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Note

Synthesis of sterically crowded derivatives of anomeric pairs of D-glucose disaccharides

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Abstract—Derivatization of carbohydrates is of considerable interest since the derivatives can be used for structural studies in the field of mass spectrometry. We report here the synthesis of a series of sterically crowded derivatives of various linkage and stereoisomeric glucose–glucose disaccharides with the impetus being to understand the effect of these derivatized groups on fragmentation of the glycosidic bond and the development of methodology for discernment of the anomeric configuration. The synthesis of per-alkylated (methyl, ethyl, propyl, butyl, and pentyl), per-esterified (acetyl, pivaloyl, mesitoyl), and per-silylated (*tert*-butyl-dimethyl silyl) glucose–glucose disaccharide derivatives has been reported.

Keywords: Derivatized disaccharides; Glucose

The synthesis of derivatized carbohydrates are of considerable interest because they can serve as synthetic intermediates in which certain functional groups can temporarily protect those hydroxyls not planned to be involved in a desired manipulation.[1] Further, they are very widely used in structural studies in biological mass spectrometry.[2] In the search for bio-based materials, polysaccharide derivatives and their properties are of continuing interest. The synthesis described in this manuscript is mainly focused on per-etherification, per-esterification, and per-silylation of linkage and stereoisomeric glucose–glucose disaccharides.

The disaccharide ethers synthesized herein are the methyl, ethyl, propyl, butyl, and pentyl ethers. Methylation has been used for confirming linkage evidence of carbohydrate chemical structure.[3–6] With increasing recognition of the significance of complex carbohydrates, roles played in the determination of specificities of hormones, immunity, biological transport, recognition, developmental biology, and in various pathological phenomena, it has become more urgent to find rapid and sensitive methods for discerning the chemical structure. For this purpose per-methylation on a microscale is important. The higher ethers of carbohydrates are also important to study since they influence the conformational behavior, making the glycosidic linkages more sterically crowded, hence comparative fragmentation and stereoisomeric information can be obtained.[2,18]

The disaccharide esters synthesized herein are the per-acetylated, per-pivaloylated, and the per-mesitoylated derivatives. Per-acetylated oligosaccharides have been used extensively in the structural determination of oligosaccharides[7–13] because they are of interest not only in relation to the conformational data of fully acetylated polymeric derivatives such as cellulose acetate,[14] but also because many naturally occurring polysaccharides particularly those of microbial origin are partially acetylated.[15] Although the biological functions of these acetyl groups are not well understood, it is known in some instances, for example, gellan, their presence greatly affects the rheological properties of aqueous solutions. Similarly, it may be possible that pivaloylated and mesitoylated derivatives will have some useful physical characteristics.

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Several approaches have been developed for the preparation of methylated derivatives. The early methods were those of Purdie and Irvine[16] and Haworth.[17] The disadvantage of the Purdie and Haworth techniques is that they require several re-methylations to obtain complete etherification. Strong bases such as sodium hydride or potassium *tert*-butoxide in dipolar aprotic organic solvents are now used. Using methyl iodide in *N*,*N*-dimethylformamide with silver oxide,[5,19] barium hydroxide,[20] or sodium hydride[21] as the basic agent, the reaction rates increased but full methylation was not achieved. The use of sodium hydride has a disadvantage in that it requires careful handling. Corey and Chaykovsky developed a new synthesis using the methyl sulfinyl carbanion, the conjugate base of dimethyl sulfoxide.[22] The Wittig type synthesis was achieved with great ease.[23,24] Hakomori adapted Corey's methylsulfinyl carbanion[25] reagent to the methylation of even complex carbohydrates. In spite of the low yields (0.3 mol of per-methylated derivative per mol of sugar), the Hakomori method has been used extensively in structural investigations of carbohydrates. The use of potassium *tert*-butoxide[26] instead of sodium hydride improved the stability of the reagent but did not substantially increase the yield of the per-methylated product.[27,28] In 1984, Ciucanu and Kerek[29] reported a simple and rapid method for the per-methylation of carbohydrates by using dimethyl sulfoxide, sodium hydroxide, and methyl iodide. This approach has the advantage of readily available starting materials, potential compatibility with a wide range of protecting groups, and a very straightforward experimental procedure. Thus, we envisioned this as an ideal method for making the different per-alkylated derivatives efficiently, as it avoids the experimental difficulties of the Hakomori method (e.g., low yields and moisture-sensitive reagents).

