# Aqueous Glycosylation of Unprotected Sucrose Employing Glycosyl Fluorides in the Presence of Calcium Ion and Trimethylamine

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S *Supporting Information*

ABSTRACT: We report a synthetic glycosylation reaction between sucrosyl acceptors and glycosyl fluoride donors to yield the derived trisaccharides. This reaction proceeds at room temperature in an aqueous solvent mixture. Calcium salts and a tertiary amine base promote the reaction with high site-selectivity for either the 3′-position or 1′-position of the fructofuranoside unit. Because nonenzymatic aqueous oligo-saccharide syntheses are underdeveloped, mechanistic studies were carried out in order to identify the origin of the selectivity,

which we hypothesized was related to the structure of the hydroxyl group array in sucrose. The solution conformation of various monodeoxysucrose analogs revealed the co-operative nature of the hydroxyl groups in mediating both this aqueous glycosyl bond-forming reaction and the site-selectivity at the same time.
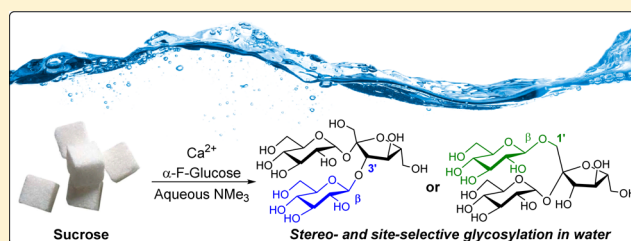
■ INTRODUCTION

The advancement of carbohydrate science depends critically on the ability to synthesize complex sugars in a highly selective manner. Tremendous successes have been achieved in carbohydrate synthesis, both in terms of efficiency and complexity in many synthetic settings.[1] Specifically, a large number of methods for the construction of the challenging glycosidic linkage has emerged. Most laboratory syntheses rely on the use of activating groups to enable glycosidic bond formation via bimolecular substitution (S_N2) or an activated oxocarbenium intermediate (S_N1).[2] The electrophilicity of these intermediates necessitates a protecting group strategy for successful coupling, avoiding reaction with other undesired hydroxyl groups.[3] It also precludes the use of aqueous solvent. Thus, protecting group-free synthetic glycosylation reactions that produce oligosaccharides under aqueous conditions are scarce.[4] Enzymatic methods using glycosyl transferases or hydrolases,[5−7] however, afford efficient and selective reactions in buffered water, employing prefashioned sugar nucleotides or non-reducing sugars as the glycosyl donors (Figure 1).[8] The

enzymatic catalysts harness considerable molecular complexity to achieve the necessary precision in the active site such that the transition state favors glycosylation between a glycosyl donor and acceptor, while hydrolysis does not conspire to prevent the coupling.

With the long-term objective of developing efficient and selective glycosylation reactions independent of enzyme specificities, we aimed to discover the requirements for nonenzymatic glycosylation reactions conducted in water and with no protecting group used at any stage.[9] Initially, we sought to search for simple glycosyl donors that would be potentially reactive at the C1-position, while exhibiting stability in water. In this context, we selected glycosyl fluorides as the donor,[10] which have been extensively studied by Jencks as model substrates involved in the hydrolysis of glycosidic bonds (Scheme 1a).[11] More precisely, Jencks showed that α-D-glucosyl fluoride (**1a**) reacts in an aqueous solution of sodium azide and acetate salts to produce the corresponding β-anomers **2a/2b**, overcoming the formation of D-glucose. However, the
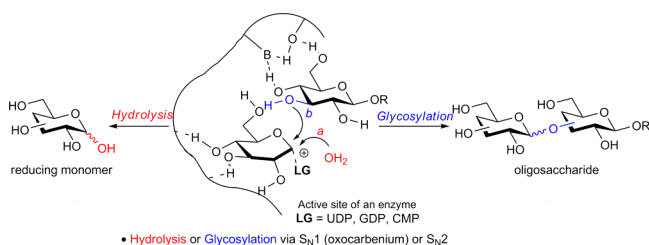
Figure 1. Competition between hydrolysis (H$_2$O (a), in red) and glycoside bond formation (acceptor (b), in blue) for a given activated donor in the active site of an enzyme.

**Scheme 1. Stereoinvertive Substitution of α-F-Glucose (1a) Using Simple Nucleophiles in Water**
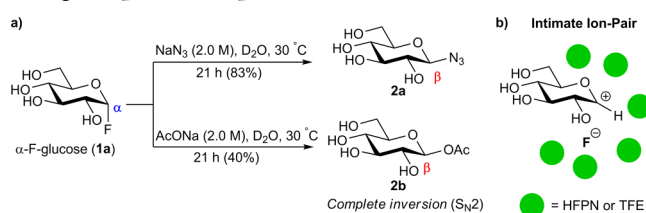
aqueous solvolysis of glycosyl fluorides in the presence of different alcohols as potential nucleophiles revealed a preference for reaction with water. No glycosylations of weakly nucleophilic alcohol acceptors were observed. These results theoretically preclude glycosylation with typical polyol acceptors in the absence of an activator (or catalyst).

