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How the Substituent at O-3 of *N*-Acetylglucosamine Impacts Glycosylation at O-4: A Comparative Study

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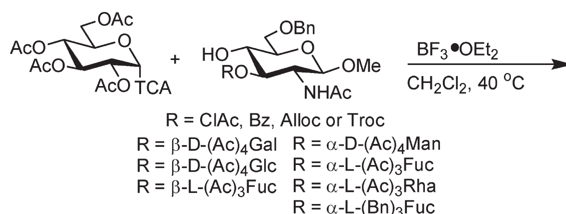

An assessment of the relative reactivities of the 4-OH of *N*-acetylglucosamine acceptors bearing simple protecting groups, *β*-linked or α-linked D or L sugars at O-3 is presented, using a per-*O*-acetylated α-D-glucosyl trichloroacetimidate donor under activation by $BF_3 \cdot OEt_2$. The presence of either an acyl or carbonate protecting group at O-3 did not impact the reactivity at O-4 with all glycosylations proceeding successfully. On the other hand, the presence of peracetylated sugars at O-3 of *N*-acetylglucosamine acceptors did impact the reactivity of the 4-OH. The acceptors with an α-D-Man, *β*-D-Gal, or *β*-D-Glc at O-3 reacted promptly. In comparison, the acceptors bearing a *β*-L-Fuc, α-L-Fuc, or α-L-Rha underwent glucosylation slowly, and unreacted acceptor was recovered from the reaction mixtures. Systematic searches carried out on the disaccharide acceptors and trisaccharide products carrying either a peracetylated *β*-D-Gal or *β*-L-Fuc at O-3 of the glucosamine residue suggest that, for these two acceptors, a conformational reorientation necessary around the fucosidic linkage contributes to the lower reactivity of the *β*-fucosylated acceptor. The acceptors bearing a *β*-linked D-Gal, D-Glc, or L-Fuc residue at O-3 each gave trisaccharide products that were mostly stable in the reaction conditions. In contrast, the α-linked residues at O-3 were rather unstable in these reaction conditions and the degradation of either the acceptors or trisaccharide products led to low glycosylation yields. In these later reactions, it was impossible to clearly assess which of the acceptor or product underwent degradation as comigration and detection issues prevented us from following these glycosylations by TLC or RP-HPLC. In contrast, the glycosylation of an acceptor carrying an α-linked perbenzylated L-Fuc residue at O-3 could be easily monitored by RP-HPLC. The data obtained when monitoring this glycosylation showed that the acceptor underwent prompt glycosylation but a decrease in the absorbance peak corresponding to the trisaccharide along with the appearance of a peak corresponding to a perbenzylated fucose hemiacetal indicated that the trisaccharide product was unstable in the reaction conditions.

Introduction

It is well-known that the hydroxyl group at C-4 of *N*-acetylglucosamine is a poor nucleophile and has reduced reactivity when compared to other acceptors in glycosylation reactions.[1−3] This lack of reactivity often makes the synthesis of biologically important oligosaccharides containing *N*-acetylglucosamine residues glycosylated at O-4 an arduous task. The low reactivity exhibited by this hydroxyl group has been attributed to steric hindrance around the O-4 position[1] as well to the formation of a hydrogen-bonding network involving the amide hydrogen.[2] In addition, poor yields also often result from the formation of stable glycosyl imidate

