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

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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 non-enzymatic aqueous oligosaccharide 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 hydroxyl group array in sucrose. The solution conformation of various mono-deoxysucrose analogs revealed the cooperative nature of the hydroxyl group in mediating both this aqueous glycosyl bond-forming reaction and the site-selectivity at the same time.

Keywords

aqueous; carbohydrate; glycosylation; glycosyl fluoride; sucrose

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 *via* 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 under aqueous conditions towards oligosaccharides are scarce.⁴ Enzymatic methods using glycosyl transferases or

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hydrolases,^{5,6,7} however, afford efficient and selective reactions in buffered water, employing pre-fashioned sugar nucleotides or non-reducing sugar 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 non-enzymatic 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 models of 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-b**, overcoming the formation of D-glucose. However, the 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 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, non-solvated 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). This strategy could also enhance the reactivity of any alcohol towards substitution. A close comparison can be made with traditional synthetic strategies that rely on an 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 non-enzymatic, chemoselective glycosylation reactions between glycosyl fluorides and sucrosyl acceptors. The unique 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 non-enzymatic 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** towards an aqueous solution of sucrose, a complex carbohydrate acceptor (Scheme 2).²² When treated with 0.5 equivalent of sucrose in the absence of any additives, compound **1a** hydrolyzes slowly and cleanly to generate α - and β -D-glucopyranose (**3a** and **3b** respectively) in 9% yield after 48 hours at room temperature. In a separate control experiment, 1.0 equiv. of $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ 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 by-products. On the other hand, addition of both $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ and trimethylamine afforded the glycosylation product **7a** in 20% yield (with respect to sucrose) after 48 hours. A close examination of both the unpurified and purified reaction mixtures by ^1H 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 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 (towards **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 CaCl_2 instead of $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$).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 Ca^{2+} and aqueous NMe_3 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 NMe_3 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 reactions conditions to give this isomer in high yields (Table 1). Initially, we found that an excess of **1a** (5 equivalents) and a high concentration of $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ (8 equivalents) 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 Ca^{2+} salts are effective at promoting glycosylation over hydrolysis of **1a** (e.g. no reaction was observed with NaNO_3 and KNO_3 , entries 2-3). A strong effect of the counterion was observed, with dissociated anions ($\text{CaBr}_2 \cdot x\text{H}_2\text{O}$ and $\text{Ca}(\text{OTf})_2$, more soluble salts) affording reactivity, whereas no reactivity was observed with insoluble or partially soluble salts containing more basic counterions (CaCO_3 or $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$, 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 equivalents of $\text{Ca}(\text{OTf})_2$, no reactivity is observed; however, with a more dilute solution (0.5 M) in sucrose, an improved 69% conversion to **7a** is obtained (c.f., entries 7,8 and 10). Interestingly, this important jump in reactivity is also observed with $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, 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 equivalents of both $\text{Ca}(\text{OTf})_2$ 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 equivalents 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 found to be more extensive (69% with 30% aq. NMe_3 , 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 by-products, silica gel or residual calcium salts are indeed removed by this method. Alternatively, peracetylation of the trisaccharide **7a** ($\text{Ac}_2\text{O}/\text{DMAP}$ 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 pentahydrate, 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 (in 76%, 59%, 55% yields respectively). With stachyose, the product and starting saccharide were found to co-elute, 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 is perhaps most impressive for stachyose (**7d**), which 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 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 starting material). Synthetic sucrose substrates were also found to be compatible with the reaction conditions (74%, **7h**; 69%, **7i**; 80%, **7j**). **7j** is also a potential precursor to aminoglycoside scaffolds, a renowned family of antibiotics²⁷ and inhibitors of the dextranucrase 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 first hydrogen bond is present in sucralose.

(1)

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 (*c. f.* 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 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 (*c.f.*, 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)