Subsequent experimentation disclosed that these reactions were found to be concerted only in the presence of strong nucleophiles. These postulates were later revisited by Chan and Bennet, who observed **1a** to react with weakly nucleophilic alcohols, such as 1,1,1-trifluoroethanol (TFE) or hexafluoro-1-propanol (HFPN), under anhydrous conditions.[12] Substitution occurs via an S_Ni-like pathway, wherein the fluoride and an oxocarbenium-like species are present in the transition state as an intimate, nonsolvated ion pair (Scheme 1b).[12,13] The capability of the leaving fluoride ion to engage in hydrogen bonding allows for a general acid/base catalysis mechanism to ensue.[12a,14] The conclusions of these reports as well as the widespread applications of glycosyl fluorides as transition-state analogue substrates (TSAS) for hydrolase enzymes grounded our interest in these monomers as potential substrates for aqueous glycosylation.[15]

Inspired by the divalent metal cation-dependent nature of many glycosyltransferases,[16] we postulated that a combined Lewis acid/Lewis base approach might provide the necessary transition-state organization to favor glycosylation of the glycosyl fluoride while outcompeting hydrolysis (Figure 2).
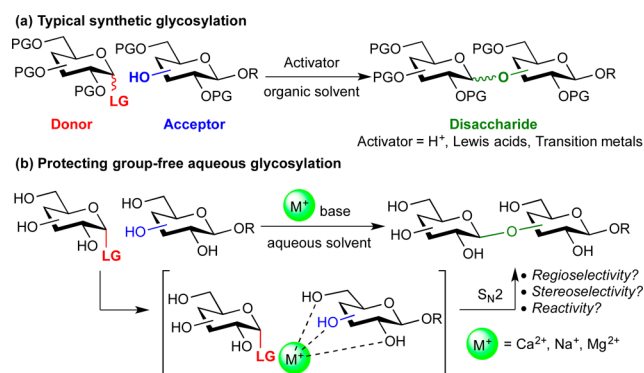
Figure 2. Synthetic glycosylation strategies for preparation of di- and oligosaccharides: (a) A fully protected donor with a suitable anomeric leaving group reacts with a partially protected acceptor to form a disaccharide in organic solvent; and (b) both water-soluble donor and acceptor react to form the same bond without the need for directing/protecting groups.

This strategy could also enhance the reactivity of any alcohol toward substitution. A close comparison can be made with traditional synthetic strategies that rely on alcohol chelation when metals are employed as catalysts for hydroxyl group functionalization in organic solvents.[1c] As part of this analysis, we were aware of the affinity of various sugars for certain water-soluble main group metal salts, including Ca^{2+}, Na^+, K^+, and Mg^{2+}, and this could be exploited to achieve complexation-induced glycosylation.[17,18]

Toward the development of such a reaction, we chose to examine sucrose as the glycosyl acceptor due to its high solubility in water, intrinsic natural abundance, and low cost. Furthermore, as a polyol, this substrate offers ample opportunity to explore site-selectivity in parallel with the development of a glycosylation reaction.[19,20] We describe

herein the successful elaboration of nonenzymatic, chemoselective glycosylation reactions between glycosyl fluorides and sucrosyl acceptors. The nature of the transformations is elucidated through independent synthesis and evaluation of eight unique deoxysucrose substrates. These experiments culminate in the delineation of the specific hydroxyl group array that is required for successful aqueous glycosyl transfer. These findings may offer a framework for the generalization of this approach beyond sucrose, providing a possible bridge between nonenzymatic glycosylation and the aqueous environments endemic to enzymatic catalysis.

■ RESULTS AND DISCUSSION

Discovery of an Aqueous Glycosylation of Sucrose. Following Jencks' and our own investigations into reactions of minimally protected carbohydrates,[11,21] we studied the reactivity of α-D-glucosyl fluoride **1a** toward an aqueous solution of sucrose, a complex carbohydrate acceptor (Scheme 2).[22] When treated with 0.5 equiv of sucrose in the absence of

