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How do various reaction parameters influence anomeric selectivity in chemical glycosylation with thioglycosides and NIS/TfOH activation?

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Abstract:

The reaction of glycosyl donor phenyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-glucopyranoside with NIS/TfOH(cat.) was systematically studied under various reaction conditions. Neither the molecular sieve pore size nor amount of NIS activator was found to have an effect on the α/β-ratio in reaction with L-menthol as glycosyl acceptor. Increasing concentration and the amount of triflic acid catalyst, however was found to increase the β-selectivity in certain cases. Also, lowering temperature was found to have a strong effect on the glycosylation outcome.

Keywords: Glycosylation, stereoselectivity, reaction parameters

Introduction:

The chemical glycosylation reaction involving an electrophilic glycosyl donor, a nucleophilic glycosyl acceptor and a suitable promoter is the tool used almost exclusively to prepare the glycosidic bond interlinking the monosaccharide units of oligosaccharides.[1–3] Given the importance of these molecules in Nature there is a good reason for attempting to understand the underlying mechanism of this reaction and improving current methodology. One of the challenges inherent to the chemical glycosylation reaction is the diastereoselectivity of the glycosylation reaction itself. Since this involves the stereochemistry of the anomeric center it is typically referred

to as anomeric selectivity. This issue has plagued[4] chemists for a long time and therefore remains to have the attention of specialists in the carbohydrate chemistry field. Different donor types (thioglycoside, trichloroacetimidate, glycosyl halide, etc.) and glycosylation conditions may be sensitive in varying degrees to perturbations of external parameters. Only a limited number of publications describe how glycosylation selectivity's change across a series of conditions for a given donor-type and results are typically only presented as one number and not as an average over multiple determinations making the level of sensitivity unclear.

Ishiwata and Ito invented a high through-put screening method for glycosylation optimization and demonstrated vast solvent screening using ^{1}H-NMR and MS with Bn-d_7 protected deuterated substrates with a SMe functionalized donor and MeOTf-activation.[5]

In a recent paper by Seeberger and co-workers in the Journal of the American Chemical Society, the authors eloquently describe how different permanent and environmental conditions can influence the anomeric selectivity of a glycosylation reaction.[6] Their main focus was on the temperature sensitivity, which could be controlled very precisely in their automated flow reactor. Donors of three types (trichloroacetimidate, *n*-butyl phosphate and thioethyl) were studied as they reacted with various electron-rich nucleophiles like EtOH or *i*PrOH and electron-poor nucleophiles like difluoroethanol or trifluoroethanol.[7]

In another recent attempt to systematically investigate the intricate nature of the glycosylation reaction, Codée and co-workers have elegantly obtained much insightful data that correlate the nucleophilicity of glycosyl acceptors like EtOH and hexafluoroisopropanol to the anomeric selectivity in glycosylation with e.g. 4,6-benzylidene protected glucosyl- and mannosyl donors.[4] Also carbohydrate-based acceptors were investigated in this study, which throughout employed SPh-functionalized donors and activation by the Ph_2SO/Tf_2O/TTBP promoter system.[8]

Much of the current insight into the glycosylation mechanism builds on and originates from highly detailed studies by Crich and co-workers.[9,10] Throughout the past two decades they have been immensely productive with the study of especially 4,6-benzylidene directed β-mannosylation, but also many other glycosylations. In general, restricting conformational flexibility can have a drastic influence on the stereochemical glycosylation outcome as also recently reviewed by Galan and co-workers.[11]

Despite the latest results and level of understanding, it is our impression that much still needs to be uncovered with regards to the fundamental reaction most chemist use to connect saccharide building blocks. There still seems to be a great deal of speculation with respect to how different reaction parameters influence anomeric selectivity and what causes the observed effects. It occurred to us that it is not always trivial to obtain reaction outcomes with sufficient reproducibility that can substantiate detailed speculation. The aim of the present study has been to learn if there are any trends that can be exploited so a higher yield of a desired isomer can be obtained and the overall synthetic efficiency increased as a result. We undertook this project well aware that a general in-depth mechanistic understanding and stereochemical control may never be obtained across all donor-types and acceptors, but additional studies could still bring previously covered effects to the surface and allow for their exploitation once revealed.

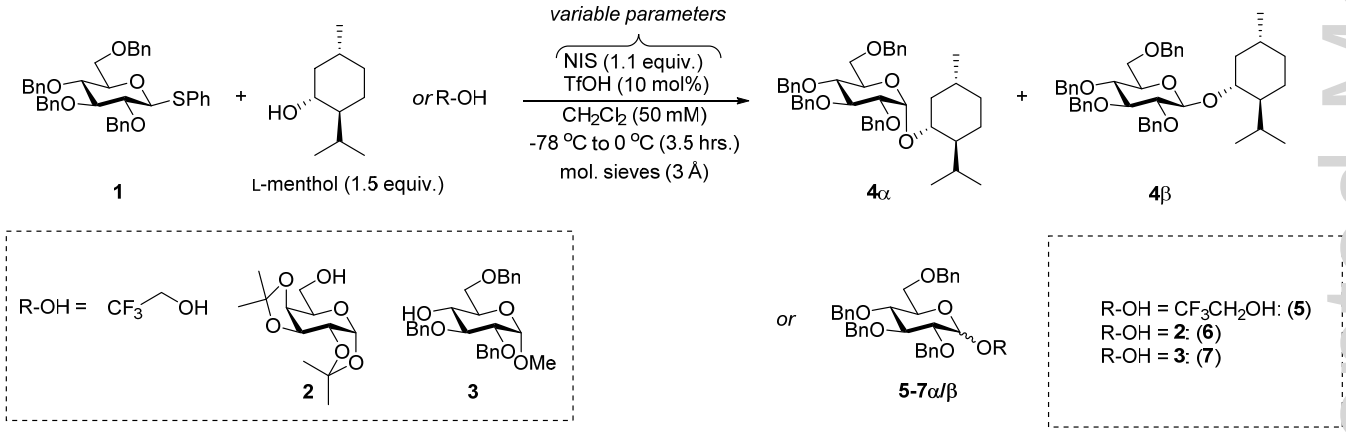
This paper describes our efforts to further illuminate how D-glucosylations in batch reactions are affected by all influential factors and this cannot necessarily be extrapolated to e.g. D-galactosylations and D-mannosylations. These are activator/catalyst concentration, substrate concentration, the presence of molecular sieves, solvent, and acceptor.

Our contribution is not meant to be a solution to the α/β-selectivity problem as we offer no new insight on this topic, but rather to point out which parameters have an influence on anomeric selectivity and with which magnitude the selectivity can change upon perturbations in reaction conditions.

