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Steroids

Aldosterone Glucuronide, an Important Biomarker: Synthesis and Structure Elucidation of Novel Isomers

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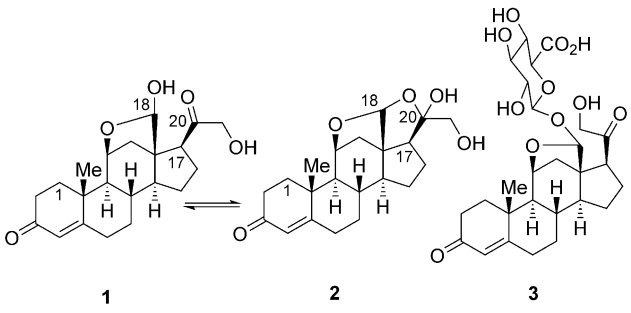
In memory of Rolf Huisgen

Abstract: Aldosterone **1** is a mineralocorticoid, it has great influence on the blood pressure and its glucuronide is an important marker for the detection of several diseases. Here, we describe the chemical synthesis of different aldosterone-18- and 20-glucuronides. Reaction of trimethylsilyl 2,3,4-triacetyl-1-β-glucuronic acid methyl ester **5b** and aldosterone diacetate **11** in the presence of TMSOTf gave the 18-α-glucuronide **9a**. The 18-β-glucuronide **15b** and the 20-β-glucuronide **16b** could be obtained by reaction of methyl 2,3,4-tri-*O*-isobutyryl-1α-glucuronate trichloroacetimidate **14** and aldosterone 21-acetate **8** in the presence of TMSOTf or $BF_3{\cdot}OEt_2$. Finally, reaction of aldosterone 21-acetate **8** and methyl 2,3,4-triacetyl-1α-glucuronate trichloroacetimidate **19** in the presence of TMSOTf gave the corresponding methyl 18-β-triacetylglucuronate **9b**, which was transformed into the desired aldosterone-18-β-glucuronide **3** by two enzymatic transformations.

Introduction

Aldosterone **1** (Figure 1) is the most important physiological mineralocorticoid being formed in the cortex of adrenal glands from cholesterol. By retention of water, tubular reabsorption of sodium and secretion of potassium into the urine, it has a major impact on the blood pressure.[1] Plasma levels of **1** are strictly regulated by a fine balance between its liberation controlled by renin as well as angiotensin and its metabolism.[2]



1 2 3

Figure 1. Aldosterone **1** as well as **2** and aldosterone-18-β-D-glucuronide **3**.

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The main metabolic pathways of **1** are the conversion into the dihydro- and tetrahydro derivatives followed by glucuronidation in the liver and independently, by the direct glucuronidation of **1** in the kidneys.[3] The latter process is believed to form the 18-β-glucuronide **3** (Figure 1). Although **1** exists as equilibrium with **2** so far, no glucuronides from the C-20-hemiacetal have been found or discussed. Alteration in the glucuronidation of aldosterone **1** results in a change of its concentration in the blood with significant consequences for the body.

For instance, inhibition of the glucuronidation by non-steroidal anti-inflammatory drugs (NSAID)[4] potentiates the adverse cardiovascular effects of aldosterone[5].The measurement of the concentration of **3** in the urine is therefore of high diagnostic value. However, establishing a high throughput quantification of **3** either by LC-MS or by an immunoassay would require an analytical standard based on **3**, but at the moment, a satisfying access to **3** does not exist.

Aldosterone-18-β-D-glucuronide **3** belongs to the group of acetalglycosides.[6] They are different from the usual glycosides where an alcohol is connected to a sugar moiety by a glycosidic bond, whereas in the acetalglycosides a hemi-acetal is bound to a sugar moiety. These types of compounds are not uncommon in nature and the largest group with such a functionality are the iridoids as loganin and the secoiridoids as secologanin.[7] The latter compound is a key intermediate in the biosynthesis of indole, cinchona, ipecacuanha and pyrroloquinoline alkaloids.