Scheme 2. Observed Reaction Pathways and Formation of a New Trisaccharide (7a)
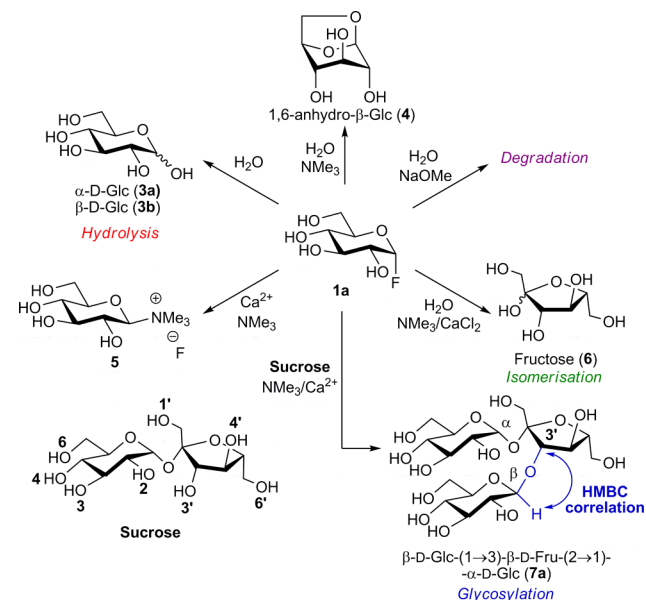
any additives, compound **1a** hydrolyzes slowly and cleanly to generate α- and β-D-glucopyranose (**3a** and **3b**, respectively) in 9% yield after 48 h at room temperature. In a separate control experiment, 1.0 equiv of $Ca(NO_3)_2 \cdot 4H_2O$ was found to accelerate hydrolysis of **1a**, while no glycosylation was observed. This behavior was found to be general, as the replacement of Ca^{2+} with other main group salts (Li^+, K^+, Na^+, Mg^{2+}, Ba^{2+}, Cs^+) or water-soluble transition metals (Cu^{2+}, Ni^{2+}, La^{3+}, Zr^{4+}) led to no reaction or to a comparable rate of hydrolysis for the fluoride **1a**. The addition of an aqueous base (NMe_3) alone also furnished the hydrolysis products **3a**/**3b**, along with the cyclization product 1,6-anhydro-β-glucose **4**.[12a] Strong bases (NaOH or NaOMe, for example) were found to give extensive amounts of compound **4** and degradation byproducts. On the other hand, addition of both $Ca(NO_3)_2 \cdot 4H_2O$ and trimethylamine afforded the glycosylation product **7a** in 20% yield (with respect to sucrose) after 48 h. A close examination of both the unpurified and purified reaction mixtures by ^{1}H NMR in D_2O revealed that the reaction

proceeded with both complete stereochemical inversion of the anomeric center of the glycosyl donor and with complete regioselectivity for the 3′ position of the fructofuranoside unit of sucrose.[22] This was confirmed by a heteronuclear multiple bond correlation (HMBC) NMR analysis of **7a** between the carbon at position C-3′ of the fructofuranose unit and the axial proton H-1″, geminal to the newly formed O-β-Glc anomeric linkage (highlighted in cyan, Scheme 2).[22]

In addition to the previously detected side-reactions (toward **3a**, **3b**, **4**), we observed the formation of a small quantity of D-fructose (Fru) (**6**). Interestingly, hydrolysis of α-D-glucosyl fluoride donor **1a** to D-glucose appears to rearrange to form D-fructose (**6**) under the reaction conditions (with CaCl₂ instead of Ca(NO₃)₂·4H₂O).[23] This precedented glucose/fructose rearrangement is also known to occur with simultaneous epimerization of D-glucose (Glc) (**3a**/**3b**) to D-mannose, which we also observed. Thus, we suspect that **6** is formed from an isomerization process and not from the decomposition of sucrose and/or **7a**. Indeed, extensive decomposition of sucrose or **7a** does not occur when they are treated with Ca²⁺ and aqueous NMe₃ in the absence of **1a**. In addition, we were able to identify and characterize the β-trimethylammonium glucosyl fluoride **5**, generated from stereoinvertive nucleophilic addition of NMe₃ to **1a**. Through control experiments, we also found that product **5** is formed when **1a** is subjected to the reaction conditions in the absence of the glycosyl donor.[22,24] The formation of any epoxide intermediate formed from **1a** was not observed.[25]

Optimization of Conditions. With the striking observation that **7a** can be formed under aqueous conditions, we decided to optimize the glycosylation reaction conditions to give this isomer in high yields (Table 1). Initially, we found that an excess of **1a** (5 equiv) and a high concentration of Ca(NO₃)₂·4H₂O (8 equiv) led to an improved efficiency such that 42% conversion to **7a** could be obtained as a single stereo- and regioisomer (entry 1, with respect to sucrose as the limiting reagent). A screen of various additives revealed that only Ca²⁺ salts are effective at promoting glycosylation over hydrolysis of **1a** (e.g., no reaction was observed with NaNO₃ and KNO₃, entries 2–3). A strong effect of the counterion was observed, with dissociated anions (CaBr₂·xH₂O and Ca(OTf)₂, more soluble salts) affording reactivity, whereas no reactivity was observed with insoluble or partially soluble salts containing more basic counterions (CaCO₃ or CaSO₄·2H₂O, entries 4–7). With salts possessing highly dissociable counterions (e.g., triflate, nitrate, iodide), we observed a marked dependence of the conversion to **7a** on the concentration of sucrose in the aqueous medium.