Results and discussion

We decided to study a specific glycosylation reaction more thoroughly and selected *N*-iodosuccinimide (NIS) activation of phenyl thio-glucopyranoside **1** with TfOH (cat.).[12] For the early stages of the project, we chose L-menthol as a model acceptor nucleophile as it is a non-hygroscopic, cheap, secondary alcohol and could be compared to previous studied done by our group (Scheme 1).[13–15] Although it is not carbohydrate-like the results may not be directly applicable to e.g. disaccharide synthesis, but the goal of the project is to search for trends in glycosylation selectivity rather than absolute values and later confirm trends with more relevant acceptors. Compared to some other activation methods of thioglycosides, NIS/TfOH is

operationally very simple and a typical mode of activation of one of the most employed glycosyl donor types. It can be viewed as a catalytic mode of activation similar to that of e.g. trichloroacetimidates that remains equi-acidic throughout the course of reaction. This is in contrast to e.g. thioglycoside activation by e.g. BSP/Tf_2O[16] or Ph_2SO/Tf_2O[8] promoted activation, where all donor is converted into glycosyl triflate or other highly reactive electrophiles before the acceptor nucleophile is added. The reaction is believed to go through an oxacarbenium ion intermediate with some degree of participation of the triflate counterion either through covalent attachment or as solvent separated/contact ion pairs.[9] Although the temperature with which a glycosylation is conducted is known to influence the anomeric selectivity, cryostats or microreactors[6] are not available to all research groups. From a practical point-of-view, it was therefore initially decided to conduct the experiments in an insulated cold bath and let the reaction temperature climb from -78 ºC (dry ice/acetone bath) to 0 ºC. This was found to reproducibly take 3.5 hours with the cold bath constructed and used for this entire project (see supporting information). Later in the project, also isothermally conducted experiments were performed.



Scheme 1. General glucosylation of L-menthol or other alcohols.

The first goal was to achieve comparable results over a triplicate of α/β-selectivity determinations. This issue was important in order to both build confidence to our results, but also allow us to see whether the observed variations are 1) outliers, 2) part of a trend, or 3) whether they are just the result of typical experimental uncertainty. Obtaining sufficient reproducibility with respect to both yield (as determined by ^{1}H-NMR using an internal standard) and especially α/β-ratios (as determined by ^{13}C-NMR of the crude reaction mixture) initially proved quite challenging. We took

this as an early indication of there being some parameters that had a significant effect on the glycosylation selectivity under the used conditions.

After some practice and especially increasing the scale to ca. 300 mg of donor (ca. 0.5 mmol) we started observing both reproducible yields and selectivity. We ascribe this to the fact that the amount of neat TfOH (10 mol%) could be added more accurately at this scale. It is not an option to make a stock solution of TfOH in CH_2Cl_2 as these are not fully miscible. The additional benefit of performing the reaction on this scale is, that there is sufficient crude product material to easily obtain a ^{13}C-NMR spectrum with excellent signal-to-noise ratio, which was used for determining the anomeric selectivity (Figure 1).[17]

The reactions were, as mentioned, performed in triplicates and conducted independently a number of times by two chemists. The results are shown in Table 1 and demonstrate a high degree of reproducibility. With respect to yield determination we estimate an uncertainty of ±5%-points. The observed β-selectivity is in accordance with a previous study, where we have reported a 1:3 α/β-ratio.[13]

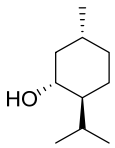
Entry	Acceptor	α/β-ratio[a]	Yield[b]	Average α/β-ratio
1		1:2.4	91%	1:2.4
2		1:2.0	102%	
3		1:2.4	99%	
4		1:2.4	102%	
5		1:2.6	101%	
6		1:2.6	94%	
7		1:2.3	97%	
8		1:2.5	94%	
9		1:2.4	85%	
10		1:2.5	94%	
11		1:2.4	89%	
12		1:2.5	86%	

Table 1. Results from glucosylation of L-menthol (1.5 equiv.) according to Scheme 1 in CH_2Cl_2 with NIS (1.1 equiv.) and TfOH (10 mol%) with respect to the donor from -78 ºC to 0 ºC over 3.5 hours. [a]Determined by ^{13}C-NMR. [b]Determined by ^{1}H-NMR using benzyl benzoate as internal standard.

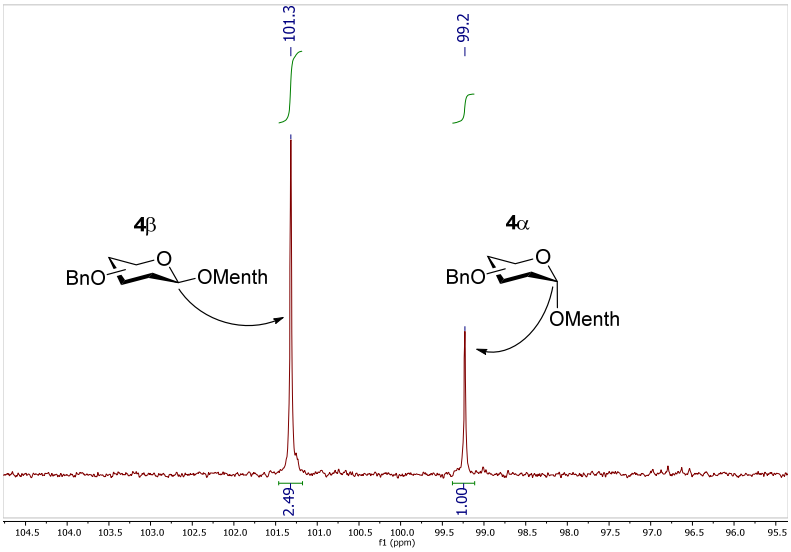


Figure 1. ^{13}C-NMR spectrum of crude glycosylation mixture showing the signals from the anomeric carbon atoms (β: 101.3 ppm, α: 99.2 ppm) in CD_3CN of **4**α/β.

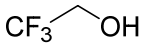
Entry	Acceptor	α/β-ratio[a]	Yield[b]	Average α/β-ratio
1		8.1:1	97%	8.1:1
2		8.0:1	94%	
3		8.1:1	78%	

Table 2. Results from glucosylation of trifluoroethanol according to Scheme 1 in CH_2Cl_2 with NIS (1.1 equiv.) and TfOH (10 mol%) with respect to the donor from -78 ºC to 0 ºC over 3.5 hours. [a]Determined by ^{13}C-NMR. [b]Determined by ^{1}H-NMR using benzyl benzoate as internal standard.

As an additional control experiment, we also included 2,2,2-trifluoroethanol as an acceptor as it is both achiral and known to induce a high level of α-selectivity in glycosylation reactions. Codée and co-workers have reported α/β 3:1 under Ph_2SO/Tf_2O/TTBP pre-activation conditions from donor **1**.[18]

After having established a satisfactory test system that returned high glycosylation yields and reproducible anomeric selectivity values as seen in Table 1, we had enough confidence to continue the project and look for trends that arise upon changes in conditions.

First, the influence of molecular sieves was investigated. Until this point all experiments had been carried out in the presence of powdered molecular sieves (3 Å) as a drying agent. As Table 3 shows, we were able to demonstrate there being no influence on anomeric selectivity as a function of desiccant pore size or its presence.

