot was observed on TLC with an R_f value lower than isopropylidene glucose (eluent benzene:methanol=4:1). Upon the completion of the hydrolysis the neutralization was performed with Na_2CO_3 solution. The solution was evaporated on a vacuum evaporator. The acetic acid was removed by the addition of 1 mL mixture of toluene:methanol=1:2, because of the formation of the ternary mixture (acetic acid:toluene:methanol=1:1:2). Afterward mono-isopropylidene glucose was extracted with ethyl acetate. It was obtained 3.8 g of the product.

Synthesis of the sodium salt of 1,2-*O*-isopropylidene-α-D-glucofuran-uronic acid (**3**)

1,2-*O*-isopropylidene-α-D-glucofuranose (**2**) (100 mg, 0.45 mmol) was dissolved in water (5-6 mL) in a round bottom flask (100 mL). pH of 8-9 was adjusted using an aqueous solution of Na_2CO_3. As a catalyst, 10% Pt on C was added. The oxidation was performed with pure oxygen with constant stirring for 24 h. Afterward, the catalyst was removed by filtration and the obtained sodium salt of glucofuran-uronic acid was not isolated; it was used in the further synthesis of obtaining γ-lactone.

Synthesis of 1,2-*O*-isopropylidene-α-D-glucofuran-urano-6,3-lactone (**4**)

The sodium salt of glucofuran-uronic acid (**3**) was acetified with the solution of sulfuric acid up to pH 2 and immediately extracted thrice with ethyl acetate in the separation flask. γ-lacton in ethyl acetate was formed. The extract was evaporated on a vacuum evaporator to dryness. 10 mg of the product were obtained, a yield of 10.1%.

Synthesis of 1,2,5-tri-*O*-acetyl-D-glucurono-6,3-lactone (**6**)

5 mL pyridine and D-glucurono-6,3-lactone (**5**) (0.9 g, 5.11 mmol) were placed in a round bottom flask (100 mL). Upon dissolution of glucurono lactone, acetanhydride was added dropwise in huge excess (5.3 mL). After 2 h at room temperature, the reaction mixture was poured into the mixture of water and ice. The resin was at the bottom of the beaker. When the ice was melted, the solution above the resin was decanted and the obtained triacetate-glucurono lactone (6) was washed with distilled water and recrystallized from isopropyl alcohol. The mobile phase for TLC was benzene: isopropyl alcohol=95:5. The solid has an NMR spectrum with spectral data in accordance with the previously reported [9].

Synthesis of 1,2,5-tri-*O*-acetyl-D-glucurono-6,3-lactone (**6**)

2.83 mL anhydride of acetic acid (ρ=1.089 g/mL) was placed in a round bottom flask (100 mL) and 4.7 mL glacial acetic acid (65-70%, ρ=1.079 g/mL), and then added 0.88 g (5 mmol) powdered D-glucurono-6,3-lactone. 0.3 mL of the catalyst was added (0.85 mL perchloric acid ρ=1.079 g/mL was added to 2.13 mL anhydride of acetic acid with cooling of the system) dropwise, so the temperature did not exceed 25 ºC. The flask with the reaction mixture was left in a water bath at 10 ºC for 1h. The crystals were removed by filtration, washed with cold water, and dried in the air. It was obtained 1.36 g (yield 90%) of 1,2,5-tri-*O*-acetyl-D-glucurono-6,3-lactone (**6**), m.p. 194 ºC.

Reaction of 1,2,5-tri-*O*-acetyl-D-glucurono-6,3-lactone (**6**) with BF_3 and $(CH_3CH_2)_3SiH$

1,2,5-tri-*O*-acetyl-D-glucurono-6,3-lactone (**6**) (300 mg, 0.99 mmol) was dissolved in 5 mL CH_2Cl_2 in a round bottom flask (100 mL) and 0.1 mL boron trifluoride etherate was added. The crystals appeared. Two products were identified using TLC of the solution above the crystals. Neutralization of a small amount of the solution (above the crystals) using the aqueous solution of $NaHCO_3$ showed on TLC one product of the same R_f value as 1,2,5-tri-*O*-acetyl-D-glucurono-6,3-lactone. To the remaining, not neutralized solution, 0.1 mL BF_3 was added. The crystals were filtered off and washed with dichloromethane. The crystals showed a melting point of 85 ºC (1-[4-(acetyloxy)-3-hydroxy-5-oxooxolan-2-yl] ethane-1,2-diyl diacetate) (**7**). Neutralization of the part of the filtrate with the aqueous solution of $NaHCO_3$ showed on TLC the presence of one product. To the

remaining filtrate (not neutralized) it was added 0.8 mL $(CH_3CH_2)_3SiH$, and after 2 h two products were identified using TLC. The reaction mixture was further warmed in a water bath using reflux with $CaCl_2$ drying tube, and then neutralized with an aqueous solution of $NaHCO_3$ and dried with anhydrous Na_2SO_4. Two products were identified using TLC. By the evaporation of the obtained solution, the oil was obtained which was chromatographed on the column.

