UC Davis

Title

Synthesis of cholesteryl-α-d-lactoside via generation and trapping of a stable β-lactosyl iodide

Permalink

Journal

ISSN

Authors

Davis, Ryan A
Fettinger, James C
Gervay-Hague, Jacquelyn

Publication Date

DOI

Peer reviewed

Synthesis of cholesteryl-α-D-lactoside via generation and trapping of a stable β-lactosyl iodide

Ryan A. Davis, **James C. Fettinger**, and **Jacquelyn Gervay-Hague**[*]
Department of Chemistry, University of California, Davis, One Shields Avenue, Davis, CA 95616, United States

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.

Keywords

H. pylori; 1,2-*cis*; Glycosyl iodide glycosylation; Mechanism

---

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 1,2-*cis* glycosides using in situ anomerization of anomeric bromides, Figure 1.[5,6] Over the years, we and others have expanded upon these findings increasing the repertoire of available α-linked-O-glycosides.[7–10] More recently, our laboratory has simplified the in situ anomerization of glycosyl iodides to enable step economical one-pot syntheses of α-linked glycolipids.[11–16] The preferred protocol relies upon electron-rich monosaccharide donors that are reacted with TMSI to first generate the α-glycosyl iodide; addition of tetrabutylammonium iodide leads to in situ anomerization, and subsequent trapping with a suitable nucleophile affords 1,2-*cis* glycosides. In this fashion, several biologically relevant glycolipids have been prepared including the immune modulating α-cholesteryl glucosides associated with *Helicobacter pylori* infection.[16–18] The pathogenicity of *H. pylori* involves bacterial uptake of host cholesterol and stereospecific biosynthesis of α-cholesteryl glucosides that lead to silencing

---

[*]Corresponding author. Tel.: +1 530 754 9577. jgervayhague@ucdavis.edu (J. Gervay-Hague).

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

of the host immune response.[19,20] As part of a campaign to search for other variants of naturally occurring steryl glycosides and to understand the molecular basis of steryl glycoside immune modulation, our lab is developing facile methods for the synthesis of various analogs including oligosaccharide-containing constructs. Herein, we report extension of glycosyl iodide glycosylation to the synthesis of α-cholesteryl lactosides.

Establishing 1,2-*cis* connectivity is especially difficult when employing hindered acceptors such as cholesterol or oligosaccharide donors, which can undergo inter-residue glycosidic bond cleavage.[21] The reactivity of glycosyl iodides is intricately linked to the protecting groups present on the donor. Ether protecting groups and especially silyl ether protecting groups are highly activating. Their electron releasing capacity has been said to 'arm' donors such as per-*O*-silyl glycosyl iodides toward nucleophilic attack. When attempting to react sterically hindered nucleophiles such as cholesterol, armed donors are preferred. However, unlike monosaccharide donors, per-*O*-silyl oligosaccharides cannot be used as donors in glycosyl iodide glycosylation because ether protecting 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, for example, at the C-2 position, it typically guides glycosylation toward 1,2-*trans* linkages (Fig. 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 co-workers[23] 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 (Fig. 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 min of the reaction during which time the β-acetate was nearly consumed. As the reaction proceeded

over the course of 24 h, the equilibrium shifted to essentially quantitative formation of the α-iodide (**4**).

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 (Fig. 3). As was observed with the *α/β* mixture, **2** was consumed within 10 min 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.

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 min 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 h 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.

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

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]

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 h 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]

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 further the synthetic campaign toward providing cholesteryl glycoside standards to aid in the discovery of novel naturally occurring cholesteryl glycosides.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