Entry	Mol. sieves	α/β-ratio[a]	Yield[b]	Average α/β
1	none	1:2.2	83%	1:2.4
2		1:2.5	99%	
3	3 Å			1:2.4[c]
4	4 Å	1:2.2	96%	1:2.3
5		1:2.3	86%	
6	5 Å	1:2.5	102%	1:2.5
7		1:2.4	95%	

Table 3. Results from glucosylation of L-menthol according to Scheme 1 in CH_2Cl_2 with NIS (1.1 equiv.) with a varying nature of the drying agent. Reactions were performed from -78 ºC to 0 ºC over 3.5 hours. [a]Determined by ^{13}C-NMR. [b]Determined by ^{1}H-NMR using benzyl benzoate as internal standard. [c]From Table 1.

Next, the effect of concentration on anomeric selectivity was investigated in the present system. Others have previously studied the concentration effect on anomeric selectivity in sialylations[19] and mannosylations.[20–22]

Reactions had been conducted with a donor concentration of 50 mM. Slightly lower and higher selectivities were found at more dilute (25 mM) and increased (100 mM) concentrations, respectively. There seems to be a clear, although mild trend, which would be somewhat more uncertain to call if fewer experiments had been performed (Table 4).

Entry	Concentration[a]	α/β-ratio[b]	Yield[c]	Average α/β
1	25 mM	1:1.9	100%	1:1.9
2		1:1.9	100%	
3		1:2.0	99%	
4	50 mM			1:2.4[d]
5	100 mM	1:3.6	99%	1:3.5
6		1:3.5	96%	
7		1:3.5	98%	

Table 4. Results from glucosylation of L-menthol according to Scheme 1 in CH_2Cl_2 with NIS (1.1 equiv.) at varying concentration of the donor[a] from -78 ºC to 0 ºC over 3.5 hours. [b]Determined by ^{13}C-NMR. [c]Determined by ^{1}H-NMR using benzyl benzoate as internal standard. [d]From Table 1.

Dichloromethane is recognized as a non-participating solvent for glycosylation, whereas ethers like Et_2O, THF or dioxane are known to induce a higher degree of axial glycoside product in opposition to acetonitrile, which is known to induce formation of the equatorial product.[23] As we believe we here have established a reproducible protocol we were interested in testing to which extent acetonitrile affected the stereochemical outcome. The glycosylation reaction was conducted as previously shown in Table 1 with the adjustment of solvent medium to contain either 25 vol% or 50 vol% acetonitrile. As reported many times, a higher degree of β-selectivity was observed in both cases. The level of β-selectivity was furthermore found to increase with increasing concentration of acetonitrile (Table 5). This was an expected trend and constitutes a non-controversial observation, but shows in the case of this specific system the size of the effect in going from neat CH_2Cl_2 (α/β: 29/71), to 25% CH_3CN in CH_2Cl_2 (α/β: 13/87) and 50% CH_3CN in CH_2Cl_2 (α/β: 6/94). Had the experiments been carried out in a less precise and confident fashion it might be reasonable to round of the selectivity values to α/β: 10/90 making 25% and 50% CH_3CN-values indistinguishable.

Entry	Solvent	α/β-ratio[a]	Yield[b]	Average α/β
1	Only CH_2Cl_2			1:2.4[c]
2	CH_2Cl_2/CH_3CN (3:1)	1:7.2	93%	1:7.0
3		1:6.7	98%	
4		1:7.2	92%	
5	CH_2Cl_2/CH_3CN (1:1)	1:14.0	99%	1:14.9
6		1:14.5	94%	
7		1:16.1	97%	

Table 5. Results from glucosylation of L-menthol according to Scheme 1 in various solvents with NIS (1.1 equiv.) and TfOH (10 mol%). Reactions were performed from -78 ºC to 0 ºC over 3.5 hours. [a]Determined by ^{13}C-NMR. [b]Determined by ^{1}H-NMR using benzyl benzoate as internal standard. [c]From Table 1.

Next, the effect of the amount of NIS was investigated. We had noticed that this parameter often varies between 1.1 equiv. to more than 2.5 equiv. in literature protocols. This could originate from the necessity to obtain full conversion of donor and possibly be ascribed to the quality of the iodonium-ion reagent. The NIS (98%+) used for this entire project, was obtained from a specific supplier and known by us in previous projects to induce full conversion with just 1.1 equiv. as also shown in Table 1.[24] It was stored at 5 °C and used without further purification.

As indicated by the result shown in Table 6 there seems to be no influence on neither the yield nor the anomeric selectivity upon increasing the excess of NIS. This could possibly be explained by it being protonated NIS (or I^+ $^-$OTf) that is the real activator and not NIS itself, which depends on the amount of TfOH instead.

Entry	NIS (equiv.)	α/β-ratio[a]	Yield[b]	Average α/β
1	1.1			1:2.4[c]
2	3.0	1:2.0	98%	1:2.4
3		1:2.6	100%	
4		1:2.5	88%	
5	5.0	1:2.3	95%	1:2.4
6		1:2.6	104%	
7		1:2.4	94%	

Table 6. Results from glucosylation of L-menthol according to Scheme 1 in CH_2Cl_2 with varying NIS and TfOH (10 mol%) with respect to the donor from -78 ºC to 0 ºC over 3.5 hours. [a]Determined by ^{13}C-NMR. [b]Determined by ^{1}H-NMR using benzyl benzoate as internal standard. [c]From Table 1.

Next, the influence of the amount of TfOH on the anomeric ratio was investigated. The results are listed in Table 7 and indicate a significant increase in β-selectivity as a function of increasing the amount of TfOH using both L-menthol (from 10% to 30%) and galactose diacetone (**2**) (from 10% to 20%). For acceptor **3** (4-OH) no effect was observed, which may be linked to the nearly unselective glycosylation at 10% TfOH.

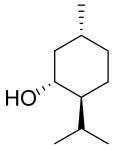
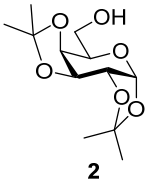
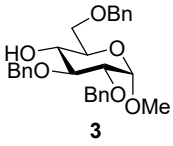
Entry	Acceptor	TfOH	α/β-ratio[a]	Yield[b]	Average α/β
1		10 mol%			1:2.4[d]
2		20 mol%	1:3.9	102%	1:3.7
3			1:4.0	98%	
4			1:3.3	95%	
5		30 mol%	1:5.2	101%	1:5.3
6			1:5.2	98%	
7			1:5.6	101%	
8	 **2**	10 mol%	1:3.5	97%	1:3.3
9			1:3.1	91%	
10			1:3.4	100%	
11		20 mol%	1:4.2	109%	1:4.2
12			1:4.3	95%	
13			1:4.3	94%	
14		30 mol%	1:4.2	96%	1:4.3
15			1:4.2	97%	
16			1:4.4	101%	
17	 **3**	10 mol%	1:1.5	80%[c]	1:1.5
18			1:1.5	84%[c]	
19			1:1.5	87%[c]	
20		20 mol%	1:1.3	76%[c]	1:1.2
21			1:1.1	73%[c]	
22			1:1.1	54%[c]	
23		30 mol%	1:1.6	88%[c]	1:1.5
24			1:1.4	102%[c]	
25			1:1.4	75%[c]	

Table 7. Results from glucosylations of L-menthol and acceptors **2**-**3** according to Scheme 1 in CH_2Cl_2 with NIS (1.1 equiv.) and varying amount of TfOH with respect to the donor from -78 ºC to 0 ºC over 3.5 hours. [a]Determined by ^{13}C-NMR. [b]Determined by ^{1}H-NMR using benzyl benzoate as internal standard. [c]Isolated yields. [d]From Table 1.