Herein, we show that an adaptation of this method works very well for the preparation of protected disaccharides with higher alkyl groups. Additionally, to the best of our knowledge per-ethylated, per-propylated, per-butylated, and per-pentylated glucose–glucose disaccharides have not been reported and can be prepared in good yields with this straightforward chemistry. The advantage of this method is the greater generality of protecting groups that can be used. However, for the higher derivatives complete etherification of the disaccharides was not obtained if the alkylating agent was added in its entirety, but only if added in incremented amounts on a daily basis for about a week.

The per-esterified derivatives prepared were the per-acetylated, per-pivaloyl, and the per-mestitoyl derivatives. Per-acetylated derivatives have been synthesized previously.[30] The hydroxyl groups of a carbohydrate material react readily with acetic anhydride in the presence of a basic catalyst like pyridine. However, the per-pivaloyl and per-mesitoyl derivatives of carbohydrates have not been reported (to the best of our knowledge) and were prepared in good yields. No difficulties were encountered in the synthesis of the acetylated and pivaloylated derivatives. For the mesitoylated derivatives a large excess of mesitoyl chloride had to be added to ensure complete derivatization of the disaccharides.

One of the most widely used protective groups for alcohols since its introduction by Corey and Venkateswarlu is the *tert*-butyl-dimethyl silyl (TBDMS) group.[31] The chemical properties of these hindered silyl ethers make them desirable intermediates for a large number of synthetic transformations involving multifunctional compounds. The established procedure for the preparation of TBDMS ethers involves reaction of an alcohol with *tert*-butyl dimethylchlorosilane (1.1 equiv) in the presence of imidazole (2.2 equiv) in *N*,*N*-dimethylformamide (DMF) at room temperature. However, this procedure was not efficient in completely silylating all the eight hydroxyls of the disaccharides. The TBDMS derivatives of disaccharides have been reported to the best of our knowledge for the first time in this manuscript using the procedure of Corey et al.[32] in which TBDMS triflate is used as the silylating agent.

This paper discusses the synthesis of sterically crowded derivatives of glucose–glucose disaccharides. The impetus for the synthesis of these carbohydrate derivatives was to test the hypothesis that the propensity of molecular fragmentation in mass spectrometers depends on the inverse of the molecular flexibility.[2] Further, to understand the effect of the alkyl groups on the fragmentation of the glycosidic bond and the development of methodology for discernment of the anomeric configuration. The mass spectrometry results of the per-alkyl derivatives along with the molecular modeling of the 1–4 and 1–6 linked disaccharides have been described elsewhere by Mendonca et al.[18,33]

1. Experimental

1.1. General

The protected disaccharide derivatives were prepared according to the methods described below. The yields are shown in Table 1 and R_f values in Table 2. Table 3 shows exact mass measurements of the various synthetic derivatized disaccharides. Figure 1 is the exact mass measurement of per-butylated maltose [D-Glc-(1→4)-α-D-Glc], which serves as a representative spectrum. The thin-layer chromatograms of the higher derivatives like pentyl however, revealed a number of spots because of the formation of partially derivatized products.

Table 1. Product yields for synthetic derivatized disaccharides (%)

Derivative	Maltose	Cellobiose	Isomaltose	Gentiobiose
Per-methylated	90.50	91.00	90.80	91.50
Per-ethylated	76.00	83.00	81.80	80.30
Per-propylated	69.00	70.00	68.10	67.20
Per-butylated	60.00	59.00	56.70	57.50
Per-pentylated	49.00	49.50	50.00	52.00
Per-acetylated	90.30	90.50	91.00	92.00
Per-pivoylated	75.80	73.50	96.80	75.50
Per-mesitoylated	61.30	63.40	62.50	60.30
Per-TBDMsilylated	69.50	70.30	68.50	69.90
	αα-Trehalose	ββ-Trehalose	Kojibiose	Sophorose
Per-methylated	92.40	90.90	87.80	90.90
Per-ethylated	78.50	77.29	73.60	72.70
Per-propylated	65.21	63.51	69.60	65.50
Per-butylated	59.10	54.78	55.60	57.39
Per-pentylated	48.50	49.27	47.00	49.27
	Nigerose	Laminaribiose		
Per-methylated	89.30	92.40		
Per-ethylated	78.50	76.00		
Per-propylated	68.60	64.60		
Per-butylated	59.10	58.20		
Per-pentylated	51.10	47.76		