(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 SIMPLE ^1H NMR technique (Secondary Isotopic Multiplets of Partially Labelled Entities; SIMPLE) in $\text{DMSO-}d_6$, 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 H-bonds initially reported for sucrose and sucralose in $\text{DMSO-}d_6$ by Davies (Table 3).³⁰ Deoxysucrose derivatives that undergo highly efficient 3'-glycosylation (**8a**, **8b**, **8g** and **8h**; *c.f.*, Table 2, entries 1, 2, 7 and 8) reveal SIMPLE ^1H NMR data that is homologous with sucrose itself. More precisely, slightly more downfield isotopic shifts for OH-2 (+85 and $+79 \times 10^{-4}$ ppm) associated with the OH-1'→OH-2 intramolecular hydrogen bonds are observed with deoxysucrose **8a** and **8b**, in comparison to sucrose ($+70 \times 10^{-4}$ 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** ($+56 \times 10^{-4}$ 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 (*c.f.*, 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 $+130 \times 10^{-4}$ ppm vs $+70 \times 10^{-4}$ 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 $+30 \times 10^{-4}$ 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 has a profound effect on the overall nucleophilicity of the sugar and its corresponding interactions with Ca^{2+} under the reaction conditions. All of these effects could influence the substrates' $\text{p}K_a$ values as well. Sucrose has an estimated $\text{p}K_a$ 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 $\text{p}K_a$ 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 $\text{Ca}(\text{OH})_2$, 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 re-optimization,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 of this alternative regioisomer were also supported by HMBC-NMR analysis²² 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 $\text{Ca}(\text{OH})_2$ is used. Since sucrose is fairly acidic, its derived calcium alkoxide may substitute the glycosyl fluoride **1a** in a conformation different from the one adopted when $\text{Ca}(\text{OTf})_2$ 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; 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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Both regioisomers (**10a** and **7a**) were found to co-elute by conventional flash chromatography. In order to get pure **10a**, a subsequent separation by semi-prep HPLC was needed (XBridge Prep Amide 5 μ L BEH glycan column using a gradient of 80% MeCN in 20% aq. NH₄OH 0.1% buffer to 50% MeCN in 50% aq. NH₄OH 0.1% buffer).

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Abbreviations

Glc	glucose
Gal	galactose
Fru	fructose
aq	aqueous
HSQC	Heteronuclear Single Quantum Correlation spectroscopy
HMBC	Heteronuclear Multiple Bond Correlation
TSAS	transition state analogue substrate
HFPN	hexafluoro-1-propanol
TFE	trifluoroethanol
SIMPLE	Secondary Isotope Multiplets of Partially Labeled Entities
UDP	uridine diphosphate
CMP	cytidine monophosphate
GDP	guanosine diphosphate

Figure 1.

Competition between hydrolysis (H_2O (a), in red) and glycoside bond formation (acceptor (b), in blue) for a given activated donor in the active site of an enzyme.

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; (b) both water soluble donor and acceptor react to form the same bond without the need for directing/protecting groups.

Scheme 1.

Stereoinvertive substitution of α -F-glucose (**1a**) using simple nucleophiles in water.

Scheme 2. Observed reaction pathways and formation of a new trisaccharide (7a)

Scheme 3. Scope of the 3'-glycosylation using $\text{Ca}(\text{OTf})_2$ optimized conditions^{a,b,c}

^aReaction conditions: $\text{Ca}(\text{OTf})_2$ (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. ^bIsolated yield (%) after flash chromatography. ^cRegio- and stereoselectivity determined by analysis of ^1H , ^{13}C , COSY, HSQC, and HMBC (see Supporting Information). ^dIsolated yield obtained after peracetylation with Ac_2O , DMAP (cat.) in pyridine. ^eReaction stirred for 2 hours at 30 °C only instead of 4 hours at 30 °C and RT o/n.

Scheme 4. Alternative Ca(OH)_2 -mediated 1'-glycosylation

Table 1
Selected optimized parameters for the glycosylation

Entry	M ⁺ or M ⁺ (equiv)	Conc. (M) ^a	α -F-Glc (equiv)	Conversion to 7a (%) ^b
1	Ca(NO ₃) ₂ (8) ^c	1.0	5.0	42
2	NaNO ₃ (8)	1.0	5.0	<5
3	KNO ₃ (8)	1.0	5.0	<5
4	CaCO ₃ (8)	1.0	5.0	<5
5	CaSO ₄ (8) ^c	1.0	5.0	<5
6	CaBr ₂ (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(NO ₃) 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.

Table 23'-Glycosylation of Various Deoxysucroses under Optimized Ca(OTf)₂ Conditions.^{a,b}

Entry	Deoxy sucrose 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

Entry	Deoxy sucrose derivative (8)	Conversion (%) ^c	Yield 9 (%) ^d
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 ¹H NMR.

^d Yield (%) isolated by flash chromatography.

Table 3
Magnitudes and Signs of Isotope Effects Observed by SIMPLE ^1H NMR analysis on **8a-8h** vs sucrose and sucralose.

Position	SIMPLE (n × 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, <i>b</i>	N.A	+128	+130	+78, +30	+63, +33
OH-3'	-22	-13	-19	<i>b</i>	<i>b</i>	0	-53	N.A.	<i>b</i>	-20
OH-1'	-43	N.A.	-50	-40	-39	0	N.A.	-96	-48	-40

^aEstimated error in magnitude is $\pm 2 \times 10^{-4}$ ppm.

^bIsotope effect (magnitude $< 10 \times 10^{-4}$ ppm) manifested as line broadening.

^cSee reference 30 for literature SIMPLE ^1H NMR data.