At a higher concentration of sucrose (>1.0 M) and with 8 equiv of Ca(OTf)₂, no reactivity is observed; however, with a more dilute solution (0.5 M) in sucrose, an improved 69% conversion to **7a** is obtained (cf., entries 7, 8, and 10). Interestingly, this important jump in reactivity is also observed with Ca(NO₃)₂·4H₂O, but to a lesser extent (42% at 1.0 M to 50% at 0.5 M, entries 1 and 9). When the reaction is conducted in the presence of 7.0 equiv of both Ca(OTf)₂ and **1a**, we found that a concentration of 0.30 M in sucrose was optimal (entry 13).[26] The equivalents of calcium salt and fluoride donor could be adjusted to 6.0 equiv without a concomitant decrease in reactivity (82%, entry 15), but further decreasing the equivalents impeded the reaction rate and lower yields of **7a** were observed.[22] Modifying the base and/or ratio of base relative to water was detrimental since hydrolysis of **1a** was

**Table 1. Selected Optimized Parameters for the Glycosylation**

sucrose (0.1 mmol)   M⁺ (I or II) / α-F-Glucose (**1a**) / 45% aq. NMe₃ / RT, 16 h   (**7a**)
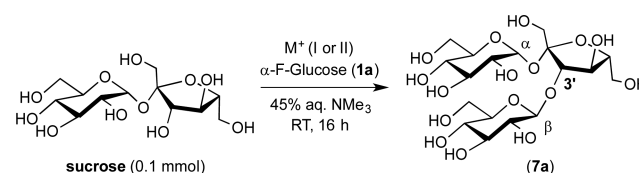
entry	M⁺ or M²⁺ (equiv)	conc. (M)[a]	α-F-Glc (equiv)	conv. to 7a (%)[b]
1	Ca(NO₃)₂ (8)[c]	1.0	5.0	42
2	NaNO₃ (8)	1.0	5.0	<5
3	KNO₃ (8)	1.0	5.0	<5
4	CaCO₃ (8)	1.0	5.0	<5
5	CaSO₄ (8)[c]	1.0	5.0	<5
6	CaBr₂ (8)[c]	1.0	5.0	40
7	Ca(OTf)₂ (8)	1.0	5.0	17
8	Ca(OTf)₂ (8)	1.5	5.0	<5
9	Ca(NO₃)₂ (8)[c]	0.50	5.0	50
10	Ca(OTf)₂ (8)	0.50	5.0	69
11	Ca(OTf)₂ (7)	0.50	7.0	71
12	Ca(OTf)₂ (7)	0.40	7.0	79
13	Ca(OTf)₂ (7)	0.30	7.0	81
14	Ca(OTf)₂ (7)	0.20	7.0	77
15	Ca(OTf)₂ (6)	0.30	6.0	82
16	Ca(OTf)₂ (6)	0.30	6.0	69[d]
17	Ca(OTf)₂ (6)	0.30	6.0	87 (80)[e,f,g]

[a]Concentration of sucrose in the aqueous solvent (M). [b]Conversion to **7a** (%) determined by ¹H NMR. [c]Hydrate of the salt was employed [d]Reaction diluted in 30% aq NMe₃ instead of 45% aq NMe₃. [e]Reaction performed at 30 °C instead of RT. [f]Reaction performed on 1.0 mmol of sucrose instead of 0.1 mmol. [g]Isolated yield in parentheses.

found to be more extensive (69% with 30% aq NMe₃, entry 16). We were able to achieve glycosylation on a practical scale by elevating the temperature to 30 °C, which afforded the desired product in 80% isolated yield (1.0 mmol of sucrose, entry 17). The product **7a** can be readily purified by column chromatography. Assessment of its purity by conventional NMR and combustion analysis demonstrated that the monosaccharides byproducts, silica gel, or residual calcium salts are indeed removed by this method. Alternatively, peracetylation of the trisaccharide **7a** (Ac₂O/DMAP in pyridine)[22] provided a derivative that is soluble in conventional organic solvents.

Reaction Scope. Encouraged by these results with sucrose, we decided to investigate the glycosylation of other sucrose-like oligosaccharides (Scheme 3). We were eager to see if the high selectivity observed for product **7a** could be translated to more complex polyols. For example, xylosucrose, raffinose pentahydate, and stachyose hydrate, oligosaccharides of biosynthetic origin and readily obtained from commercial suppliers, afforded the desired glycosylated products **7b**, **7c**, and **7d** in practical yields (76%, 59%, 55%, respectively). With stachyose, the product and starting saccharide were found to coelute, and peracylation (with Ac₂O/DMAP in pyridine) of the mixture was necessary in order to isolate the pure product.