In general, the desired glucuronide **3** can be formed by two different mechanisms.[6] First the hydroxy group of the hemiacetal moiety in **1** can undergo a nucleophilic attack at C-1 of a oxycarbenium ion **4** (Figure 2) formed from the corresponding bromo or trichloroacetimidato glucuronate. In a second ap-

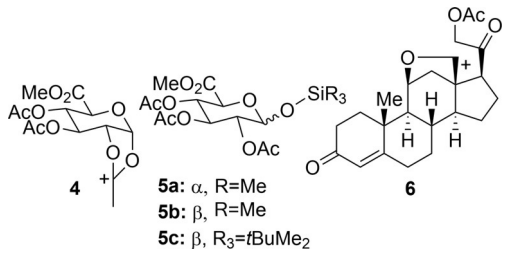

Figure 2. Mechanisms for the synthesis of aldosterone-18-β- and 18-α-glucuronides.

proach a nucleophilic attack of the oxygen of the $OSiR_3$ group of a silyl glucuronate **5a–c** could take place at carbocation **6** (Figure 2) formed from the hemiacetal **1** with retention of configuration at C-1 of the sugar moiety (formalistic view).

Acetalglycosides are usually more acid labile than the common glycosides. Thus, the glucuronide **3** is hydrolyzed at pH 1–2.5; on the other hand, it shows increased stability against β-D-glucuronidase.

An enzymatic glucuronidation of aldosterone **1** to give **3** has been accomplished by C. Girard et al.[8] and K. M. Knights et al[4] using cell lysates; however, **3** has not been isolated due to the very small amount formed in this process and was only determined by LC-MS/MS. Moreover, its chemical synthesis has also caused severe problems so far. The first synthetic investigations have been performed by Underwood and Frye[9] using a Koenigs-Knorr reaction of aldosterone 21-acetate **8**, which however did not give the desired derivative of compound **3**. Later, Carpenter and Mattox[10] again used a Koenigs-Knorr reaction of **8** with a twentyfold excess of methyl acetobromoglucuronate **7** in the presence of a twentyfold excess of Ag_2CO_3 via the formal carbocation **4** as a proposed intermediate. They obtained a maximum of 10% of the desired aldosterone-18-β-D-glucuronate **9b** and in addition the 18-α-D-glucuronate **9a** as well as the 1-acetoxy-glucuronic acid methyl ester derivative **10**. The authors mentioned in their publication that the reproducibility of their process is low. We have repeated the work of Carpenter and Mattox using a synthetically acceptable approach with only a twofold access of **7** and were not able to isolate any of the desired aldosterone-18-β-D-glucuronate **9b**.

Results and Discussion

Since the Koenig–Knorr reaction of **7** and **8** gave only a low yield of **9b** (Figure 3) in a hardly reproducible way as described by Carpenter et al.[10] we have investigated new approaches for the synthesis of aldosterone glucuronides. For this purpose, we used a procedure being developed by us for the preparation of iridoid glucosides using a trimethylsilyl tetraacetyl-1β-D-glucoside.[6] For the synthesis of **3** we employed the corresponding trimethylsilyl 2,3,4-triacetyl-1β-D-glucuronic acid methyl ester **5b**, which is easily accessible from 2,3,4-triacetyl-1β-D-glucuronic acid methyl ester using HMDS and TMSCl in dichloromethane and pyridine at 0 °C.[11]

As a second reaction partner we used aldosterone diacetate **11** (Figure 4), which can be obtained from aldosterone **1** by

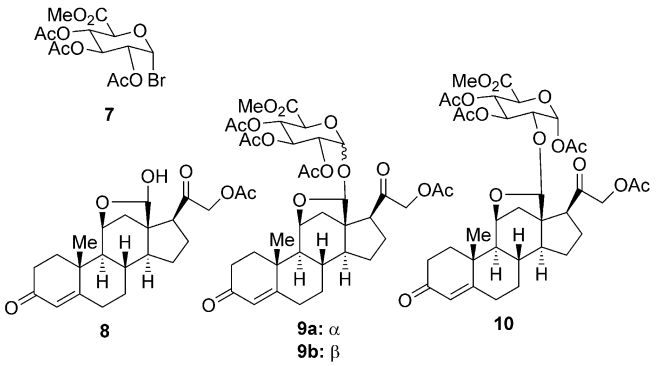

Figure 3. Koenigs–Knorr Reaction of **7** and **8**.

