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Synthesis and Structure of Novel Potentially Bioactive Amphiphilic -*O*-(*N*)-Glycosides †

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Abstract: Herein we present the synthesis and structural analysis of novel -*O*-(*N*)-glycosides. The biphasic reaction of NHS and acetobromo-α-D-glucose or D-galactose gives the β anomer glycoside in a straightforward manner. Further hydrazinolysis and condensation with decanal afforded the desired products. Their complete structures, including the anomeric and *E*/*Z* double bond configurations, were determined by spectroscopic analysis.

Keywords: amphiphilic carbohydrates; -*O*-(*N*)-glycosides; NMR

1. Introduction

Carbohydrate-based surfactants are molecules with a sugar hydrophilic moiety and a hydrophobic tail, usually a hydrocarbon chain such as a fatty acid or alcohol. These glycosides have been synthesized in the last few decades and their structures were analyzed in terms of supramolecular arrangements as described in the literature [1–3]. Furthermore, they were proposed as potential therapeutic active molecules. This bioactive profile it's based on their capability to interact with the lipid bilayer of biological membranes due to the lipophilicity exhibited by the glycoside's aglycone [4]. In this work, we show the design and synthesis of carbohydrate derivatives with amphiphilic properties including a bioactive moiety, a Schiff base. Oxime ether function is an important structural motif in many famous drug scaffolds. Many antiviral agents have the -C=N-O- function in their structures, for example, the kinase inhibitor "enviroxime" and the recently reported "EIDD-2801" glycoside, which has shown broad anti-influenza virus activity [5].

2. Materials and Methods

2.1. General

Commercially available reagents were directly used without further purification. All sugar derivatives precursors were prepared by known methods, carefully purified, and fully characterized by ^{1}H and ^{13}C NMR. Reactions were monitored by TLC on silica gel (Merck 60 F254 plates), with detection by UV light (254 nm) or by charring with sulfuric acid. Flash chromatography was performed using silica gel (230–400 mesh). ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AVIII (600 and 150 MHz, respectively) in CDCl₃. HSQC and COSY spectra were used to establish peak assignments in ^{1}H and ^{13}C NMR.

2.2. N-Hydroxysuccinimide Glycosylation

To a stirred mixture of acetobromo-α-D-glucose (1 mmol) and Na_2CO_3 solution in CH_2Cl_2 (10 mL, 1M) was added N-hydroxysuccinimide (3 mmol) followed by TBAHS (1 mmol) phase transfer catalyst. The mixture was stirred at 25 °C until the reaction was completed as indicated by TLC. Then, CH_2Cl_2 (10 mL) was added and washed twice with 10 mL of water and brine. The organic layer was dried and concentrated in vacuo. The corresponding product was purified by flash column chromatography to obtain the pure glycoside derivative.

2.3. Hydrazinolysis Procedure

To a stirred solution of the aforementioned compound (0.5 mmol) in 10 mL of 1:1 CH_2Cl_2-MeOH mixture was added hydrazine hydrate (1.5 mmol) at room temperature. The reaction was monitored by TLC, and after 30 min quenched with 5 mL of saturated $NaHCO_3$ solution. The mixture was extracted with 3 × 10 mL of CH_2Cl_2. The organic layers were washed with brine, dried, and concentrated in vacuo. Products **1** (D-glucose derivative) and **2** (D-galactose derivative) were carefully purified by column chromatography to obtain the corresponding -O-NH_2 β-glycoside.

2.4. Synthesis of Oxime Derivatives

The aminooxygycoside (1 mmol) and 1.2 eq of decanal were dissolved in dried CH_2Cl_2 (2 mL) then one drop of concentrated HCl was added. The mixture was stirred for 24 h at ambient temperature until the reaction was completed as indicated by TLC, then quenched with water (3 mL) and the aqueous phase was extracted with CH_2Cl_2 (3 × 10 mL), neutralized with $NaHCO_3$ solution and washed twice with water. The combined organic layers were dried and concentrated under reduced pressure. The residue was dried under high vacuum. The purification was performed by flash column chromatography (hexane/EtOAc 8:2) to afford the desired compounds 3 and 4.

