Synthesis of Cholesteryl- α -D-lactoside via Generation and Trapping of a Stable β -Lactosyl Iodide

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ABSTRACT: The generation of β -lactosyl iodide was carried out under non-*in-situ*-anomerization, metal free conditions by reacting commercially available β -per-O-acetylated lactose with trimethylsilyl iodide (TMSI). The β -iodide was surprisingly stable as evidenced by NMR spectroscopy. Introduction of octanol or cholesterol under microwave conditions gave high yields of α -linked glycoconjugates. Careful analysis of the reaction products and mechanistic considerations suggest an acid catalyzed rearrangement that provides α -linked glycosylation products with a free C2-hydroxyl. Accessibility to these compounds may further advance glycolipidomic profiling of immune modulating bacterial derived-glycans.

This work is dedicated to the memory and inspiration of Professor Harry Wasserman.

Carbohydrate chemists are continually challenged to control the stereochemistry of glycosidic bond formation. Although methodologies exist to provide 1,2-*trans* linkages with great confidence,¹⁻³ procedures for achieving 1,2-*cis* glycosylations are limited.⁴ As early as 1975, Lemieux and co-workers reported efficient routes to

groups also render the interglycosidic bond susceptible to cleavage into smaller units when treated with TMSI.^{2,3,22} In stark contrast, ester protecting groups are referred to as “disarming” due to inductive effects; and while they stabilize interglycosidic linkages toward degradation, ester-protected glycosyl iodides are considerably less reactive than their ether protected counterparts. Moreover, if the ester is proximal to the anomeric center, e.g. at the C-2 position, it typically guides glycosylation toward 1,2-*trans* linkages (**Figure 1**). Considering these factors, the ideal oligosaccharide donor would have ester protecting groups on the hydroxyls remote from the anomeric center to protect against interglycosidic cleavage and an ether protecting group at the C-2 hydroxyl to activate the donor toward 1,2-*cis* attack. In fact, a monosaccharide corollary to this design was successfully employed by Demchenko and coworkers²³ wherein 3,4,6-tri-*O*-benzoyl-2-*O*-benzyl- β -glucosyl bromide underwent reaction with carbohydrate acceptors giving α -stereoselectivity. Importantly, a relatively “disarmed” β -glycosyl bromide was intercepted to achieve 1,2-*cis* connectivity. This work inspired us to explore the possibility of using ester-protected glycosyl iodides to synthesize cholesteryl- α -lactoside. Previous mechanistic studies in our lab had shown that β -glycosyl iodides could be generated from per-*O*-acetylated galactose and glucose encouraging us to consider per-*O*-acetylated lactose as a possible donor.²⁴ We were hopeful that the increased reactivity of the iodide relative to the bromide might allow us to use per-acetylated donors and avoid multiple protecting group manipulations. Thus, the formation of the β -iodo-lactoside was explored with the intent of developing conditions to trap the reactive intermediate with cholesterol in order to achieve 1,2-*cis* glycosylation.

Initial studies were carried out on a 1:3 α : β -mixture of per-*O*-acetylated lactose and the progress of the reaction was studied by NMR (**Figure 2**). Immediately upon introduction of TMSI into the NMR tube, the β -lactosyl acetate (**2**) began to react generating the β -anomeric iodide (**3**) due to anchimeric assistance from the C-2 acetate.²⁴ However, the reaction of lactose was significantly slower than previously studied monosaccharides, which turned out to be fortuitous because the lifetime of the β -iodo-lactoside (**3**) was significantly extended. As shown in **Figure 2**, the α -acetate (**1**) was essentially unreactive for the first 15 minutes of the reaction during which time the β -acetate was nearly consumed. As the reaction proceeded over the course of 24 hours, the equilibrium shifted to essentially quantitative formation of the α -iodide (**4**).

Figure 2. NMR analysis of a 1:3 α:β-mixture of per-*O*-acetylated lactose reacting with TMSI.

The generation of **3** using pure per-*O*-acetylated-β-lactoside (**2**) continued by reacting **2** with TMSI in deuterated chloroform in order to monitor iodide formation via NMR spectroscopy (**Figure 3**). As was observed with the α/β mixture, **2** was consumed within 10 minutes giving pure **3**, which was stable enough at ambient temperature to persist for a few hours before completely anomerizing to the α-iodide (**4**). Having established conditions for generating a relatively long-lived β-lactosyl iodide, investigations continued with the introduction of acceptor alcohols.

Figure 3. β-per-*O*-acetylated **2** can cleanly generates **3**.

Initial reactions of **3** with either octanol (2 equiv) or cholesterol (2 equiv) under microwave conditions afforded good glycoconjugate yields that favored 1,2-*cis* linkages and α-selectivity (**Scheme 1**). At the same time, these findings were surprising, as the major α-products **5** and **8** were missing the C-2 acetate. The reaction of **3** with octanol was completed in 30 minutes and afforded 87% overall yield of α-glycosides (44:1, α:β) in ~5:1 ratio of **5**:**6**. Similarly, the reaction with cholesterol produced a 71% yield of α-glycosides (7:1, α:β) in approximately the same ratio ~5:1 ratio of **8**:**9**, albeit the reaction required 2 hours with the more hindered acceptor. In the latter reaction, significant amounts of acetylated cholesterol (**13**) and 3-β-iodo-5-cholestene (**14**) were also recovered.