One explanation of the significant increase in β-selectivity in the case of L-menthol and galactose diacetone (**2**) could originate from the more forcing conditions and the fact that the experiments were conducted under gradient warming. Reactions performed with e.g. 30 mol% TfOH were found to initiate at a lower temperature as indicated by the development of colour due to the formation of iodine, than those conducted with 10 mol% catalyst. This observation also correlates with the higher level of β-selectivity observed at higher concentrations (Table 4).

We have previously observed how the more reactive per-*O*-benzylether protected glucosyl donor (**1**) gave an improved β-selectivity over the less reactive per-*O*-p-chlorobenzyl protected analogue under gradient warming. This was then also speculated to be a result of different activation temperatures, which was supported by result from glycosylations carried out under isothermal conditions (-10 °C), which were found to give the same level of selectivity.[13]

Under the assumption, that the α/β-selectivity is a direct result of an S_N2-type displacement of an anomerically bound triflate an additional explanation could be given. Both the rate of interconversion between α- and β-triflate and the position of equilibrium will be expected to be altered by temperature.[25] A higher reaction temperature would favour more of the β-triflate in equilibrium according to the principle of Le Châtelier and a faster equilibrium, which would result in more α-glycoside and an eroded selectivity in the present case, which is in accordance with the findings. This effect would additionally be strengthened by the common ion effect, which would increase the amount of covalently attached triflate over the S_N1 path going through an oxacarbenium ion/triflate ion pair.

It appears clear from the results of Table 7 that the effect of increasing the amount of TfOH does not generally provide an improved selectivity. The reason for results of galactose diacetone (**2**) being unaffected in going from 20 mol% to 30 mol% could be a result of the smaller percentage increase (50%) than going from 10 mol% to 20 mol% (100%). One may also expect there to be a maximum selectivity that can be reached with a given acceptor nucleophile, and that this was obtained at around 1:4.2. The selectivity in glycosylation of **3** appears to be unaffected by changes in the amount of TfOH. Again, this may be a result of the maximum level of selectivity, which, at the limit (100 mol% TfOH) will be comparable to the result obtained through BSP/Tf_2O or

Ph_2SO/Tf_2O. In actual fact, Kim and Seeberger have reported an unselective reaction (α/β 1:1) with BSP/Tf_2O/TTBP activation.[26]

Until this point, all reactions had been performed using the same cooling bath with acetone/dry ice starting at -78 °C. Once all reagents had been added, a set of tweezers were used to remove the remaining lumps of dry ice and let the reaction mixture slowly warm until 0 °C over 3.5 hours. This way, the reaction initiates once the cooling bath temperature reaches a certain point. This *modus operandi* is frequently used in oligosaccharide synthesis to secure the lowest possible reaction temperature, which could result in the best possible selectivity for the reaction in question. It additionally makes it unnecessary to know a specific reaction temperature and to use a potentially expensive cryostat capable of holding a fixed temperature. It is our firm impression that the warming in our case occurs reproducibly as also indicated by the small degree of variation across different experiments. The drawback with the procedure could be, however, that different reaction parameters may change the temperature at which a certain reaction occurs, and that this in turn may influence the stereochemical outcome.

The glycosylation reaction with varying amounts of TfOH was next tested under isothermal conditions using a cryostat at -50 °C in order to separate the effect of the reaction temperature and the amount of TfOH. This temperature was chosen since the development of colour from the formation of iodine was observed at around this temperature with a TfOH load of 10 mol%, which indicates that reaction is occurring. The results of Table 8 still show an increased selectivity for the kinetic β-product as a function of catalyst, albeit to a lesser extent than the data collected under gradient warming conditions.

Entry	TfOH	α/β-ratio[a]	Yield[b]	Average α/β
1	10 mol%	1:5.2	100%	1:5.2 (1:2.4)[c]
2		1:5.1	97%	
3		1:5.3	101%	
4	20 mol%	1:5.6	100%	1:5.6 (1:3.7)[c]
5		1:5.7	100%	
6		1:5.6	104%	
7	30 mol%	1:6.1	103%	1:6.3 (1:5.3)[c]
8		1:6.3	102%	
9		1:6.5	105%	

Table 8. Results from glucosylation of L-menthol according to Scheme 1 in CH_2Cl_2 with NIS (1.1 equiv.) and varying amount of TfOH with respect to the donor at -50 ºC over 5 hours. [a]Determined by ^{13}C-NMR. [b]Determined by ^{1}H-NMR using benzyl benzoate as internal standard. [c]Conducted at -78 ºC to 0 ºC over 3.5 hours for comparison (Table 7).

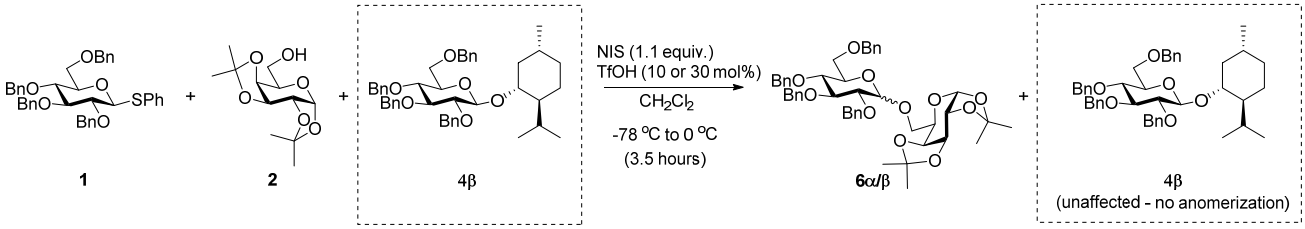
To investigate the influence on glycosylation selectivity of performing the reaction at various constant temperatures (isothermal) was next investigated (Table 9).

Entry	Temperature	α/β-ratio[a]	Yield[b]	Average α/β
1	-50 °C			1:5.2[c] (1:2.4)[d]
2				
3				
4	-40 °C	1:3.1	98%	1:3.1
5		1:3.1	100%	
6		1:3.1	92%	
7	-10 °C	1:1.3	98%	1:1.3
8		1:1.3	99%	
9		1:1.3	98%	

Table 9. Results from glucosylation of L-menthol according to Scheme 1 in CH_2Cl_2 with NIS (1.1 equiv.) and TfOH (10 mol%) with respect to the donor. [a]Determined by ^{13}C-NMR. [b]Determined by ^{1}H-NMR using benzyl benzoate as internal standard. [c]From Table 8. [d]Conducted at -78 ºC to 0 ºC over 3.5 hours for comparison (Table 1).

The glycosylation selectivity with L-menthol as acceptor was found to depend strongly on the temperature and much more so than on the amount of TfOH catalyst. Temperature being a major parameter in influencing anomeric outcome is in accordance with observations by Seeberger and co-workers.[6] Although gradient warming is convenient this shows that a vastly improved selectivity can be obtained through temperature control.