3. Results and Discussion

The biphasic reaction between *N*-hydroxysuccinimide and acetobromo-α-D-glucose or D-galactose gives the β-O-(*N*)-glycoside in a straightforward manner. Further hydrazinolysis and condensation with decanal, under acid catalysis, give the desired products. (Figure 1)
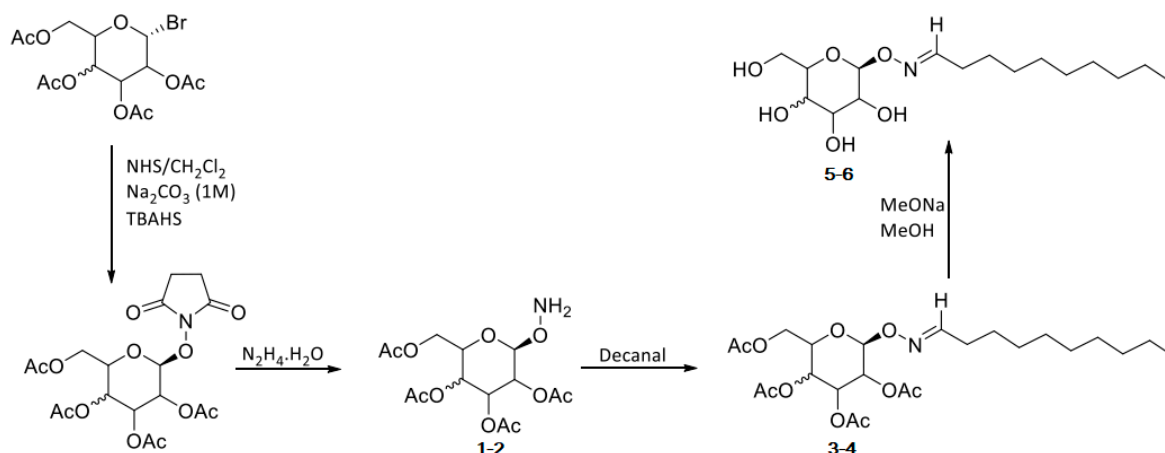

Figure 1. Multistep synthesis of amphiphilic glycosides Schiff bases from acetobromo-α-D-glucose and D-galactose.

In all cases, we obtained the products in good yields and high anomeric stereoselectivity. The values of the $^3J_{H1-H2}$ coupling constants were measured and all of them were around 8–9 Hz. This undoubtedly indicates the beta configuration at the anomeric center.

Their structures were determined and fully characterized by ¹H and ¹³C NMR spectroscopy and were as expected. The oxime ether glycosidic linkage is a Schiff base moiety. The *E* isomer is the thermodynamically more stable and consequently the major isomer obtained. ¹H NMR spectra of nonpurified products reveal the presence of a minor byproduct. An isomeric compound was detected in a 3:2 ratio in both the D-glucose and D-galactose derivatives. Particularly, the oximyl proton could be clearly differentiated, and we present δ values obtained in Table 1.

Table 1. Oximyl proton displacements for compounds **3** and **4**, the values were extracted from ¹H NMR spectra of isomeric mixtures.

Product	δ1 (ppm)	δ2 (ppm)
3	6.81	7.51
4	6.80	7.53

The complete ¹H and ¹³C NMR experiments demonstrate that this minor product was the *Z* isomer. The assignment of the *E/Z* configuration may be done from the ¹H NMR spectra of the oxime protons. It is well reported that the oximyl protons of (*E*)-oxime ethers are deshielded relative to those of (*Z*)-oxime ethers [6].

After flash column chromatography with 15–25 µm silica gel, it was possible to separate both isomers. Pure *E* and *Z* products were obtained and the NMR analysis confirms ours mentioned hypothesis. Comparing the data extracted from ¹H NMR spectra, with those reported previously by us and others we may consider that the *E* configuration isomer was the major product obtained [7] (Figure 2).
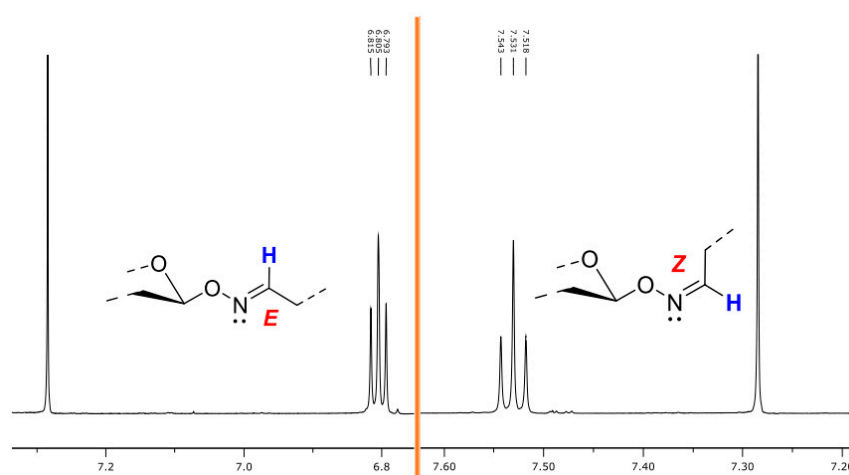