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side products.[3] The most common method to overcome both the low reactivity at this position and the formation of glycosyl imidates is to replace the *N*-acetyl group with, for instance, an *N*-phthalimido (*N*-Phth), *N*-trichloroethoxycarbonyl (*N*-Troc), or azido substituent. More recently Crich and Vinod have also reported that the simultaneous protection of the *N*-acetyl group and O-3 with an oxazolidone allowed for efficient glycosylation to occur at O-4 of *N*-acetylglucosamine.[4] Although rare, there are reports in the literature of successful glycosylations at O-4 of *N*-acetylglucosamine glycosyl acceptors.[5] In fact, we have recently described reaction conditions[6] that allow such successful glycosylations to take place. Glucosylation at O-4 of an *N*-acetylglucosamine monosaccharide acceptor, using the α-trichloroacetimidate of peracetylated glucopyranose as a donor, proceeded without the concomitant formation of glycosyl imidates and in more than 90% yield when carried out under activation with excess $BF_3 \cdot OEt_2$ (2 equiv) at room temperature. We have hypothesized[6] that in these conditions 1 equiv of $BF_3 \cdot OEt_2$ interacted noncovalently with the nucleophilic *N*-acetyl group and that the second one promoted glycosylation. Therefore, we concluded that as long as donor and acceptor could withstand activation with multiple equivalents of $BF_3 \cdot OEt_2$ at elevated temperatures, glycosylations at O-4 of a glucosamine acceptor with a trichloroacetimidate donor may proceed in synthetically useful yields. However, the result described above constitutes only one successful example of such glycosylation and there is limited literature to clarify when glycosylation at O-4 of *N*-acetylglucosamine is feasible. In this paper, we report our results when attempting to glucosylate at O-4 of *N*-acetylglucosamine in various mono- and disaccharide acceptors using the reaction conditions mentioned above. We have examined the impact that the substituent at O-3 of *N*-acetylglucosamine acceptors has on the reactivity of the neighboring 4-OH by attempting glycosylations of acceptors bearing various protecting groups or sugar residues at O-3. The disaccharide acceptors include both D and L series sugars, linked at O-3 through either α or β glycosidic bonds. Thus, we report that while simple acyl groups at O-3 allow for such reactions to proceed in high yield, having a sugar residue at O-3 may impact negatively and drastically the outcome of the glycosylation. We show that yields are sometimes reduced due to a lower reactivity at O-4 but also in some cases due to the fast degradation of product in these rather harsh conditions. Indeed, trends indicate that the efficiency of the glycosylation at O-4 is affected by the structure of the sugar residue at O-3 (D vs. L) and that the stability of the trisaccharide formed is affected by the stereochemistry of the glycosidic linkage at O-3 (α vs. β). Finally, we describe the use of RP-HPLC to monitor glycosylation of an acceptor bearing a perbenzylated residue at O-3.

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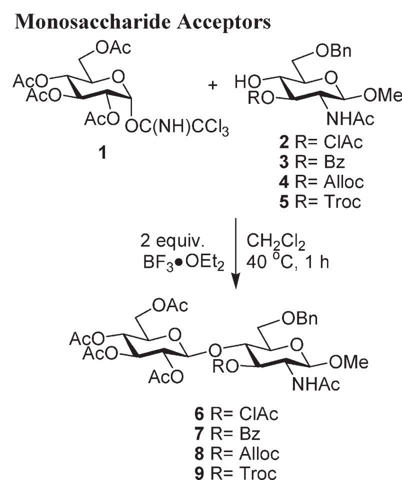
TABLE 1. Glucosylation of Monosaccharide Acceptors [a]

entry	acceptor	product	yield (%)
1	**2**	**6**	88
2	**3**	**7**	76
3	**4**	**8**	87
4	**5**	**9**	90

[a] Reagents and conditions: $BF_3 \cdot OEt_2$ (2 equiv), **1** (5 equiv), CH_2Cl_2, 40 °C, 1 h

SCHEME 1. Glycosylation of Monosaccharide Acceptors



Results and Discussion

In this study the known[7] trichloroacetimidate **1** was used to attempt glycosylation of four monosaccharide acceptors (**2**–**5**), three disaccharide acceptors that carried a β-linked glycoside at O-3 (**10**–**12**), and four disaccharide acceptors that carried an α-linked glycoside at O-3 (**17**–**19**, **23**). While acceptors **2**,[6] **3**,[8] **10**,[9] **19**,[10] and **23**[11] are known, the preparation of monosaccharide acceptors **4** and **5** as well as that of disaccharide acceptors **11**, **12**, **17**, and **18** is described in the Supporting Information for this paper.

Glycosylation of Monosaccharide Acceptors (Scheme 1, Table 1). We have reported[6] that under activation with 2 equiv of $BF_3 \cdot OEt_2$, the α-trichloroacetimidate **1** (5 equiv) could be easily coupled to the 3-chloroacetylated acceptor **2**. Indeed whether the reaction was left to proceed at room temperature for 1 h or stirred at 40 °C for 10 min, the disaccharide **6** has been isolated in about 90% yield.[6] In this study, all the glycosylations were first attempted by using 5 equiv of donor **1** and 2 equiv of $BF_3 \cdot OEt_2$ and were left to proceed for 1 h at 40 °C. As expected, these conditions led to an excellent yield (88%) of disaccharide **6** when coupling donor **1** with chloroacetate acceptor **2** (Table 1, entry 1). In fact the results presented in Table 1 show that each of the coupling reactions involving donor **1** and the monosaccharide acceptors (**2**–**5**) proceeded successfully. There was essentially no difference between the relative reactivities of the acceptors bearing an Alloc, or Troc (**4** and **5**, entries 3 and 4)