Table 2. R_f values for synthetic derivatized disaccharides

Derivative	Maltose	Cellobiose	Isomaltose	Gentiobiose
Per-methylated	0.34	0.16	0.35	0.28
Per-ethylated	0.49	0.62	0.68	0.66
Per-propylated	0.28	0.18	0.44	0.22
Per-butylated	0.24	0.21	0.28	0.24
Per-pentylated	0.15	0.25	0.30	0.28
Per-acetylated	0.78	0.43	0.45	0.67
Per-pivaloylated	0.28	0.21	0.33	0.29
Per-mesitoylated	0.31	0.17	0.34	0.30

1.2. Alkylation

The underivatized disaccharide (5 mg, 0.015 mmol) was introduced into a dried tube. DMSO (2 mL) was added and the solution stirred till it dissolved. Powdered sodium hydroxide (200 mg, 5 mmol) was added and the suspension was stirred for 10 min. Finally methyl iodide (0.21 g, 1.4 mmol) was added and the solution was stirred at room temperature for 24 h. The product was extracted with chloroform (3 mL), washed several times with distilled water (4×10 mL), and dried with sodium sulfate and then at high vacuum to yield a yellowish oil. The same reagents were used for preparation of the higher derivatives from ethyl to pentyl with differences in the proportions of alkyl iodide added. For ethylation 500 μL of ethyl iodide was added in 100 μL (0.19 g, 1.25 mmol) aliquots over a period of 5 days after which the products were extracted in chloroform as for methylation. For propylation 600 μL of propyl iodide was added in 100 μL (0.17 g, 1.03 mmol) increments over a period of 6 days after which the product was extracted. Butylation was carried out over a period of 7 days in 100 μL (0.16 g, 0.87 mmol) increments. Preparation of the ethyl, propyl, and butyl derivatives were all carried out at room temperature. The pentyl derivatives were synthesized by first heating the reaction mixture at 55–60 °C for 24 h and then for 8 days at room temperature. Two milliliters of pentyl iodide was added to the reaction mixture in aliquots of 200 μL (0.3 g, 1.5 mmol) for 9 days, after which the product was extracted with chloroform.

1.3. Acetylation

The underivatized disaccharide (10 mg, 0.02 mmol) was introduced in a dried flask. It was dissolved in dry pyridine (1 mL). To this Ac_2O (1.5 mL) was added and the reaction mixture was then stirred, heated to 60 °C, and stirred for 24 h. The product was then extracted with ethyl acetate (5 mL) and washed with water (10 mL). It was then washed with 10% HCl (2×10 mL) to remove the pyridine. It was further washed with $NaHCO_3$

Table 3. Exact measurements of derivatized disaccharides

Derivative	Maltose	Cellobiose	Isomaltose	Gentiobiose
Per-methylated	455.264	455.251	455.264	455.264
Per-ethylated	589.362	589.361	589.363	589.355
Per-propylated	701.476	701.463	701.472	701.476
Per-butylated	813.594	813.593	813.583	813.596
Per-pentylated	925.708	925.670	925.701	925.699
Per-acetylated	701.250	701.485	701.195	701.350
Per-pivaloylated	1037.241	1037.425	1037.007	1037.140
Per-mesitoylated	1533.479	1533.453	1533.461	1533.468
Per-TBDMsilylated	1277.390	1277.450	1277.658	1277.700
	αα-Trehalose	ββ-Trehalose	Kojibiose	Sophorose
Per-methylated	477.254	477.254	477.254	477.255
Per-ethylated	589.376	589.373	589.367	589.372
Per-propylated	701.509	701.486	701.488	701.485
Per-butylated	813.582	813.598	813.615	813.505
Per-pentylated	925.704	925.698	925.737	925.735
	Nigerose	Laminaribiose		
Per-methylated	477.257	477.254		
Per-ethylated	589.366	589.377		
Per-propylated	701.483	701.489		
Per-butylated	813.600	813.599		
Per-pentylated	925.718	925.738		