We have previously investigated whether pure menthyl β-glucopyranoside **4β** anomerizes under the reaction conditions (NIS, 1.1 equiv; TfOH 10 mol%) by conducting a glycosylation with donor **1** and acceptor **2** under the standard conditions in its presence.[13] For this project, a similar experiment with 30 mol% TfOH was conducted with a similar result (Scheme 2). Since menthyl β-glucopyranoside (**4β**) was not affected we conclude that all reported selectivity values are the result of the glycosylation outcome and not influenced by post-glycosylation anomerization towards the thermodynamic α-product.



Scheme 2. Investigation of degree of product anomerization (see ref. 13).

We were next curious to investigate whether simultaneously changing two different reaction parameters that each induce better β-selectivity would further improve the selectivity for the equatorial isomer. As shown in Table 10 both increasing the amount of TfOH from 10 mol% to 30 mol% and using CH_2Cl_2/acetonitrile 1:1 as reaction medium indeed afforded an even better level of β-selectivity although there is only a small actual difference between 1:14.9 and 1:19.7. A careful optimisation with regards to reaction temperature may increase the selectivity even further, but this was not investigated.

Entry	Solvent (TfOH)	α/β-ratio[a]	Yield[b]	Average α/β
1	Only CH_2Cl_2 (10 mol%)			1:2.4[c]
2	CH_2Cl_2/CH_3CN (1:1) [10 mol%]			1:14.9[d]
3	Only CH_2Cl_2 [30 mol%]			1:5.3[e]
4	CH_2Cl_2/CH_3CN (1:1) [30 mol%]	1:18.1	93%	1:19.7
5		1:18.2	91%	
6		1:22.8	91%	

Table 10. Results from glucosylation of L-menthol according to Scheme 1 in various solvents with NIS (1.1 equiv.) and TfOH (10 mol%). Reactions were performed from -78 ºC to 0 ºC over 3.5 hours. [a]Determined by ^{13}C-NMR. [b]Determined by ^{1}H-NMR using benzyl benzoate as internal standard. [c]From Table 1. [d]From Table 10. [e]From Table 8.

Conclusion

Chemical glycosylation will remain a reaction with great practical importance as it can supply glycobiologists with homogeneous samples for biological evaluation. Its synthetic efficiency is intimately linked to the control of the stereochemical outcome of the glycoside forming reaction and it is widely accepted that each glycosylation reaction is a new challenge.[27] We have systematically investigated one of the most typical glycosyl donor types and their activation with the NIS/TfOH promoter system and learned that obtaining reproducible selectivity results is not trivial, which should be kept in mind in cases where detailed speculation is given.

The amount of NIS or the molecular sieve pore size were found not to influence glycosylation selectivity with L-menthol whereas concentration was found to have some degree of influence. Our selectivity determinations, however, clearly show how various nucleophiles (glycosyl acceptors) have different degrees of sensitivity to temperature and TfOH loading. This sensitivity was found to be highest for galactose diacetone (**2**) and especially L-menthol, while secondary alcohol **3** was insensitive.

With our setup and triplicate measurements, we furthermore were able to establish to which degree accurate temperature control can increase selectively compared to reactions conducted under gradient warming conditions.

From a practical point-of-view we believe the NIS/TfOH (cat.)-method has a lot to offer, even with the more convenient gradient warming approach used here. In this paper we showed how the β-selectivity can be pushed from 1:2.4 to 1:19.7 by combining the effects of a higher catalyst load and the presence of acetonitrile as co-solvent.

Despite the influence of intrinsic parameters such as glycosyl acceptor structure, using the ideas and thoroughness presented here a lot can be learned about how external parameters can be manipulated in order to increase anomeric selectivity. Although the task may seem immense since other donors varying in both protecting group pattern and stereochemistry undoubtedly will behave differently, valuable insight can certainly be gained.

Experimentals

General Remarks. All reagents were used as purchased without further purification. High purity NIS was bought from Chempur (004499, N-iodosuccinimide/98%+). TfOH was bought from Sigma-Aldrich (158534, reagent grade/98%+). Dry solvents were taken from a solvent purification system. Glassware used for water-free reactions were dried for 12 h at 120 °C or flame dried before use. Molecular sieves were bought from Fluka (3Å, 95664, powder) and activated. Columns were packed with silica gel 60 (230−400 mesh) as the stationary phase. TLC plates were visualized by cerium (IV) sulphate/ammonium molybdate in 10% aq. sulphuric acid and heating until spots appeared. ^{1}H NMR and ^{13}C NMR spectra were recorded on the same 400 MHz spectrometer. Chemical shifts (δ) are reported in ppm relative to the residual solvent signal ($CDCl_3$ or CD_3CN). High-resolution mass spectral (HRMS) data were obtained on an electrospray (ES) mass spectrometer analyzing time-of-flight. NMR assignments were based on DEPT-135, COSY and HSQC NMR experiments.

Phenyl 2,3,4,5-tetra-*O*-acetyl-1-thio-β-D-glucopyranoside (S1)

β-D-Glucose pentaacetate (19.865 g, 0.051 mol) was dissolved in anhydrous CH_2Cl_2 (100 mL) and cooled to 0 °C. Thiophenol (10.5 mL, 0.102 mol, 2 eq.) and $BF_3 \cdot OEt_2$ (19.0 mL, 0.154 mol, 3 eq.) was added and the reaction mixture was stirred at rt for 22 h. Sat. aq. $NaHCO_3$ was added and the water phase was extracted with CH_2Cl_2. The combined organic phases were washed with brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. Upon addition of Et_2O product precipitated immediately as a white solid. The crude product was used without further purification.

R_f (EtOAc/Pentane 2:1) 0.57, HRMS (ES) calcd. for $C_{21}H_{30}NO_8S^+$ m/z 458.1479, found m/z 458.1498.

Phenyl 2,3,4,5-*O*-benzyl-1-thio-β-D-glucopyranoside (1)

Crude **S1** was dissolved in MeOH (500 mL). Sodium methoxide was added until pH 10. The reaction mixture was stirred overnight at rt, neutralized with amberlite IR120, filtered and concentrated *in vacuo*. The crude mixture was dissolved in anhydrous DMF (150 mL) under a N_2 atmosphere and cooled to 0 °C. Benzylbromide (36.3 mL, 0.305 mol, 6 eq.) was added followed by sodium hydroxide (60 % in mineral oil) (16.31 g, 0.408 mol, 8 eq.). The reaction mixture was stirred at rt overnight and quenched with MeOH and H_2O. The aqueous phase was extracted with EtOAc. The combined organic phases were washed with H_2O. The crude product was partly purified by flash column chromatography (EtOAc/Pentane 1:10 → EtOAc/Pentane 1:1). Impurities were crystallized from Et_2O and filtered off. **1** was crystallized from CH_2Cl_2/Pentane as white needles (28.828 g, 0.046 mol, 90% over 3 steps).