Strikingly, under the optimized Ca(OTf)₂ conditions, we obtained exclusively the 3′-glycosylated products with no significant quantities of products derived from alternate sites of glycosylation (by ¹H NMR analysis of the unpurified reaction mixture). This finding is perhaps most impressive for stachyose, the starting oligosaccharide providing **7d** in 55%, which

**Scheme 3. Scope of the 3′-Glycosylation Using Ca(OTf)$_2$ Optimized Conditions**[a,b,c]
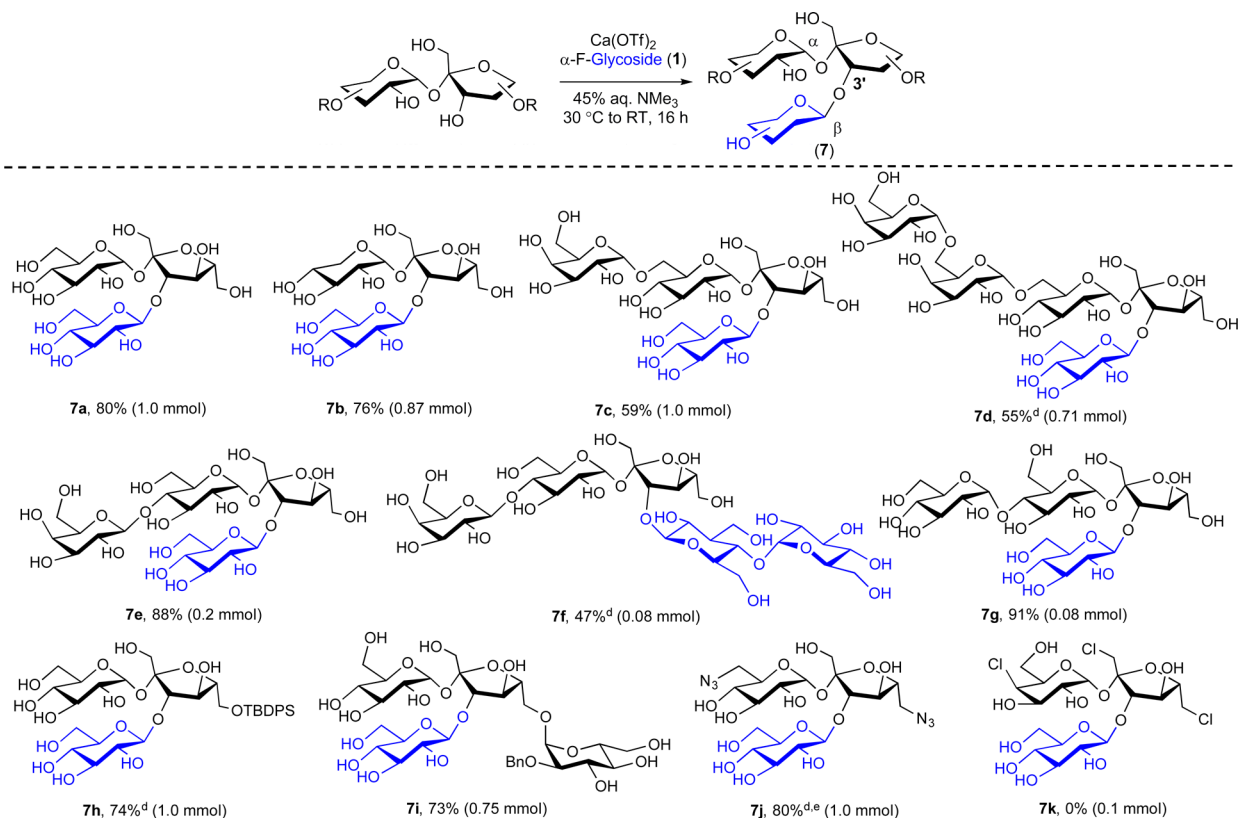
[a]Reaction conditions: Ca(OTf)$_2$ (6.0 equiv), α-F-Glycoside (**1a** or **1b**) (6.0 equiv), 0.30 M in sucrose derivative in 45% aq NMe$_3$, 30 °C, 4 h then RT o/n. [b]Isolated yield (%) after flash chromatography. [c]Regio- and stereoselectivity determined by analysis of ^{1}H, ^{13}C, COSY, heteronuclear single quantum correlation spectroscopy, and HMBC (see Supporting Information). [d]Isolated yield obtained after peracetylation with Ac$_2$O, DMAP (cat.) in pyridine. [e]Reaction stirred for 2 h at 30 °C only instead of 4 h at 30 °C and RT o/n.