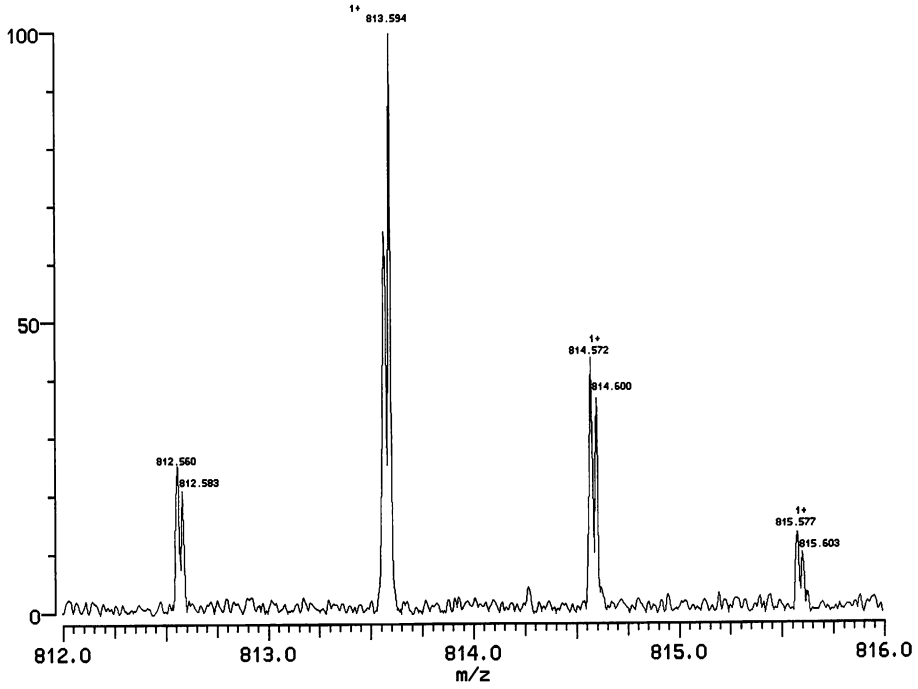

Figure 1. Exact mass measurement (813.594) of per-butylated maltose [D-Glc-(1→4)-α-D-Glc].

(2 × 10 mL) and finally with water (10 mL). It was then dried under Na_2SO_4 under high vacuum.

1.4. Pivaloylation

The underivatized disaccharide (5 mg, 0.015 mmol) was introduced into a dry flask. It was dissolved in dry pyridine (230 μL). Dimethyl amino pyridine (DMAP) (17.6 mg, 0.0049 mmol) was added to catalyze the reaction. Finally, trimethylacetyl chloride (0.18 g, 1.46 mmol) was added to the reaction mixture. The reaction mixture was then stirred at room temperature for 2 days. The product was then extracted with ethyl acetate (10 mL) and washed with water (2 × 10 mL),

followed by 10% HCL (2×10 mL), then $NaHCO_3$ (2×10 mL) and finally again with water (10 mL). The product was an oil, which was vacuum rotary evaporated and then dried under high vacuum.

1.5. Mesitoylation

The underivatized disaccharide (100 mg, 0.29 mmol) was introduced into a dry, two-necked flask under Ar. Dry pyridine (10 mL) was added and the solution stirred until it dissolved. Dimethyl amino pyridine (350 mg, 2.9 mmol) was then added followed by trimethylbenzoyl chloride (5.34 g, 29.2 mmol). The solution was heated to 80 °C and stirred for 3 days. The product was then extracted with ethyl acetate (20 mL) and washed with water (10 mL) followed by 10% HCL (2×10 mL), then satd aq $NaHCO_3$ (2×10 mL) and finally by water (10 mL). The product was then rotary evaporated and finally dried with vacuum to yield a yellowish solid.

1.6. Silylation

The underivatized sugar (100 mg, 0.29 mmol) was introduced in a dry flask under Ar. Dry methylene chloride (10 mL) was added and the solution stirred until most of the sugar dissolved. Dry 2,6-lutidine (0.75 g, 6.9 mmol) was then added followed by *tert*-butyl-dimethyl triflate (0.93 g, 3.5 mmol). On addition of the triflate the undissolved sugar dissolved completely and the reaction mixture became clear. The reaction mixture was stirred at room temperature for a day. The product was extracted with ether (20 mL) and washed with water (20 mL). The aqueous layer was then extracted with ether (3×10 mL). The product in the combined ether extracts was rotary evaporated and dried under high vacuum to yield a clear oil.

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