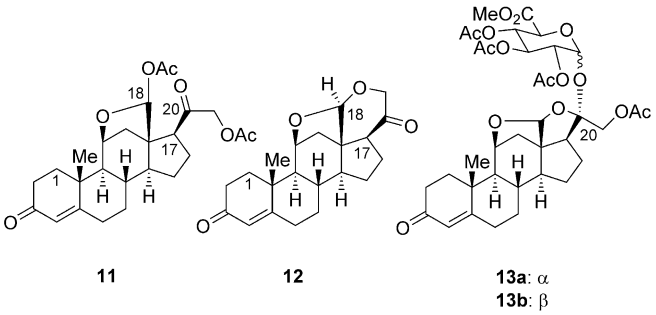

Figure 4. Aldosterone derivatives.

treatment with acetic anhydride and pyridine in 90% yield. Reaction of **5b** and **11** in the presence of TMSOTf at −10 °C in dry dichloromethane gave the aldosterone-18-α-glucuronide **9a** in 38% yield and the known 18,21-anhydroaldosterone **12** in 5% yield; however, aldosterone-18-β-glucuronide **9b** and the also possible aldosterone-20-glucuronides **13** were not found. It can be assumed that the formation of aldosterone-18-α-glucuronide **9a** is due to an isomerization of the β-trimethylsilyl sugar **5b** under the reaction conditions to give the α-trimethylsilyl sugar **5a**. To avoid such an isomerization, we prepared the β-*t*-butyldimethylsilyl sugar **5c** from the corresponding β-hydroxy sugar using TBDMSCl in 56% yield at 20 °C. Unfortunately, the reaction of **5c** with aldosterone diacetate **11** in the presence of TMSOTf did not lead to the desired compound; the major product with 60% yield, besides a very small amount of the α-glucuronide **9a**, was 18,21-anhydroaldosterone **12**;[12] here, one can assume that the system is too bulky to give the glycosides. Finally, we treated **9a** with $BF_3 \cdot OEt_2$ to allow an isomerization to give at least a small amount of the β-glucuronide **9b**; however, **9b** was not obtained but a small amount of **10**.

Since all attempts to obtain the β-glucuronide of aldosterone **9b** using a silyl sugar had failed, we investigated the glycosidation of **8** using the trichloroacetimidate[13] of methyl 2,3,4-tri-*O*-isobutyryl-D-glucuronate **14**, since Brown et al.[14] and Stachulski et al.[15] have shown that by replacing acetyl with isobutyryl groups in the donor transacylations as a major side reaction can be avoided and moreover the formation of β-glycosides can be improved due to steric reasons. Indeed, re-

action of **8** with **14** in the presence 20 mol-% of TMSOTf at −40 °C in dry DCM under an argon atmosphere gave 14% of the desired protected (*18R*)-aldosterone-18-β-glucuronic acid methyl ester **15b** (Figure 5). Moreover, 8% of the so far unknown (*18R, 20R*)-aldosterone-20-β-glucuronic acid methyl ester **16b** was also obtained. In addition, the 1-glucuronic acid methyl ester isobutyrate **17** was isolated in 12% yield and 25% of the starting material **8** could be recovered. The aldosterone-18-α-glucuronic acid methyl ester **15a** and the aldosterone-20-α-glucuronic acid methyl ester **16a** were not found. Interestingly, using TMSOTf at −10 °C gave a completely different picture. Under these conditions β-glucuronides were not obtained. The major product was the 18,21-anhydroaldosterone **12** with 50%; in addition, 12% of **17** and 3% of **15a** were found. Instead of TMSOTf also $BF_3{\cdot}OEt_2$ could be used as a Lewis acid at −10 °C. Here, 5.2% of **15b** and 7.2% of **16b** were obtained. In addition, 10% of **17** and 50% of **8** were isolated. The final steps in the preparation of **3** from **15b** would have been the hydrolysis of the methyl ester and the cleavage of the three isobutyrate groups. Two major problems in these transformations had to be considered. First, the elimination of the isobutyrate group at C-4′ at the sugar and secondly the isomerization at C-17 in the aldosterone moiety.[16]

To avoid the elimination, we first hydrolyzed the methyl ester using an excess of NaOH in dioxane/water at room temperature for 30 min. The corresponding acid **18** was obtained in 45% yield (Figure 6), which probably underwent an isomerization at C-17. However, compound **18** was not further investigated, since the hydrolysis of the isobutyrate moieties in **18** could not be accomplished without destroying the molecule.

The isomerization at C-17 was also a major dilemma in the synthesis of **3** by Carpenter et al.[10] These authors solved the problem in a rather complicated procedure by transforming the obtained glucuronide into a double semicarbazone, followed by removal of the acetyl moieties and cleavage of the methyl ester as well as final acid catalyzed hydrolysis of the two semicarbazone moieties. Unfortunately, no NMR data of **3** prepared by this procedure have been reported; moreover, NMR data of **3** do not exist in the literature at all.