possesses 14 distinct hydroxyl groups as candidate acceptor sites, each in a different chemical environment. Moreover, the reaction conditions could be transposed accordingly to lactosyl fructofuranoside (88%, **7e**) and erlose (91%, **7g**), two other saccharides possessing a O-β-Gal (glactose, Gal) and O-α-Glc linkage, respectively, at the 4-position of the Glc unit of the sucrose backbone. α-Fluoro-D-glucose (**1a**) could also be effectively replaced with α-fluoro-D-maltose (**1b**) in the presence the of lactosyl fructofuranoside, providing the pentamer **7f** in 47% yield (the remainder of the mass consisting of unreacted starting material). Synthetic sucrose substrates were also found to be compatible with the reaction conditions (74%, **7h**; 69%, **7i**; 80%, **7j**). The trisaccharide **7j** is also a potential precursor to aminoglycoside scaffolds, a renowned family of antibiotics[27] and inhibitors of the dextransucrase enzymes of microorganisms responsible for dental caries.[28] However, sucralose, an approved no-calorie sweetener,[29] could not be converted into the corresponding trisaccharide **7k**.

Mechanism-Driven Studies. The differences between sucrose and sucralose are subtle; chlorine atoms replace the hydroxyl groups at positions 4 (Glc), 1′ (Fru), and 6′ (Fru). The chlorine at position 4 (Glc) of sucralose is also in the inverted axial configuration in contrast to the sucrose equatorial hydroxyl group.

In search of an explanation for this striking regioselectivity exhibited by sucrose-like compounds and the absence of reactivity for sucralose, we encountered reports by Davies comparing the intramolecular hydrogen-bonding networks

within various sucrose derivatives.[30] A conclusion from these studies is that sucrose possesses two prominent and competing conformations (**A** and **B**) in DMSO-d_6 (eq 1). These
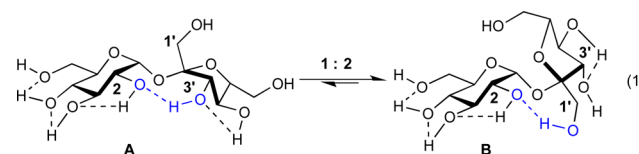
conformations arise from a strong intramolecular hydrogen bond between the 1′-hydroxyl (Fru) and 2-hydroxyl (Glc) and between the 3′-hydroxyl (Fru) and 2-hydroxyl (Glc). However, the substitution of OH-1′ with a Cl atom precludes the latter interaction, and only the former hydrogen bond is present in sucralose. These differences in the hydrogen-bonding network of sucrose and sucralose as well as the observed impact of the various hydroxyl groups on the effectiveness of glycosylation led us to interrogate each of them individually. In order to do so, we performed a complete deoxygenation scan by single hydroxyl group deletion present in sucrose. We synthesized each of the deoxysucroses (**8a**−**8h**) following either reported literature procedures or conventional orthogonal protecting group strategies (see Supporting Information for extensive details).[22,31] We then submitted them to the optimized 3′-glycosylation conditions (cf., conditions of Scheme 3).

Remarkable and nearly binary results were observed with all permutations (Table 2). 2-Deoxysucrose (**8d**), 1′-deoxysucrose (**8e**), and 3′-deoxysucrose (**8f**) are completely inactive under

Table 2. 3′-Glycosylation of Various Deoxysucroses under Optimized Ca(OTf)2 Conditions[a,b]

Entry	Deoxysucrose derivative (**8**)	Conversion (%)[c]	Yield **9** (%)[d]
1	6-Deoxysucrose (**8a**)	92	90
2	4-Deoxysucrose (**8b**)	88	82
3	3-Deoxysucrose (**8c**)	<5	N.D.
4	2-Deoxysucrose (**8d**)	0	0
5	1′-Deoxysucrose (**8e**)	0	0
6	3′-Deoxysucrose (**8f**)	0	0
7	4′-Deoxysucrose (**8g**)	65	60
8	6′-Deoxysucrose (**8h**)	100	93

[a]Each color denotes a position where an hydroxyl group was selectively removed. [b]Reaction conditions: deoxysucrose (0.214 mmol), Ca(OTf)2 (6.0 equiv), α-F-Glucose (6.0 equiv), 0.30 M in deoxysucrose in 45% aq NMe3, 30 °C, 4 h then RT, o/n. [c]Conversion (%) determined by ^{1}H NMR. [d]Yield (%) isolated by flash chromatography.

the reaction conditions. On the contrary, 6-deoxysucrose (**8a**), 4-deoxysucrose (**8b**), and 6′-deoxysucrose (**8h**) all give nearly full conversions and yields for the 3′-glycosylated trimers (90%, 82%, and 93% yields, respectively, for **9a**, **9b**, and **9h**). 3-Deoxysucrose (**8c**) and 4′-deoxysucrose (**8g**) exhibit intermediate activity, the former giving very low conversion (<5%).