References and notes

1. Halcomb RL, Danishefsky SJ. J Am Chem Soc. 1989; 111:6661.

2. Hsieh HW, Schombs MW, Gervay-Hague J. J Org Chem. 2014; 79:1736. [PubMed: 24490844]

3. Hsieh HW, Davis RA, Hoch JA, Gervay-Hague J. Chem Eur J. 2014; 20:6444. [PubMed: 24715520]

4. Demchenko AV. Curr Org Chem. 2003; 7:35.

5. Lemieux RU, Hendriks KB, Stick RV, James K. J Am Chem Soc. 1975; 97:4056.

6. Lemieux RU, Driguez H. J Am Chem Soc. 1975; 97:4069. [PubMed: 51033]

7. Chernyak A, Oscarson S, Turek D. Carbohydr Res. 2000; 329:309. [PubMed: 11117314]

8. Baeschlin DK, Chaperon AR, Charbonneau V, Green LG, Ley SV, Lücking U, Walther E. Angew Chem, Int Ed. 1998; 37:3423.

9. Shingu Y, Nishida Y, Dohi H, Kobayashi K. Org Biomol Chem. 2003; 1:2518. [PubMed: 12956070]

10. Shingu Y, Nishida Y, Dohi H, Matsuda K, Kobayashi K. J Carbohydr Chem. 2002; 21:605.

11. Hadd MJ, Gervay J. Carbohydr Res. 1999; 320:61.

12. Du W, Gervay-Hague J. Org Lett. 2005; 7:2063. [PubMed: 15876055]

13. Du W, Kulkarni SS, Gervay-Hague J. Chem Commun. 2007:2336.

14. Kulkarni SS, Gervay-Hague J. Org Lett. 2008; 10:4739. [PubMed: 18798644]

15. Schombs M, Park FE, Du W, Kulkarni SS, Gervay-Hague J. J Org Chem. 2010; 75:4891. [PubMed: 20387787]

16. Davis RA, Lin C-H, Gervay-Hague J. Chem Commun. 2012:9083.

17. Davis RA, Fettinger JC, Gervay-Hague J. J Org Chem. 2014; 79:8447–8452. [PubMed: 25093454]

18. Nguyen HQ, Davis RA, Gervay-Hague J. Angew Chem, Intl Ed. 2014; 53:13400–13403.

19. Lee SJ, Lee BI, Suh SW. Proteins. 2011; 79:2321. [PubMed: 21557320]