Scheme 1. Glycosylation with octanol and cholesterol.

Importantly, both α -linked compounds (**8**, **9**) could be deacetylated using sodium methoxide to afford cholesteryl α -D-lactoside **15** (Scheme 2).

Scheme 2. Deacetylation of cholesteryl α -lactosides **8** or **9**.

A plausible mechanistic explanation for the observed products is presented in **Scheme 3** and depends upon acid catalyzed rearrangement of the oxocarbenium resulting from anchimeric collapse onto the β -lactosyl iodide to give **16**. Attack by the nucleophile at the acetate carbon rather than the anomeric center leads to formation of orthoacetates (**17**). Protonation of the C-2 oxygen to give **18** with concomitant introduction of iodide at the anomeric center would produce **19** or alternatively oxocarbenium formation to produce **20**. Finally, nucleophilic

attack on the highly reactive β -iodide would lead to the observed major products, **5** or **8**. The other two major α -glycosides, **6** and **9** (**Scheme 1**), may result from direct nucleophilic attack on β -iodide **3**. Similarly, the minor amounts of β -products, **7** and **10**, may result from direct displacement of the α -iodide **4**. It should be noted, that the reactivity of **19** is presumed to be much higher than **3** because it does not have an electron withdrawing acetate at the proximal C-2 position. Studies in our lab have repeatedly shown that the reactivity of glycosyl iodides is inversely correlated with the number of acetate groups on the pyranose.^{3,12,15,22}

Scheme 3. Proposed mechanism of HI catalyzed orthoester rearrangement to α -linked 2-hydroxy glycosylation products **5** and **8**.

Achieving 1,2-*cis* glycosylation in the reaction of cholesterol addition to per-*O*-acetylated- β -lactosyl iodide contrasts earlier studies on monosaccharides. In 2008, Murakami, Sato, and Shibakami reported reacting α -iodo-2,3,4,6-*O*-tetraacetyl glucopyranoside with zinc halides and observed a 25-50% yield of β -linked product along with 15-20% yield of the 2-hydroxy product as an α : β -mixture.²⁵ The same reaction when performed on α -iodo-2,3,4,6-*O*-tetrabenzoyl glucopyranoside gave mostly 1,2-*cis* products with the C-2 benzoate remaining intact, however the reaction did not work when employing cholesterol as an acceptor. The reactions reported herein do not require metal catalysis, result in 1,2-*cis* glycosylation and are high yielding even with hindered cholesterol acceptors. It is noteworthy that Murakami and co-workers also observed 30-40% of acetylated acceptor, further supporting the acid catalyzed rearrangement mechanism of the orthoester (**Scheme 3**), which depicts the departure of acetylated acceptor as iodide attacks. Indeed, a small amount of acetylated cholesterol (**13**) was isolated from the reaction mixture, but the 3- β -iodo-5-cholestene (**14**) was the major byproduct obtained. It is likely that compound **14** was

generated by HI or TMSI catalyzed substitution of **13**, explaining the small amounts of isolated acetylated cholesterol **13**. To support this hypothesis, **13** was treated with TMSI under the same microwave conditions for 2 hours and a 73% yield of **14** was obtained. Retention of stereochemistry at C-3 was confirmed from X-ray crystallography (**Scheme 4**). In related studies, formation of 3- β -iodo-5-cholestene (**14**) was observed in the reaction of cholesterol with HI,²⁶ aluminum iodide,²⁷ and treatment of 3- β -*O*-mesyl-5-cholestene with TMSI.^{28,29} Retention of stereochemistry at C-3 results from a non-classical cyclopropylcarbinyl cation generated from homoallylic stabilization of initial carbocation **22** (**Scheme 4**).³⁰ Upon formation of cyclopropylcarbinyl cation (**23**) iodide attacks from the top face generating **14** (**Scheme 4**). The same reaction performed with dihydrocholesterol gave the inverted iodide as the major byproduct presumably due to S_N2 displacement of the acetate.³¹

Scheme 4. Reaction and mechanism of acetylated cholesterol (**13**) with TMSI to afford 3- β -iodo-5-cholestene (**14**).

The salient features of this research include the NMR characterization of the β -iodolactoside (**3**) carried out at ambient temperature, showing its unusual longevity and slow equilibration to the α -lactosyl iodide (**4**). Efficient trapping of the more reactive β -iodide anomer (**3**) via anchimeric assistance and then acid catalyzed rearrangement of the orthoester resulting from nucleophilic addition offers a new synthetic protocol for achieving 1,2-*cis* glycosylation of oligosaccharides. Importantly, the protocol accommodates sterically demanding nucleophiles such as cholesterol. Being able to carry out 1,2-*cis* glycosylations with per-*O*-acetylated sugars has the potential to rapidly access structurally diverse cholesteryl glycoside analogs because many per-*O*-acetylated oligosaccharides

are commercially available and even more are readily accessible by a one-step protection. To date, the cholesteryl glycosides isolated from natural sources have consisted of primarily monosaccharides. Generation of cholesteryl α -lactoside and higher order oligosaccharide constructs will furthered the synthetic campaign toward providing cholesteryl glycoside standards to aid in the discovery of novel naturally occurring cholesteryl glycosides.

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Supplementary Data.

Complete experimental data including NMR, MS, and X-ray diffraction data are available online at:

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