Data Analysis. An understanding of the conformation of sucrose and its deoxygenated derivatives would offer insight into the remarkable regioselectivity observed in this glycosylation reaction. Toward this end, we studied the solution structures of all species (cf., eq 1), looking for differences that correspond to a specific hydroxyl group deletion.[30] For example, we suspected that elimination of the 2-Glc hydroxyl might have a profound effect on the sucrose conformation in solution (OH replaced for H, in red, eq 2).
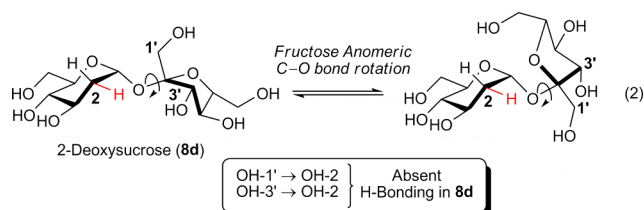
Davies and O'Leary used equilibrium isotope effects to elucidate intramolecular hydrogen-bonding arrays in polyol

substrates.[30,32] Thus, we elected to examine qualitative correlations among sucrose, sucralose, and deoxysucroses **8a**–**8h** (Table 3). The nature of these solution conformations was probed using the secondary isotopic multiplets of partially labelled entities (SIMPLE) ^{1}H NMR technique in DMSO-d_6, in which intermolecular H-to-D exchange between substrates (or with solvent) is slow.[30] The SIMPLE phenomenon observed by Davies for sucrose at OH-2, OH-1′, and OH-3′ is amplified at these sites (highlighted in blue, eq 1) and is explained by invoking several cooperative hydrogen bonds between the other OH groups that are present. Consequently, when these interactions are absent, as in the case of OH-to-Cl substitutions in sucralose, the SIMPLE effects are attenuated.[29]

For the purpose of our study, we compared the equilibrium isotopic perturbations associated with the predominant OH-1′ → OH-2 and OH-3′ → OH-2 hydrogen bonds initially reported for sucrose and sucralose in DMSO-d_6 by Davies (Table 3).[30] Deoxysucrose derivatives that undergo highly efficient 3′-glycosylation (**8a**, **8b**, **8g**, and **8h**; cf., Table 2, entries 1, 2, 7, and 8) reveal SIMPLE ^{1}H NMR data that is homologous with sucrose itself. More precisely, slightly more downfield isotopic shifts for OH-2 (+85 and +79 × 10⁻⁴ ppm) associated with the OH-1′ → OH-2 intramolecular hydrogen bonds are observed with deoxysucrose **8a** and **8b** (+85 and +79 × 10⁻⁴ ppm), in comparison to sucrose (+70 × 10⁻⁴ ppm). Notably, these substrates provide somewhat faster reactions than sucrose under identical Ca-mediated glycosylation conditions. Yet the magnitude of the corresponding isotopic shift decreases for substrate **8c** (+56 × 10⁻⁴ ppm), and it is absent altogether for 2-deoxysucrose **8d**. This may reflect a weaker hydrogen bond in the case of **8c** and the absence of critical hydrogen bonding for **8d**. Accordingly, the glycosylation for those substrates was found to be greatly inhibited (cf., Table 2, entries 3 and 4). Notably, other poor substrates for the glycosylation reactions also reflect significantly altered intramolecular hydrogen-bonding arrays in comparison to sucrose.

For example, **8e** and **8f** exhibit isotopic shifts at OH-2 roughly two times higher than those observed for sucrose (+128 and +130 × 10⁻⁴ ppm vs +70 × 10⁻⁴ ppm). Moreover, only one hydrogen bond induces a SIMPLE effect for **8e** and **8f**. This pattern is also observed for sucralose; the single OH-3′ → OH-2 network exhibits a lower but significant +30 × 10⁻⁴ ppm isotopic shift with respect to the OH-2 (due to Cl atoms lowering the amount of cooperative hydrogen bonds present).[30a] Thus, it seems likely that the hydrogen-bonding network present in sucrose plays a critical role in determining the reactivity and selectivity this glycosylation method. Deletion of the hydroxyl group at positions 2-Glc, 1′-Fru and 3′-Fru not only alters the hydrogen-bonding network but also has a profound effect on the overall nucleophilicity of the sugar and its corresponding interactions with Ca²⁺ under the reaction conditions. All of these effects could influence the substrates'

Table 3. Magnitudes and Signs of Isotope Effects Observed by SIMPLE ^{1}H NMR Analysis on 8a−8h vs Sucrose and Sucralose

position	SIMPLE ($n \times 10^{-4}$ ppm)[a]									
	sucrose[c]	sucralose[c]	8a	8b	8c	8d	8e	8f	8g	8h
OH-2	+70, +32	+30	+85, +22	+79, +26	+56[b]	N.A.	+128	+130	+78, +30	+63, +33
OH-3′	−22	−13	−19	[b]	[b]	0	−53	N.A.	[b]	−20
OH-1′	−43	N.A.	−50	−40	−39	0	N.A.	−96	−48	−40