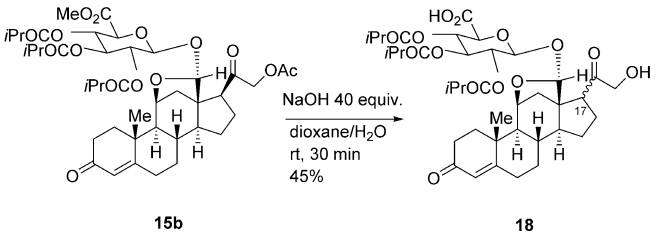

Figure 6. Synthesis of the acid **18**.

Based on our comprehensive knowledge acquired in our rather long enterprise of making (*18R*)-aldosterone-18-β-D-glucuronic acid **3** we finally came up with a straight forward synthesis of **3**, in which the final two steps namely the cleavage of the methyl ester and the removal of the protecting groups at the sugar and the acetate at the aldosterone moiety were accomplished using two enzymatic transformations. Moreover, we also developed a reproducible glycosylation of aldosterone 21-acetate **8** using the trichloroacetimidate of methyl 2,3,4-triacetyl-D-glucuronate **19** (Figure 7). Thus, reaction of **8** and **19** in the presence of 20 mol-% of TMSOTf at −40 °C in dry DCM under an argon atmosphere gave the desired (*18R*)-aldosterone-18-β-D-glucuronic acid methyl ester **9b** in 10% yield together with a mixture of 20-β- and 20-α-glucuronide **13b** and **13a** in 5% yield, which could not be separated. The rearranged compound **10** was formed with 12% yield and 30% of the starting material **8** could be recovered. Finally, **9b** was

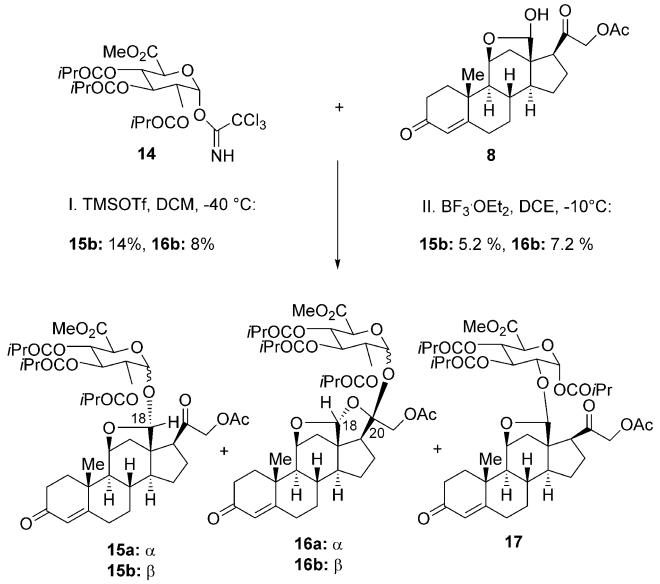

Figure 5. Reaction of **8** and **14** to give aldosterone-18- and 20-β-glucuronides **15b** and **16b**.

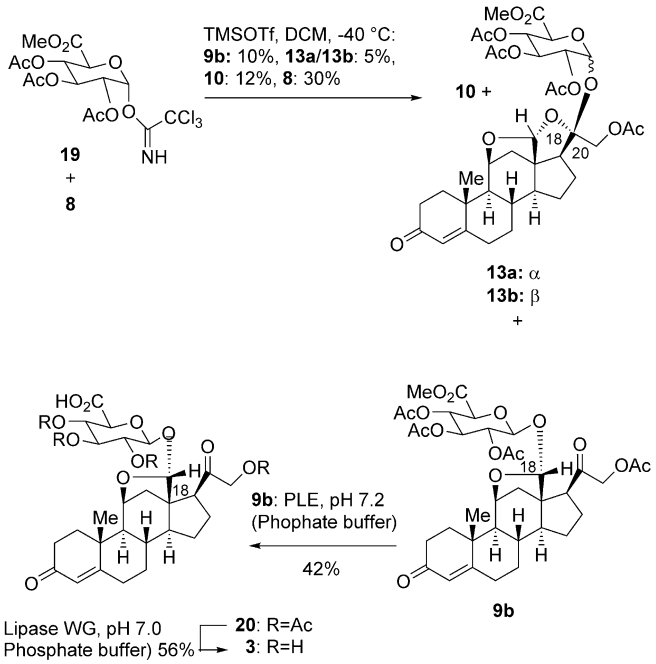

Figure 7. Synthesis of (*18R*)-aldosterone-18-β-D-glucuronic acid **3**.

transformed into the desired aldosterone-18-β-glucuronide **3** by treatment with PLE esterase[17] to give the corresponding acid **20** followed by reaction with lipase WG[18] to remove the acetyl moieties avoiding any isomerization at C-17 with 24% overall yield.