20. Kobayashi M, Lee H, Nakayama J, Fukuda M. Curr Drug Metab. 2009; 10:29. [PubMed: 19149511]

21. Demchenko AV. Synlett. 2003:1225.

22. Hsieh HW, Schombs MW, Witschi MA, Gervay-Hague J. J Org Chem. 2013; 78:9677. [PubMed: 23980653]

23. Kaeothip S, Yasomanee JP, Demchenko AV. J Org Chem. 2011; 77:291. [PubMed: 22136383]

24. Gervay J, Nguyen TN, Hadd MJ. Carbohydr Res. 1997; 300:119.

25. Murakami T, Sato Y, Shibakami M. Carbohydr Res. 2008; 343:1297. [PubMed: 18406402]

26. Shoppee CW, Summers GHR. J Chem Soc. 1952:1786.

27. Broome J, Brown BR, Summers GHR. J Chem Soc. 1957:2071.

28. Sun Q, Cai S, Peterson BR. Org Lett. 2009; 11:567. [PubMed: 19115840]

29. Ortega N, Feher-Voelger A, Brovetto M, Padron JI, Martin VS, Martin T. Adv Synth Catal. 2011; 353:963.

30. Lambert JB, Ciro SM. J Org Chem. 1996; 61:1940.

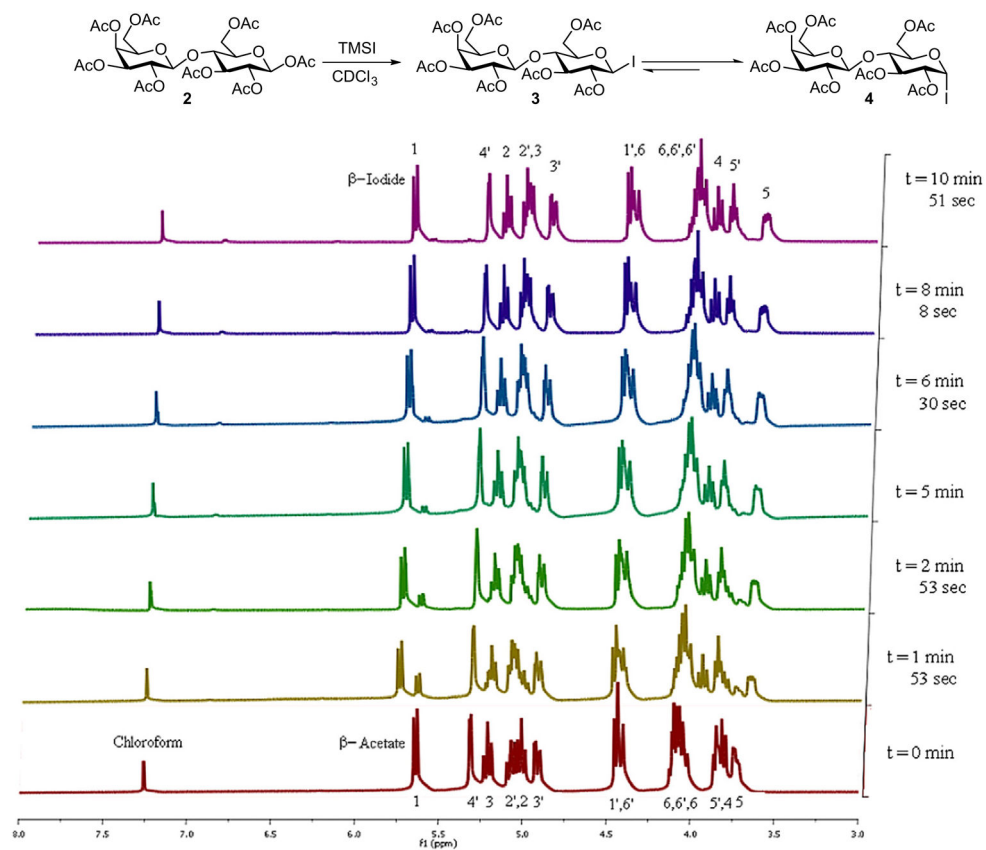31. Davis, RA. PhD Dissertation. University of California; Davis: 2012.
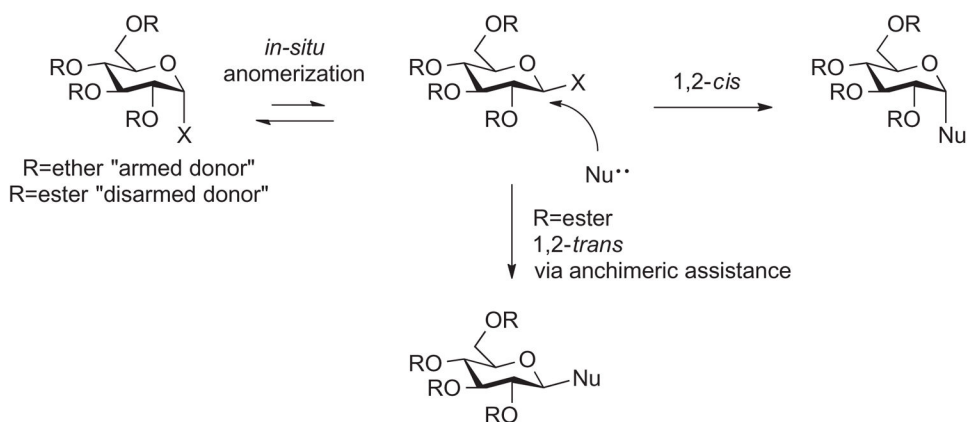
Figure 1.
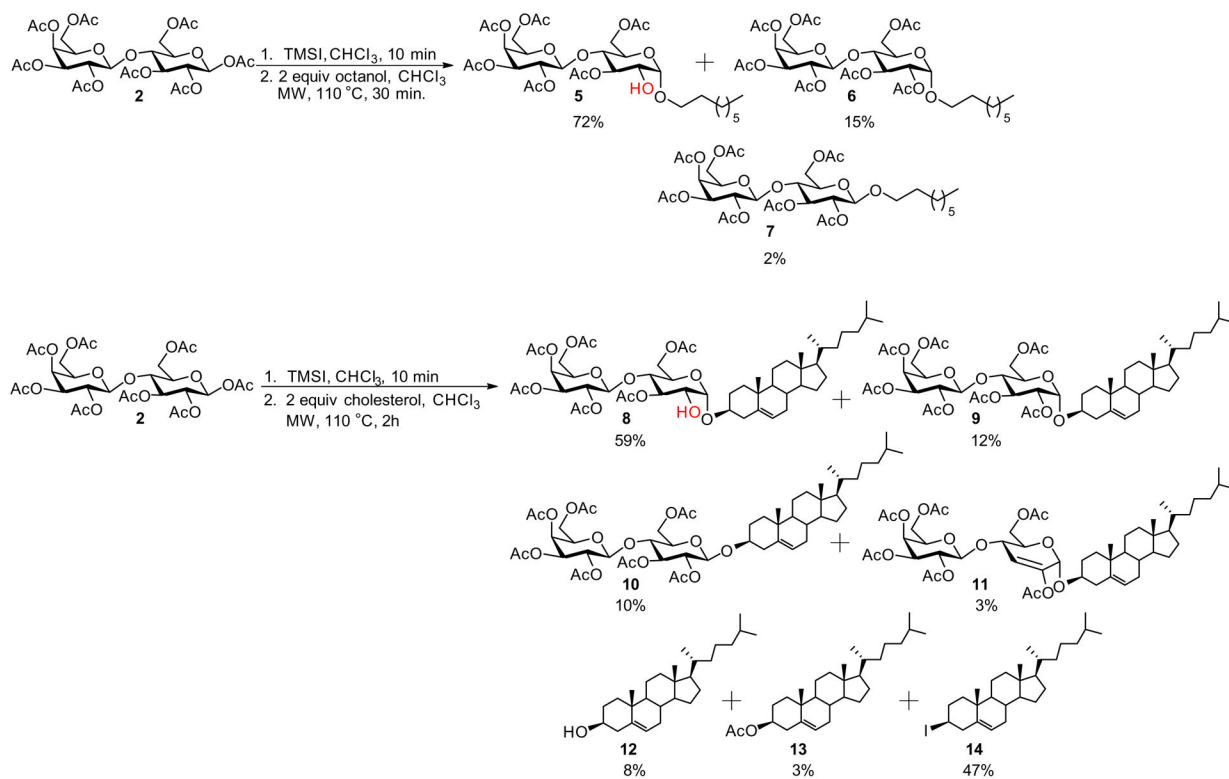In situ anomerization to achieve 1,2-*cis* connectivity.
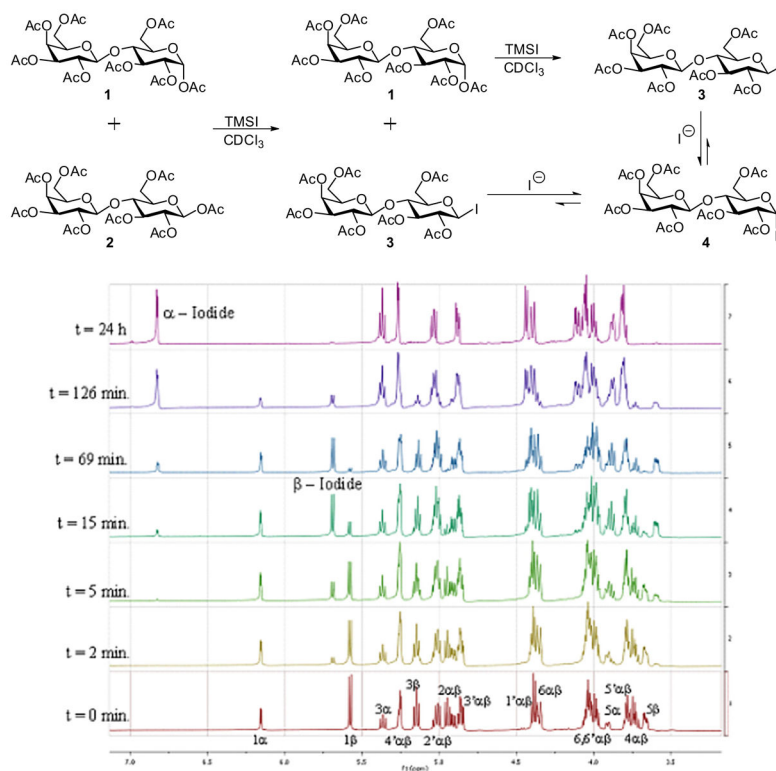
Figure 2.
NMR analysis of a 1:3 *α*/*β*-mixture of per-*O*-acetylated lactose reacting with TMSI.
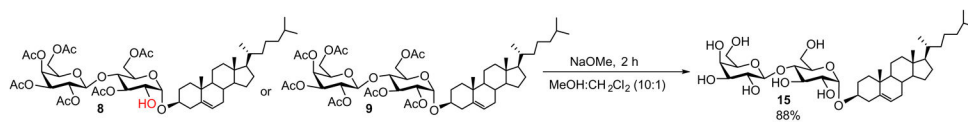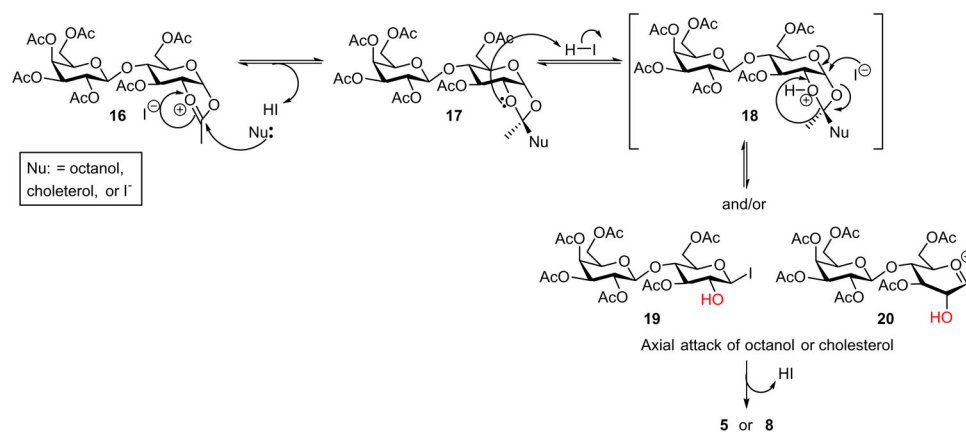
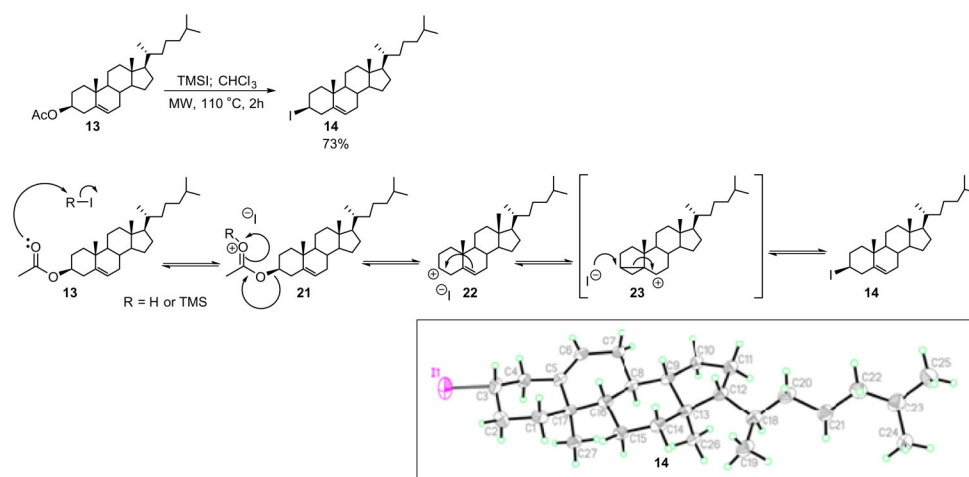**Figure 3.**
β-Per-*O*-acetylated **2** can cleanly generates **3**.

Scheme 1.
Glycosylation with octanol and cholesterol.

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

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

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