R_f (EtOAc/Pentane 1:10) 0.31, $[\alpha]_D^{298K}$ + 1.8 (*c* 1.0, $CHCl_3$) lit. + 3[28], Mp (uncorr.) 92.0-93.4 °C (CH_2Cl_2/Pentane) lit. 91-92 °C.[1]

^{1}H-NMR (400 MHz, $CDCl_3$) δ_H 7.64-7.58 (m, 2H, Ar*H*), 7.45-7.20 (m, 23H, Ar*H*), 4.96-4.83 (m, 2H, C*H*HPh), 4.76 (d, 1H, *J* 12 Hz, C*H*HPh), 4,70 (d, 1H, *J* 8 Hz, H1), 4.67-4.54 (m, 3H, C*H*HPh), 3.85-3.64 (m, 4H, H6, H4, H3), 3.58-3.50 (m, 2H, H2, H5). ^{13}C-NMR (101 MHz, $CDCl_3$) δ_C 138.5, 138.4, 138.1, 133.9 (Ar), 132.1-127.6 (Ar*H*), 87.6 (C1), 86.9 (C3), 80.9 (C2), 79.2 (C5), 77.9 (C4), 76.0 (*C*H_2Ph), 75.6 (*C*H_2Ph), 75.2 (*C*H_2Ph), 73.5 (*C*H_2Ph), 69.1 (C6). HRMS (ESI) calcd. for $C_{41}H_{46}NO_4S^+$ m/z 650.2953, found m/z 650.2956. Spectral values were in accordance with literature.[13]

Methyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyranoside (S2)

Methyl α-D-glucopyranoside (19.982 g, 0.103 mol) was dissolved in dry DMF (120 mL) under a N_2 atmosphere. Benzaldehyde dimethylacetal (27 mL, 0.185 mol, 1.8 eq.) and (+)-10-camphor sulfonic acid (2.401 g, 0.103 mol, 0.1 eq.) was added to the solution and the reaction was stirred at 50 °C for 3.5 h. The reaction mixture was cooled to rt and anhydrous DMF (350 mL) was added. Benzyl bromide (36.8 mL, 0.309 mol, 3 eq.) and sodium hydride (60 % in mineral oil) (14.832 g, 0.618 mol, 6 eq.) was added to the reaction mixture and stirred at rt overnight. The reaction was diluted with EtOAc and quenched with MeOH and H_2O. The water phase was extracted with EtOAc. The combined organic phases were washed with water and brine, dried over $MgSO_4$, filtered and concentrated. The product was partly purified by flash column chromatography (EtOAc/Pentane 1:9 → 1:6) to give **S2** as white crystals (24.023 g, 45.61 mmol, 51%). The impure fractions were recrystallized from CH_2Cl_2/pentane to give an overall yield of 59% (28.006 g, 53.18 mmol).

R_f (Pentane/EtOAc 9:1) 0.30, $[\alpha]_D^{298K}$ -27.8 (*c* 1.0, $CHCl_3$) lit. -31.5[29], Mp 82.9-84.3 °C (CH_2Cl_2/Pentane), lit. 93 °C.[29]

^{1}H-NMR (400 MHz, $CDCl_3$) δ_H 7.45-7.33 (m, 2H, Ar*H*), 7.32-7.10 (m, 14H, Ar*H*), 5.44 (s, 1H, OC*H*O), 4.81 (d, J_{gem} 11.4 Hz, 1H, C*H*HPh), 4.75 (d, J_{gem} 12.2 Hz, 1H, C*H*HPh), 4.73 (d, J_{gem} 11.4 Hz, 1H, CH*H*Ph), 4.59 (d, J_{gem} 12.2 Hz, 1H, CH*H*Ph), 4.48 (d, $J_{1,2}$ 3.6 Hz, 1H, H1), 4.16 (dd, $J_{6a,6b}$ 10.4 Hz, $J_{6a,5}$ 4.8 Hz, 1H, H6a) 3.94 (t, $J_{3,2/4}$ 9.2 Hz, 1H, H3), 3.72 (td, $J_{5,4/6b}$ 10.0 Hz, 1H, H5), 3.60 (app. t, 1H, H6) 3.49 (t, 1H, H4), 3.45 (dd, 1H, H2), 3.29 (s, 3H, OC*H*$_3$). ^{13}C-NMR (101 MHz, $CDCl_3$) δ_C 138.8, 138.2, 137.5 (*C*$_{Ar}$), 129.0-126.1 (*C*H$_{Ar}$), 101.3 (O*C*HO), 99.3 (C1), 82.2 (C4), 79.2 (C2), 78.7 (C3), 75.5 (*C*H$_2$Ph), 73.9 (*C*H$_2$Ph) 69.1 (C6), 62.4 (C5), 55.5 (*C*H$_3$). HRMS (ESI) calcd. for $C_{28}H_{30}O_6H^+$ m/z 463.2115, found m/z 463.2133. Spectral values were in accordance with literature.[29]

Methyl 2,3,6-tri-*O*-benzyl-α-D-glucopyranoside (3)

S2 (12.641 g, 27.3 mmol) was dissolved in dry CH_2Cl_2 under a N_2 atmosphere. Triethylsilane (22.0 mL, 0.138 mol, 5 eq.) was added and the reaction mixture was cooled to 0 °C. Trifluoroacetic acid (10.5 mL, 0.138 mol, 5 eq.) was added. The reaction mixture was stirred at rt for 16 h. Triethylsilane (4.4 mL, 27.5 mmol, 1 eq.) and trifluoroacetic acid (2.1 mL, 27.4 mmol, 1 eq.) was

added and the reaction mixture was stirred for 0.5 h. $NaHCO_3$ was added and the reaction mixture was diluted with CH_2Cl_2. The organic phase was washed with $NaHCO_3$ and brine, dried over $MgSO_4$, filtered and concentrated. The product was purified by flash column chromatography (Pentane/EtOAc, 4:1 → 3:1) to give **3** as a colorless oil (8.552 g, 18.411 mmol, 67 %).

R_f (Pentane/EtOAc, 3:1) 0.26, $[\alpha]_D^{298K}$ +16.2 (*c* 1.0, $CHCl_3$) lit. 12.5.[29]

^{1}H-NMR (400 MHz, $CDCl_3$) δ_H 7.56-7.22 (m, 15H, Ar*H*), 5.05 (d, J_{gem} 11.6 Hz, 1H, C*H*HPh), 4.81 (d, J_{gem} 12.0 Hz, 1H, C*H*HPh), 4.78 (d, J_{gem}11.6 Hz, 1H, CH*H*Ph), 4.74-4.66 (2x d, J_{gem} 12.0 Hz, $J_{1,2}$ 3.6 Hz, 2H, CH*H*Ph, H1), 4.63 (d, J_{gem} 12.2 Hz, 1H, C*H*HPh), 4.58 (d, J_{gem} 12.2 Hz, 1H, CH*H*Ph), 3.83 (t, $J_{3,2/4}$ 8.8 Hz, 1H, H3), 3.78-3.61 (m, 4H, H4, H5, H6b, H6a), 3.58 (dd, $J_{2,3}$ 9.6 Hz, 2H, H2), 3.43 (s, 3H, OC*H*$_3$). ^{13}C-NMR (101 MHz, $CDCl_3$) δ_C 138.9, 138.1, 138.1 (C_{Ar}), 128.7-127.7 (*C*H$_{Ar}$), 98.3 (C1), 81.5 (C3), 79.6 (C2), 75.5 (*C*H$_2$Ph), 73.6 (*C*H$_2$Ph), 73.2 (*C*H$_2$Ph), 70.7 (C4/C5), 69.9 (C4/C5), 69.5 (C6), 55.3 (*C*H$_3$). HRMS (ESI) calcd. for $C_{28}H_{32}O_6NH_4^+$ m/z 482.2537, found m/z 482.2558. Spectral values were in accordance with literature.[29]