The structure elucidation of the new compounds was performed using mass spectrometry and 2D NMR spectroscopy. The β-configuration of the glycosidic bond in the β-glucuronides **3**, **9b**, **15b** and **16b** (Figure 8) was deduced from the coupling constant of the doublet for 1′-H of 7.9 Hz at $\delta = 4.39$ for **3** and of 7.9–8.3 Hz of the signals at $\delta = 4.97$ to 5.36 for **9b**, **15b** and **16b**. The doublets at $\delta = 5.29$–5.33 with $J = 4.3$ to 4.4 Hz clearly confirm the α-configuration at the sugar moiety in the compounds **9a** and **15a**. In the ^{13}C NMR spectra of the 18-glucuronides two signals for the carbonyl moieties C-3 and C-20 are seen for example, at $\delta = 198.0$ and 203.7 for **15a** and at $\delta = 198.0$ and 204.4 for **15b**, whereas in the 20-glucuronides as **16b** only one signal for a carbonyl group at $\delta = 198.0$ is observed. In the latter compound also an upfield shift for the signals of the methylene group C-21 is observed from $\delta = 4.61$ and 4.77 with $J = 16.0$ Hz in **15b** to $\delta = 4.07$ and 3.90 with $J = 11.7$ Hz in **16b**. In the spectra of the final compound **3** two doublets at $\delta = 4.44$ and 4.24 with $J = 17.8$ Hz are observed for 21-H_2. The CO-groups resonate at $\delta = 211.4$ for C-20, $\delta = 198.2$ for C-3 and $\delta = 170.3$ for the glucuronic acid moiety. Clearly, the signals for the acetates and the glucuronic acid methyl ester at $\delta = 167.2$ in the precursor **9b** do not exist anymore.

Most difficult was the determination of the configuration at C-18 and C-20 in **3**, **9b**, **15b** and **16b** as well as **9a** and **15a**, which was finally achieved by extensive NOESY experiments. Although **3** is a known compound, the configuration at C-18 had not been determined so far. In the 18-glucuronides the (*18R*)-configuration was proven by a strong interaction of 18-H with 8-H, a moderate with 15β-H and a weak interaction with the 19-Hs of the methyl group. In the 20-glucuronides the (*20R*)-configuration was confirmed by a NOE-signal between the 21-Hs and 17α-H, whereas these glucuronides have also the *R*-configuration at C-18, showing the same NOE signals for 18-H as in the case of the 18-glucuronides. Finally, we have also determined the so far unknown configuration at C-18 of the known 18,21-anhydroaldosterone **12** as (*18S*) using NOESY experiments.

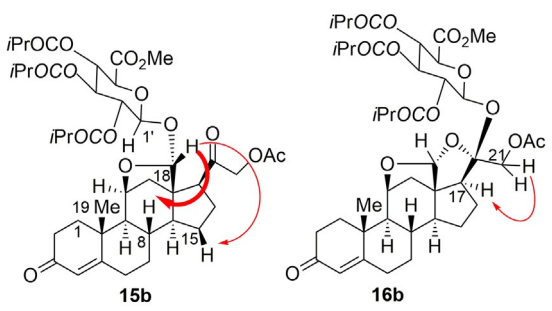

Figure 8. Stereochemical assignment of 18- and 20-glucuronides by NOE signals.

Conclusion

In summary, a reproducible synthesis of the important biomarker (*18R*)-aldosterone-18-β-D-glucuronic acid **3** has been developed via reaction of aldosterone 21-acetate **8** and the trichloroacetimidate of the methyl 2,3,4-tri-*O*-acetyl-D-glucuronic acid methyl ester **19** in the presence of TMSOTf at −40 °C. The final cleavage of the methyl ester and the hydrolysis of the acetyl moieties was achieved using PLE and Lipase WG in two enzymatic reactions avoiding any isomerization at C-17. The so far unknown configuration at C-18 in **3** has been determined by NOESY experiments showing a significant NOE between 18- and 8-H. Moreover, novel (*18R*, *20R*)-aldosterone-D-glucuronides have been prepared for the first time.

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Conflict of interest

The authors declare no conflict of interest.

Keywords: aldosterone · enzymatic hydrolysis · glucuronides · Koenigs–Knorr reaction · silyl glycosides

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