Reaction of 1,2,5-tri-*O*-acetyl-D-glucurono-6,3-lactone (**6**) with $LiBH_4$ and BF_3

1,2,5-tri-*O*-acetyl-D-glucurono-6,3-lactone (**6**) (300 mg, 0.99 mmol) was placed in a round bottom flask (250 mL) and dissolved in 40 mL methylene chloride and added 6 mg $LiBH_4$ and 0.5 mL boron-trifluoride etherate. The reaction mixture was refluxed in the nitrogen stream with continuous stirring on the magnetic stirrer for 24 h. The reaction was monitored using TLC which showed a very weak intensity of the desired reaction. Due to this fact, 0.2 mL boron trifluoride etherate was added and mixing was continued for the next 6 h. The solid appeared. The reaction mixture was neutralized with the aqueous solution of $NaHCO_3$. After neutralization, the extraction was performed with methylene chloride (4-5 times) and the extracts were dried with anhydrous Na_2SO_4. The organic layer was evaporated, and the oil was obtained. TLC showed two products which we separated by silica-gel column chromatography (granulation less than 0.063 mm). The substance with a higher Rf value was obtained with a yield of 37.5 mg (m.p.=183 °C), and the substance with a lower R_f value with a yield of 79.4 mg (m.p.=163 °C). The elution was performed with the mixture benzene:isopropanol=97.5:2.5.

Reaction of 1,2,5-tri-*O*-acetyl-D-glucurono-6,3-lactone (**6**) with I_2 and $(CH_3CH_2)_3SiH$

1,2,5-tri-*O*-acetyl-D-glucurono-6,3-lactone (**6**) (0.88 g, 2.91 mmol) was placed in a round bottom flask (100 mL) and previously prepared agent: 5 mL CH_2Cl_2 was placed in Erlenmeyer flask, then 6.5 mL $(CH_3CH_2)_3SiH$, and to this, 0.05 g I_2 was added. When iodine lost its colour, it was measured with a 5 mL reagent and transferred to the round bottom flask with 1,2,5-tri-*O*-acetyl-D-glucurono-6,3-lactone (**6**). The mixture was refluxed for 12 h with magnetic stirring. Two products were identified using TLC and then extracted using diethyl ether and water. The separated diethyl ether layer was titrated with $Na_2S_2O_3$, dried with anhydrous Na_2SO_4, and evaporated on a vacuum evaporator. The mass of the obtained crystals was 0.7 g with m.p.=180 °C.

Reaction of D-glucurono-6,3-lactone (5) with $CuCl_2 \cdot 2H_2O$ in acetone-synthesis of 1,2-*O*-isopropylidene-D-glucurono-6,3-lactone (**4**)

880 mg (5 mmol) D-glucurono-6,3-lactone was placed in the round bottom flask (100 mL) and dissolved into 20 mL of dry acetone with the addition of 0.8525 g copper(II)chloride dihydrate. The reaction mixture was refluxed for 8 h, after which the neutralization was performed using a saturated aqueous solution of $NaHCO_3$. The solid was filtered off, and the filtrate was dried using anhydrous Na_2SO_4, and then the solid phase was separated from the liquid. After the evaporation, 1.5 g of crude product was obtained. The crude product was put on the column and eluted with chloroform:acetone=30:1. After the chromatography, 0.9504 g of the substance (88%) was obtained, 1,2-*O*-isopropylidene-D-glucurono-6,3-lactone (**4**).

Reaction of 1,2-*O*-isopropylidene-D-glucurono-6,3-lactone (**4**) with benzoyl chloride in pyridine-synthesis of 5-*O*-benzoyl-1,2-*O*-isopropylidene-D-glucurono-6,3-lactone (**8**)

1,2-*O*-isopropylidene-D-glucurono-6,3-lactone (**4**) (0.216 g, 0.999 mmol) was dissolved in pyridine and added 0.2 mL benzoyl chloride on cold. The mixture was kept in the fridge for 24 h. After that, a small quantity of aqueous solution of $NaHCO_3$ was added and evaporated using a vacuum evaporator. The residue was dissolved in ethyl acetate and washed three times with water; the aqueous layer was discarded each time. The ethyl acetate layer was dried with anhydrous Na_2SO_4. The liquid phase was separated from the solid and evaporated on a vacuum evaporator. After the evaporation 0.4 g of crude substance was left, which was purified on the silica-gel column with the eluent n-hexane: isopropyl alcohol=9:1. After the chromatography, 0.3200 g 5-*O*-benzoyl-1,2-*O*-isopropylidene-D-glucurono-6,3-lactone was obtained (**8**).