[a]Estimated error in magnitude is ±2 × 10⁻⁴ ppm. [b]Isotope effect (magnitude <10 × 10⁻⁴ ppm) manifested as line broadening. [c]See ref 30 for literature SIMPLE ^{1}H NMR data.

pK_a values as well. Sucrose has an estimated pK_a value of 12.6 in water, and the most acidic position was calculated by Houdier and Pérez to be at the 2-hydroxyl (Glc).[33] This unusually high acidity for a polyol (versus a simple alcohol) is thought to be a result of this complex hydrogen-bonding network.[20b−d,34,35] We suspect that the pK_a of certain deoxysucrose substrates is altered in comparison to native sucrose as a result of the hydrogen-bonding network perturbation (as observed by SIMPLE NMR). The specifics of the interaction of the sucrosyl hydroxyl group array under our reaction conditions may also prove highly context dependent. For example, studies of sucrose and C-sucrose analogs show differing affinity for Ca^{2+} in methanol solvent.[36] The present aqueous conditions, in the presence of trimethylamine could well alter the equilibrium to favor Ca^{2+}-sucrose adducts of unique reactivity, which seems consistent with our deoxysucrose scan glycosylation results.

Implications for Alternate Regioselectivity. It is not straightforward to alter the site-selectivity of the present aqueous glycosylation with sucrose as the acceptor. For example, we found that when the reaction is performed with the basic and partially soluble $Ca(OH)_2$, a moderate level of reactivity is still observed (42% yield), albeit to a much more complicated mixture of trisaccharides products. A close look into product distribution showed that the major product formed is the 1′-glycosylated regioisomer **10a** over the 3′-glycosylated product **7a** (in a ratio of roughly 55:45). After reoptimization,[22] **10a** could be obtained in increased yield (65% yield, **10a:7a** ratio of 70:30), and appreciable amounts of this pure trisaccharide could be isolated by prep-HPLC (Scheme 4).[37] The connectivity and relative stereochemistry

Scheme 4. Alternative $Ca(OH)_2$-Mediated 1′-Glycosylation
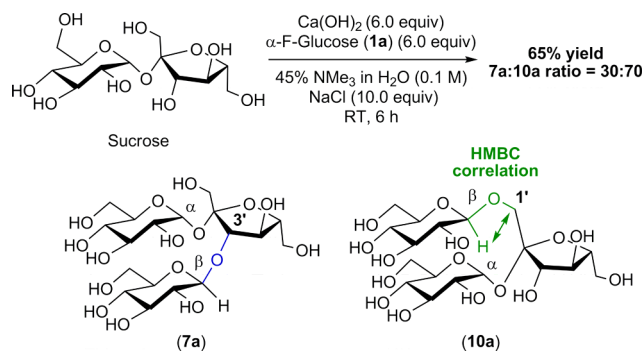
of this alternative regioisomer were also supported by HMBC-NMR analysis[22] between the carbon at position C-1′ of the fructofuranose unit and the axial Glc proton H-1″ (highlighted in green, Scheme 4).

Contrary to **7a**, product **10a** was recently isolated by the fermentation of plants extract and comparison of the reported spectroscopic data to ours confirmed its identity.[38] The regioselectivity for the formation of **10a** is consistent with a consideration of the conformational analysis.[30] As noted earlier, the 1′-hydroxyl group is hydrogen bonded to the 2-hydroxyl oxygen. The switch of selectivity may be a manifestation of a conformational change when $Ca(OH)_2$ is used. Since sucrose is fairly acidic, its derived calcium alkoxide may interact or react with the glycosyl fluoride **1a** in a conformation different from the one adopted when $Ca(OTf)_2$ is used.

CONCLUSION

This glycosylation boasts several unique features: the reaction is carried out under completely aqueous conditions and high levels of glycosylation of sucrose and several of its analogs are observed, and the glycosylation proceeds with complete stereoinversion at the anomeric center of the glycosyl donor as well as complete regioselectivity of the acceptor. From a practical perspective, all of the reagents used are inexpensive, readily available compounds, and the procedure itself is experimentally simple. The glycosylation products would be difficult to access using any currently reported glycosylation methods, and their synthesis is yet unreported in the literature. More broadly, the mechanistic basis for the unique reactivity foreshadow well for the exploration of substrates beyond sucrose. Could other metal ion/basic additive/carbohydrate combinations be found that allow for the related aqueous glycosylations of other substrates so that the scope could be expanded? This critical question is the focus of ongoing studies in our laboratories.

ASSOCIATED CONTENT

ⓢ Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/jacs.5b13384.

> Additional figures, experimental details and characterization (PDF)
> Spectra for all synthesized compounds (PDF)

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Notes
The authors declare no competing financial interest.

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