Standard glycosylation protocol:

Donor (1 eq. (ca. 300 mg, 0.5 mmol)), acceptor (1.5 equiv.), benzylbenzoate (0.5 equiv.) and freshly activated molecular sieves (3 Å, 50 mg/mL solvent) was added to a special designed screw-top vial and dissolved in anhydrous CH_2Cl_2 (donor conc. 0.05 M) under an Ar atmosphere. The reaction mixture was stirred for 1 h at rt and then cooled to -78 °C for 15 min in a dry ice/acetone bath. NIS (1.1 equiv.) and TfOH (0.10 equiv.) were added at -78 °C. TfOH was added using 10 µL Hamilton syringe by inserting the tip of the syringe into the solution to ensure full addition of the acid. The reaction was allowed to heat to 0 °C at which Et_3N was added (ca. 0.1 mL) until a color change to orange was observed. The mixture was filtered, washed with 10% aq. $Na_2S_2O_3$, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude reaction product was analyzed by ^{13}C-NMR and ^{1}H-NMR.

L-menthyl 2,3,4,6-tetra-*O*-benzyl-α/β-D-glucopyranoside (4α/β)

4α: Appearance: Colorless oil. R_f (Pentane/EtOAc 10:1) 0.43. $[\alpha]_D^{300K}$ +33.2 (*c* 1.0, $CHCl_3$), lit. +31 (*c* 1.0, $CHCl_3$)[13]. ^{1}H-NMR (400 MHz, $CDCl_3$) δ_H 7.44-7.02 (m, 20H, C*H*$_{Ar}$), 5.02 (d, $J_{1,2}$ 3,6 Hz, 1H, H1), 4.98 (d, J_{gem} 10.8 Hz, 1H, C*H*HPh), 4.83 (2x d, J_{gem} 10.8 Hz, J_{gem} 10.8 Hz, 2H,

C*H*HPh, CH*H*Ph), 4.75-4.58 (m, 3H, C*H*HPh, C*H*HPh, CH*H*Ph), 4.46 (2x d, J_{gem} 12.4 Hz, J_{gem} 10.8 Hz, 2H, CH*H*Ph, CH*H*Ph), 4.07-3.91 (m, 2H, H3, H5), 3.75 (dd, $J_{6a,6b}$ 10.8 Hz, $J_{6a,5}$ 4.0 Hz, 1H, H6a), 3.69-3.59 (m, 2H, H6b, H4), 3.55 (dd, $J_{2,3}$ 9.6 Hz, 1H, H2), 3.45-3.30 (m, 1H, OCH-menthyl), 2.49-2.34 (m, 1H), 2.12 (m, 1H), 1.67-1.60 (m, 2H), 1.46-1.22 (m, 3H), 1.13-0.77 (m, 13H), 0.70 (d, *J* 6.8 Hz, 3H). ^{13}C-NMR (101 MHz, $CDCl_3$): δ_C 138.9-127.5 (C_{Ar}), 98.6 (C1), 82.0, 80.9, 80.5, 78.1, 77.2, 75.5, 75.1, 73.5, 73.2, 70.3, 68.6, 48.8, 43.1, 34.3, 31.8, 24.6, 22.9, 22.3, 21.1, 16.1. HRMS (ESI) calcd. for $C_{44}H_{54}O_6NH_4^+$ m/z 696.4259, found m/z 696.4286. Spectral were in accordance with previously found values.[13]

4β: Appearance: White solid. R_f (Pentane/EtOAc 10:1) 0.57. $[\alpha]_D^{300K}$ -17.4 (*c* 1.0, $CHCl_3$), lit. -16 (*c* 1.0, $CHCl_3$)[13]. Mp (uncorr.) 79.0-80.5 °C, lit.76.5-78.8 °C.[13] ^{1}H-NMR (400 MHz, $CDCl_3$): δ_H 7.38 – 7.22 (m, 19H, C*H*$_{Ar}$), 7.22 – 7.16 (m, 1H, C*H*$_{Ar}$), 4.94 (2xd, J_{gem} 10.7 Hz, 2H, C*H*HPh, C*H*HPh), 4.80 (2x d, J_{gem} 10.8 Hz, 2H, C*H*HPh, CH*H*Ph), 4.68 (d, J_{gem} 10.8 Hz, 1H, CH*H*Ph), 4.64 – 4.50 (m, 3H, CH*H*Ph, CH_2Ph), 4.47 (d, $J_{1,2}$ 7.8 Hz, 1H, 1H), 3.69 (d, *J* 3.2 Hz, 2H, H6ab), 3.67-3.55 (m, 2H, H3, H4), 3.50 (td, J_{vic} 10.6 Hz, J_{vic} 4.2 Hz, 1H, OCH-menthyl), 3.45–3.36 (m, 2H, H2, H5), 2.42-2.27 (m, 1H), 2.19 – 2.07 (m, 1H), 1.66 (m, 2H), 1.42-1.20 (m, 2H), 1.08 – 0.86 (m, 9H), 0.82 (d, *J* 6.8 Hz, 3H). ^{13}C-NMR (101 MHz, $CDCl_3$): δ_C 138.9-127.7 (C_{Ar}), 100.9 (C1), 85.1, 82.3, 78.1, 77.9, 75.8, 75.2, 75.0, 74.9, 73.8, 69.4, 48.2, 41.1, 34.6, 31.6, 31.1, 25.4, 23.3, 22.4, 21.2, 16.1. HRMS (ESI) calcd. for $C_{44}H_{54}O_6NH_4^+$ m/z 696.4259, found m/z 696.4287. Spectral values were in accordance with previously found values.[13]

2,2,2,-Trifluoroethyl 2,3,4,6-tetra-*O*-benzyl-α/β-D-glucopyranoside (5α/β)

α/β: 8:1. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.37-7.15 (m, 152H, C*H*$_{Ar}$), 7.14-7.04 (m, 18H, C*H*$_{Ar}$), 4.94 (d, J_{gem} 10.8 Hz, 8H, C*H*HPhα), 4.90 (d, J_{gem} 11.0 Hz, 1H, C*H*HPhβ), 4.89 (d, J_{gem} 10.7 Hz, 1H, C*H*HPhβ), 4.82-4.71 (m, 32H), 4.64 (d, J_{gem} 10.8 Hz, 1H, CH*H*Phβ), 4.61-4.37 (m, 28H), 4.23-4.11 (m, 1H), 3.95 (t, *J* 9.3 Hz, 8H, α), 3.89-3.77 (m, 15H), 3.75-3.51 (m, 42H), 3.50-3.38 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ_C 138.8-134.6 (C_{Ar}), 129.9-119.7 (*C*H$_{Ar}$), 103.7 (C1β), 97.9 (C1α), 84.4, 81.8, 81.7, 79.7, 77.3, 75.9, 75.3, 75.2, 75.1, 73.6, 71.0, 68.6, 68.2, 66.3, 66.0, 64.8 (q, 1C, $^2J_{C,F}$ 35 Hz, *C*H$_2$CF$_3$α). HRMS (ESI) calcd. for $C_{36}H_{37}F_3O_6NH_4^+$ m/z 640.2880, found m/z 640.2910. Spectral values were in accordance with previously found values.[30]