Reaction of 1,2-*O*-isopropylidene-D-glucurono-6,3-lactone (**4**) with acetanhydride in pyridine-synthesis of 5-*O*-acetyl-1,2-*O*-isopropylidene-D-glucurono-6,3-lactone (**9**)

1,2-*O*-isopropylidene-D-glucurono-6,3-lactone (**4**) (0.216 g, 0.999 mmol) was dissolved in pyridine and added 0.2 mL acetanhydride on cold. The mixture was kept in the fridge for 24 h. After that, a small quantity of saturated aqueous solution of $NaHCO_3$ was added and evaporated on a vacuum evaporator. The residue was dissolved in ethyl acetate and washed three times with water; each time the aqueous layer was discarded. The ethyl acetate layer was dried with anhydrous Na_2SO_4. The liquid phase was separated from the solid and evaporated on a vacuum evaporator. After the evaporation, 0.3 g of crude substance was left, which was purified on the column of silica-gel with the eluent n-hexane: isopropyl alcohol=9:1. After the chromatography, 0.258 g 5-*O*-acetyl-1,2-*O*-isopropylidene-D-glucurono-6,3-lactone was obtained (9), m.p.=93 °C. The obtained ^1H and 2D COSY NMR spectra of compound 9 are available in the Supplementary Material.

Results and discussion

The synthesis of D-glucurono-6,3-lactone was achieved starting from readily available α-D-glucose. α-D-glucose was firstly transformed into 1,2,5,6-di-*O*-isopropylidene-α-D-glucofuranose (**1**) in 56% yield, where selective deprotection with 40% CH_3COOH led to 1,2-*O*-isopropylidene-α-D-glucofuranose (**2**). Then, we used Pt/C as a catalyst and introduced pure oxygen to get sodium salt of 1,2-*O*-isopropylidene-α-D-glucofuranuronic acid (**3**). The addition of sulphuric acid up to pH=2 gave the closure of the lactone ring (Figure 1). This protected derivative of D-glucurono-6,3-lactone could be an important starting material in carbohydrate synthesis. Its synthesis from not-so-easily available and cheap D-glucoheptonolactone was previously published [10].

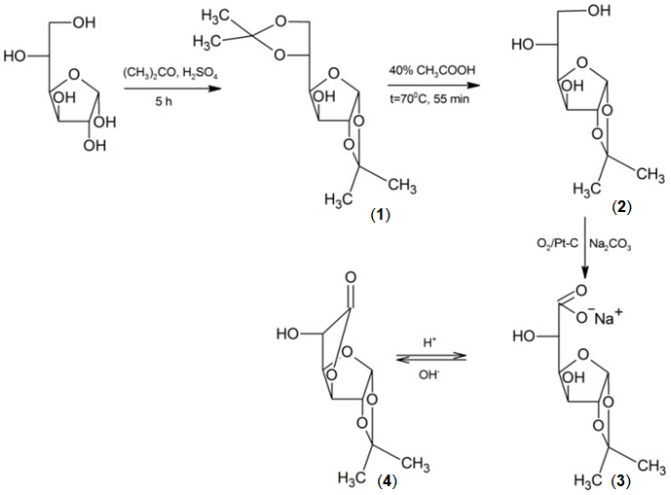

Figure 1. Synthetic route of obtaining 1,2-*O*-isopropylidene-α-D-glucurono-6,3-lactone (**4**) from α-D-glucose.

A peracetylated derivative of glucuronolactone, 1,2,5-tri-*O*-acetyl-D-glucurono-6,3-lactone (**6**), is used widely in the synthesis of glucurono lactone derivatives [11]. In the first attempt of the synthesis of 1,2,5-tri-*O*-acetyl-D-glucurono-6,3-lactone (**6**), we used pyridine and acetanhydride [12]; we got a colored reaction mixture that showed several substances. The best results were obtained using acetanhydride with perchloric acid as a catalyst [13]. The yield of synthesized 1,2,5-tri-*O*-acetyl-D-glucurono-6,3-lactone (6) was above 90%.

The reaction of 1,2,5-tri-*O*-acetyl-D-glucurono-6,3-lactone (**6**) with BF_3 and $(CH_3CH_2)_3SiH$ (Figure 2) gave the new product (**7**) characterized by ^1H NMR spectrum available in the Supplementary Material (Fig. 1). Combinations of BF_3 with $(CH_3CH_2)_3SiH$, and I_2 and $(CH_3CH_2)_3SiH$ with 1,2,5-tri-*O*-acetyl-D-glucurono-6,3-lactone (**6**) did not give the product characterized by ^1H NMR spectrum and presented in Fig. S1.