Figure 2. Partial ¹H NMR spectra for both *E* and *Z* isomers of **3a** and **3b**.

With the method optimized, after Zemplén deacetylation we synthesized four new -*O*-(*N*)-glycosides bearing a hydrophobic tail at the anomeric position as seen in Figure 3 [8].
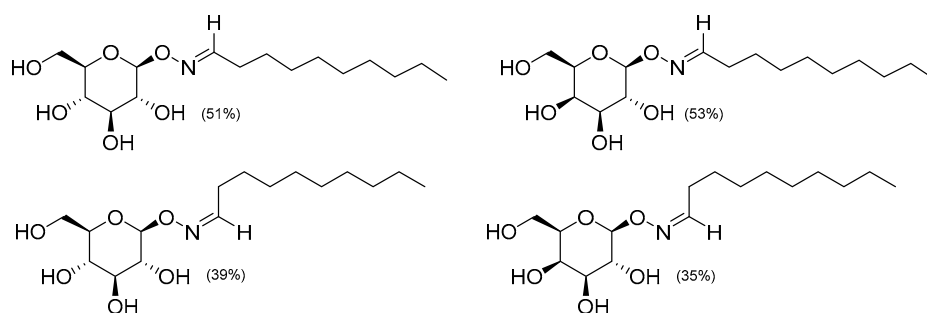

Figure 3. New amphiphilic -*O*-(*N*)-β-glycosides. Overall yields in brackets.

The glycosides synthetized were particularly stable. As previously described in the literature [9], the stability towards hydrolysis of the oximes is higher than simple hydrazones because of the electronegativity differences in the heteroatom (χ_O = 4.5 vs. χ_N = 3.0). No acetal or oxime hydrolysis products were detected after several days in the aqueous solution. Moreover, the protonation of the anomeric oxygen, to catalyze the acetal hydrolysis in an exocyclic cleavage, is also not favored. If an alternative endocyclic cleavage mechanism may be performed, the rate-limiting step involves the delocalization of the electrons on oxygen over the glycosidic bond. This delocalization is completely disfavored [10].

4. Conclusions

A simple and efficient route for the synthesis of novel amphiphilic carbohydrate-derived oxime ethers has been described. The products were obtained in good overall yields and with high anomeric and *E/Z* stereocontrol. Both the *E* and *Z* isomers were obtained pure after careful chromatography work and a complete structural analysis was done, compiling interesting data that may be used for setting future correlations. We recently initiated investigations to evaluate the surfactant properties and cytotoxic effects of the novel compounds prepared.

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8. Representative NMR data. Compound **4a**. ^{1}H: δ 7.53 (t, J =6.3 Hz, 1H, H7); 5.43 (dd, J =3.4, 1.1 Hz, 1H, H4); 5.32 (dd, J =10.4, 8.4 Hz, 1H, H2); 5.15 (d, J =8.45 Hz, 1H, H1); 5.10 (dd, J =10.35, 3.4 Hz, 1H, H3); 4.20 (d, J =6.8 Hz, 2H, H6); 4.03 (td, J =6.84, 1.20 Hz, 1H, H5); 2.24-1.27 (m, 16H, H 8-15); 2.17 (s, 3H, CH$_3$); 2.08 (s, 3H, CH$_3$); 2.06 (s, 3H, CH$_3$); 2.01 (s, 3H, CH$_3$); 0.90 (t, 3H, H16). ^{13}C: 170.35 (C=O); 170.28 (C=O); 170.14 (C=O); 169.50 (C=O); 155.11 (C7); 102.35 (C1); 71.15 (C3); 70.71 (C5); 67.54 (C2); 66.86 (C4); 61.01 (C6); 31.90-22.66 (CH$_2$); 20.80-20.59 (CH$_3$); 14.11 (C16).
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