2,3,4,6-Tetra-*O*-benzyl-α/β-D-glucopyranosyl-(1→6)-[1,2:3,4]-di-*O*-isopropylidene-α-D-galactopyranose (6α/β)

α/β: 1.1:1. ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 7.42-7.39 (m, 2H, C*H*$_{Ar}$), 7.36-7.21 (m, 34H, C*H*$_{Ar}$), 7.14-7.10 (m, 4H, C*H*$_{Ar}$), 5.55 (d, $J_{1,2}$ 5.0 Hz, 1H, H1β), 5.50 (d, $J_{1,2}$ 5.0 Hz, 1H, H1α), 5.04 (d, J_{gem} 11.2 Hz, 1H, C*H*HPhβ), 4.98 (d, $J_{1',2'}$ 3.6 Hz, 1H, H1'α), 4.96 (d, J_{gem} 11.0 Hz, 1H, C*H*HPhα), 4.94 (d, J_{gem} 11.1 Hz, 1H, C*H*HPhβ), 4.82-4.65 (m, 8H, 2x C*H*$_2$Phα, C*H*HPhβ, 2x CH*H*Phβ), 4.62-4.56 (m, 4H, C*H*HPhα, C*H*HPhβ, H4α, H4β), 4.51 (d, J_{gem} 12.5 Hz, 1H, CHHPhβ), 4.49 (d, J_{gem} 11.2 Hz, 1H, CH*H*Phβ), 4.47 (d, J_{gem} 10.9 Hz, 1H, CHHPhα), 4.45 (d, J_{gem} 12.2 Hz, 1H, CH*H*Phα), 4.44 (d, $J_{1',2'}$ 7.8 Hz, 1H, H1'β), 4.34 (dd, $J_{3,2}$ 7.9 Hz, $J_{3,4}$ 2.0 Hz, 1H, H3α), 4.31-4.28 (m, 2H, H2α, H2β), 4.23 (dd, $J_{3,2}$ 7.9 Hz, $J_{3,4}$ 1.9 Hz, 1H, H3β), 4.15 (dd, $J_{6a,6b}$ 10.7 Hz, $J_{6a,5}$ 3.7 Hz, 1H, H6aβ), 4.08 (ddd, $J_{5',4'}$ 7.3 Hz, $J_{5',6a'}$ 3.5 Hz, $J_{5',6b'}$ 1.7 Hz, 1H, H5'β), 4.05-4.00 (m, 1H, H5'α), 3.97 (t, $J_{3',4'/2'}$ 9.4 Hz, 1H, H3'α), 3.83-3.54 (m, 12H, H2'α, H3'β, H4'α, H5α, H5β, H6α, H6'α, H6bβ, H6'β), 3.48-3.40 (m, 2H, H2'β, H4'β), 1.52 (s, 3H, C*H*$_3$α), 1.49 (s, 3H, C*H*$_3$β), 1.44 (s, 6H, C*H*$_3$α, C*H*$_3$β), 1.32-1.28 (4x s, 12H, 2x C*H*$_3$α, 2x C*H*$_3$β). ^{13}C NMR (101 MHz, $CDCl_3$) δ_C 138.8-138.1 (*C*$_{Ar}$), 128.8-127.6 (*C*H$_{Ar}$), 109.5 (β-O*C*O-), 109.3 (α-O*C*O-), 108.7 (α-O*C*O-), 108.7 (β-O*C*O-), 104.5 (C1'β), 97.2 (C1'α), 96.5 (C1β), 96.4 (C1α), 84.7 (H3'β), 82.1 (H3'α), 81.8 (H2'β), 79.9 (H2'α), 77.9 (H5β), 77.7, 75.8, 75.8, 75.1, 74.9 (C4'β), 74.5, 73.6, 73.6, 72.5, 71.6, 70.9, 70.9, 70.8, 70.8, 70.6, 70.4, 69.8 (C6β), 68.9 (C6'β), 68.5 (C6α/C6'α), 67.5 (C5'β), 66.3 (C6α/C6'α), 65.8 (H5'α), 26.3 (*C*H$_3$α), 26.2 (*C*H$_3$α), 26.2 (*C*H$_3$β), 26.1 (*C*H$_3$β), 25.2 (*C*H$_3$β), 25.1 (*C*H$_3$α), 24.8 (*C*H$_3$α), 24.6 (*C*H$_3$β). HRMS (ESI) calcd. for $C_{46}H_{54}O_{11}Na^+$ m/z 805.3558, found m/z 805.3558. Spectral values were in accordance with previously found values.[13]

Methyl 2,3,6-tri-*O*-benzyl-4-*O*-(2,3,4,6-tetra-*O*-benzyl-α/β-D-glucopyranosyl)-α-D-glucopyranoside (7α/β)

α/β 1:1.4. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.53-7.02 (m, 93H, C*H*$_{Ar}$), 5.73 (d, $J_{1',2'}$ 3.6 Hz, 1H, H1'α), 5.12 (d, J_{gem} 11.2 Hz, 1H), 5.07 (d, *J* 11.6 Hz, 1H), 4.94-4.86 (m, 3H), 4.86-4.70 (m, 12H), 4.66-4.50 (m, 13H), 4.50-4.38 (m, 6H), 4.29 (d, *J* 12.4 Hz, 1H), 4.17-4.04 (m, 3H), 4.03-3.81 (m, 8H), 3.79-3.45 (m, 17H), 3.45-3.29 (m, 10H, 2x C*H*$_3$). ^{13}C NMR (101 MHz, $CDCl_3$) δ_C 139.6-134.5 (*C*$_{Ar}$), 129.8-126.8 (*C*H$_{Ar}$), 102.6 (C1'β), 98.5 (C1β), 97.8 (C1α), 96.7 (C1'α), 84.9, 82.9,

82.1, 80.5, 80.2, 79.5, 78.8, 77.7, 77.4, 76.7, 75.7, 75.6, 75.5, 75.2, 75.0, 75.0, 74.9, 74.5, 73.7, 73.5, 73.4, 73.3, 73.2, 72.2, 71.0, 70.0, 69.5, 69.0, 68.1, 67.9, 55.4, 55.2, 53.5, 29.8. Spectral values were in accordance with previously found values.[13]

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Table of Content:

Achieving the highest possible selectivity in a glycosylation where both the α- and β-anomer are potential isomeric products is a central challenge in oligosaccharide synthesis. Our practically angled manuscript describes which parameters have (and don't have) an influence on the anomeric outcome and with which magnitude the selectivity varies as a function of changing reaction parameters.