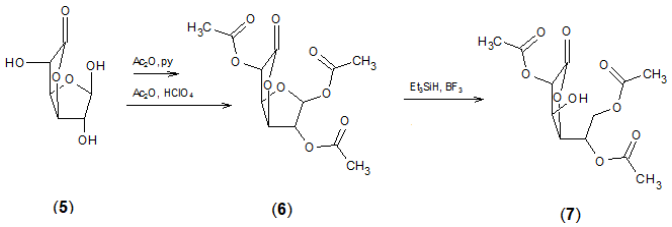

Figure 2. Synthetic route of obtaining **7** from D-glucurono-6,3-lactone (**5**).

D-glucurono-6,3-lactone (**5**) was treated with acetone and $CuCl_2 \cdot 2\ H_2O$ and refluxed for 8 h. We got 1,2-isopropylidene-D-glucurono-6,3-lactone (**4**) in high yield (88%). This is the first synthesis of isopropylidene derivative in this way, and with high yield and high purity so that no additional purification is necessary. To the isopropylidene derivative, we attached additional protection in the form of the acetyl-protecting group. The acetyl derivative was obtained in quantitative yield, and its melting point and NMR spectra were not reported before. The respected ^1H NMR spectrum of the obtained derivative 5-O-acetyl-1,2-*O*-isopropylidene-D-glucurono-6,3-lactone (**9**) shows at δ=2.24 ppm singlet of three protons from the acetyl group (Fig. S2). Similarly, the benzoyl derivative was obtained in high yield (Figure 3), although its synthesis was reported earlier [14].

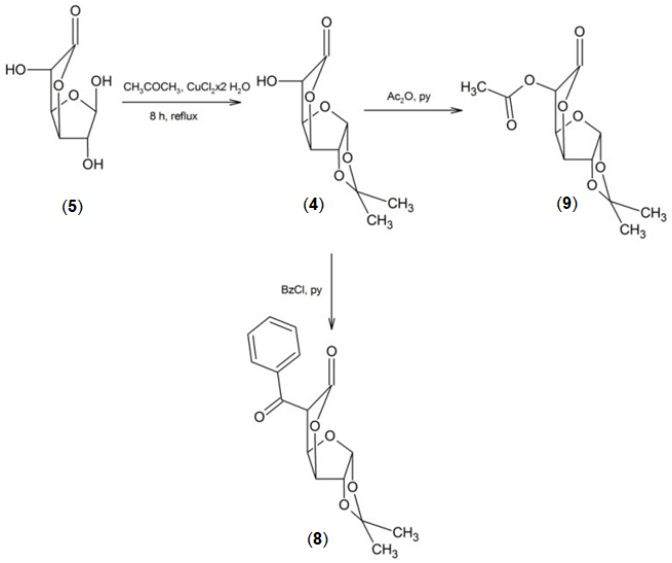

Figure 3. Synthetic route of derivatives (**4, 8** and **9**) of D-glucurono-6,3-lactone (**5**).

Conclusion

1,2,5-tri-*O*-acetyl-D-glucurono-6,3-lactone was obtained in high yield (90%) in the reaction of acetic-anhydride in glacial acetic acid using $HClO_4$ as an acid catalyst. Using BF_3 and $(CH_3CH_2)_3SiH$ on 1,2,5-tri-*O*-acetyl-D-glucurono-6,3-lactone we obtained the desired product (based on ^1H NMR spectra). The protection reactions to get acetyl and benzoyl derivatives went smoothly in quantitative yields. A very high yield (88%) was obtained for the first time with $CuCl_2 \cdot 2\ H_2O$ and acetone for the

isopropylidene derivative of D-glucurono-6,3-lactone.

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Izvod

D-GLUKURONO-6,3-LAKTON KAO INTERESANTNO JEDINJENJE U SINTETIČKOJ HEMIJI SRODNIH META UGLJENIM HIDRATIMA

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Izopropiliden acetali i ketali su česte zaštitne grupe u hemiji ugljenih hidrata, posebno u organskoj sintezi. Prinos ovih derivata mora biti visok. Pored starog, dat je i novi način sinteze ovih derivata u visokim prinosima i efikasnim uklanjanjem. Acetil i benzoil grupe su česte zaštitne grupe, i njihovo korišćenje u hemiji ugljenih hidrata, posebno na derivatu D-glukurono-6,3-laktona je pokazano. Reakcije 1,2,5-tri-*O*-acetil-D-glukurono-6,3-laktona sa BF_3 i $(CH_3CH_2)_3SiH$, $LiBH_4$, redom i I_2 su takođe date.

Ključne reči: izopropiliden; acetil; Lewis-ova kiselina