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SCHEME 2. Glucosylation of Acceptors Bearing a β-Linked Sugar at O-3

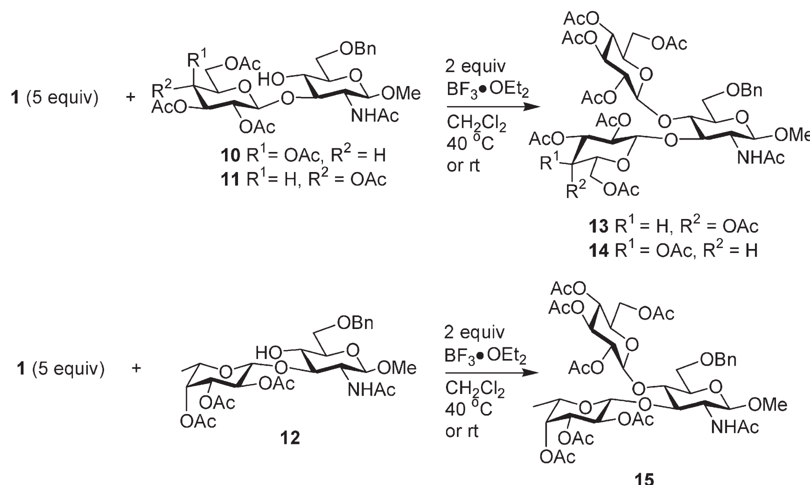


TABLE 2. Glucosylation of Acceptors Bearing a β-Linked Sugar at O-3[a]

entry	acceptor	temp (°C)	time (h)	product	yields (%) product	yields (%) acceptor
1	**10**	40	1	**13**	93	
2	**11**	40	1	**14**	88	
3	**10**	rt	1	**13**	88	
4	**11**	rt	1	**14**	91	
5	**10**	40	18	**13**	61	
6	**12**	40	1	**15**	63[b]	24[b]
7	**12**	rt	4	**15**	63[b]	18[b]

[a]Reagents and conditions: $BF_3 \cdot OEt_2$ (2 equiv), **1** (5 equiv), CH_2Cl_2. [b]Acceptor and product were isolated as a mixture, the yield was determined by NMR.

when compared to that of the chloroacetate **2**, and the three couplings gave the disaccharides **8**, **9**, and **6** in yields of around 90%. In contrast, the acceptor **3** bearing a benzoyl group at O-3 gave a significantly lower but still acceptable 76% yield of the disaccharide **7** (Table 1, entry 2), possibly due to either electronic or steric effects of the phenyl ring. Globally, acceptors carrying an acyl or carbonate group at O-3 of *N*-acetylglucosamine reacted very well toward glycosylation at O-4 with donor **1** activated with 2 equiv of $BF_3 \cdot OEt_2$ at 40 °C.

Glycosylation of Disaccharide Acceptors Carrying a β-Linked Residue at O-3 (Scheme 2, Table 2). We then focused our attention on the coupling of the glucosyl α-trichloroacetimidate **1** with O-4 of *N*-acetylglucosamine acceptors that carried a β-linked D or L sugar residue at O-3 (**10**−**12**).

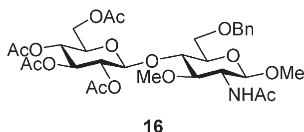
These glycosylation reactions were first attempted by using the same conditions as described above (5 equiv of **1**, 2 equiv of $BF_3 \cdot OEt_2$, 1 h at 40 °C). As can be seen in Table 2 (entries 1 and 2), the glucosylation of acceptors **10** and **11** that carry β-D-galactosyl and glucosyl residues, respectively, at O-3 gave the corresponding trisaccharides **13** and **14** in approximately 90% yield.

Even at room temperature (Table 2, entries 3 and 4), acceptors **10** and **11** were promptly glycosylated, and after 1 h of reaction the trisaccharides **13** and **14** were isolated in ~90% yield. Interestingly, when the coupling of **1** and **10** was left to proceed at 40 °C for 18 h, the yield of trisaccharide **13** dropped to 61% suggesting that it was slowly undergoing degradation when left in these conditions (2 equiv of $BF_3 \cdot OEt_2$) for such an extended time.

Surprisingly, the glucosylation of acceptor **12** that carries a β-L fucosyl residue at O-3 proceeded with much greater difficulty than that of acceptors **10** and **11** that both carry a β-D residue at O-3. While an analytical sample of the pure trisaccharide **15** was isolated after multiple chromatographies, comigration of acceptor **12** and trisaccharide **15** on normal phase silica gel precluded the monitoring of these reactions by TLC. Thus, the two compounds were isolated together by flash chromatography and ^1H NMR was used to determine the yields reported in Table 2 (entries 6 and 7). Thus, after 1 h at 40 °C, the glucosylation of acceptor **12** (Table 2, entry 6) gave a 7:3 mixture of trisaccharide **15** (63%) and unreacted acceptor **12** (24%) supporting that the β-fucosylated acceptor **12** was less reactive than acceptors **10** and **11** carrying respectively a β-linked D galactose or glucose at O-3. Identical results within experimental error were obtained when the glucosylation of β-fucosylated acceptor **12** was left to proceed at room temperature for 4 h (Table 2, entry 7). While in all cases the trisaccharides were formed in acceptable yields, these results suggest that the glycosylation at O-4 of *N*-acetylglucosamine acceptors that carry a β-linked D sugar residue at O-3 is easier than that of acceptors that carry a β-linked L unit at O-3.

A Possible Explanation to the Slower Glycosylation of the β-L-Fucosylated Acceptor 12 vs. that of the β-D-Galactosylated Acceptor 10. As with all chemical processes, the reactivity of substrates in glycosylation reactions is largely controlled by electronic and steric effects leading to the relative stabilization of starting materials, transition states, intermediates, and products. While quantum mechanical calculations are best suited to investigate the detailed electronic behaviors that led to the observed differences in reactivity between, for example, the β-D-galactosylated acceptor **10** and the β-L-fucosylated acceptor **12**, the size of these molecules precludes the use of such high-level calculations. One may suggest that the two disaccharide acceptors **10** and **12** are quite similar in their electronic behaviors since they both display a β-linked galactose-type sugar residue at O-3. Indeed, electronically these acceptors only differ by the presence of an additional acetyl substituent at C-6 of this galactose residue in acceptor **10** while acceptor **12** is

deoxygenated at this position. In contrast, while the galactose residue in acceptor **10** belongs to the D hexoses family and thus adopts a $^4C_1$ conformation, the fucose residue in acceptor **12** belongs to the L hexoses family and thus adopts a $^1C_4$ conformation. Therefore, it is reasonable to hypothesize that conformational differences may contribute to the difference in reactivity toward glycosylation that was observed between the β-D-galactosylated acceptor **10** and the β-L-fucosylated acceptor **12**. Indeed, differences in the conformations of the acceptors may make O-4 less accessible in acceptor **12** than in acceptor **10** or differences in the conformations of the products may make trisaccharide **15** less stable and thus more difficult to obtain than trisaccharide **13**. It is generally accepted that branched trisaccharides are much less flexible than the corresponding individual disaccharides. Thus, we wondered if the lowest energy conformations that are favored in the more flexible disaccharide analogues **10**, **12**, or **16** were still accessible in the branched analogues **13** and **15**, or if any glycosidic torsion could no longer achieve the most favored orientation identified in the simple disaccharides. To test this hypothesis, we carried out systematic searches with Sybyl8.0 on compounds **10**, **12**, **13**, and **15** and as well as on disaccharide **16** in implicit CH_2Cl_2 and using the Tripos[12] force field with the inclusion of the PIM parameters[13] for carbohydrates. The acetate C−O−C−C torsions were rotated in 180° increments and based on the study by González-Outeiriño et al.[14] the secondary acetate H−C−O−C torsions were rotated in 30° increments from −60° to +60° while the same torsions in primary acetates were rotated in 30° increments from 0 to 360°. With use of the known[15] preferred orientations of the acetamido group in *N*-acetylglucosamine, the H−C−N−C and C−N−C−C torsions were rotated in 180° increments. The hydroxymethyl and benzyl groups rotating bonds were sampled with 120° increments reflecting their expected staggered preferences and the O−H group was rotated in 120° increments to include both H-bonding and non-H-bonding orientations. Most of the computational effort was expended on rotations around the glycosidic linkages as the Φ and Ψ torsions were rotated in 10° increments. Only the conformations found within 10 kcal·mol^{-1} of the lowest energy conformation were retained and clustered based on the Φ and Ψ torsions.



The lowest energy representatives of each family were further minimized and the torsion angles measured for the final global minima identified for the disaccharides and trisaccharides are listed in Table 3. We first compared the conformational preference of the glycosidic β-D-Glc-(1→4)-GlcNAc bond in the protected disaccharide **16** to the same linkage in trisaccharides **13** and **15**. The Φ/Ψ maps for this glycosidic linkage in disaccharide **16** as well as in trisaccharides **13** and **15** are given in the Supporting Information and were essentially identical with one another with only an apparent restricted mobility around this β-D-Glc-(1→4)-GlcNAc bond in the trisaccharides. As seen in Table 3, the Φ/Ψ torsions found for the global minimum **A** identified for disaccharide **16** (entry 3) were essentially conserved in the global minima found for trisaccharides **13** (Φ^2/Ψ^2, entry 4) and **15** (Φ^2/Ψ^2, entry 5). Therefore, we concluded that the presence at O-3 of the galactose or fucose residues in the disaccharide acceptors **10** and **12** did not prevent the glucose residue introduced at O-4 of the *N*-acetylglucosamine from adopting the most favored glycosidic torsions found in disaccharide **16**.

TABLE 3. Glycosidic Torsions in Disaccharides 10, 12, 16 and Trisaccharides 13, 15 Global Minima

		torsion angles[a]			
entry	compd	Φ^1	Ψ^1	Φ^2	Ψ^2
1	**10**	−72	96		
2	**12**	80	−48		
3	**16**			−64	−107
4	**13**	−73	119	−66	−130
5	**15**	142	145	−72	−109

[a] Φ^1, Ψ^1 = β-Gal/Fuc-(1→3)-GlcNAc; Φ, Ψ^2 = β-Glc-(1→4)-GlcNAc.

Next, we assessed the impact that the presence of a glucose residue at O-4 of GlcNAc had on the glycosidic torsions of the O-3 galactose or fucose residues. Panels a and b of Figure 1 show the Φ/Ψ maps for the β-D-galactosyl- and β-L-fucosyl-(1→3)-GlcNAc glycosidic bonds for the disaccharides **10** and **12**, respectively, while panels c and d of Figure 1 show the Φ^1/Ψ^1 maps for the same glycosidic linkages in trisaccharide **13** and trisaccharide **15**, respectively. It is evident from Figure 1a,b that the β-D-galactosylated acceptor **10** is much less flexible than the β-L-fucosylated acceptor **12**. Indeed while within 3 kcal·mol^{-1} of the global minimum only one local minimum (Figure 1a, **B**) was found for acceptor **10**, three such local minima (Figure 1b, **B**−**D**) were identified for the fucosylated analogue **12**. As could be expected, the two acceptors **10** and **12** adopt global minima (Table 3, entries 1 and 2; Figure 1a,b) that favor the (−) or (+) exoanomeric orientation, respectively, and in both cases allow the formation of a hydrogen bond between the galactose or fucose ring oxygen and the GlcNAc 4-OH.

When comparing the Φ/Ψ map obtained for the β-D-Gal-(1→3)-GlcNAc glycosidic bond in acceptor **10** (Figure 1a) with the Φ^1/Ψ^1 map obtained for the same bond in trisaccharide **13** (Figure 1c) one observes a considerable loss of flexibility around this bond with the local minimum **B** found for the disaccharide no longer found in the branched trisaccharide. However, as can be seen in these maps as well as in Table 3 (compare entries 1 and 4) and Figure 2a, the global minimum (**A**) adopted by this linkage in disaccharide **10** (Figure 2a, gray structure) is essentially conserved in trisaccharide **13** (Figure 2a, black structure) with only a rotation of less than 30° around Ψ^1 to accommodate more comfortably the glucose residue introduced at the vicinal O-4. In sharp contrast, a very different picture emerges when comparing the Φ/Ψ map obtained for the β-L-Fuc-(1→3)-GlcNAc glycosidic bond in acceptor **12** (Figure 1b) with the

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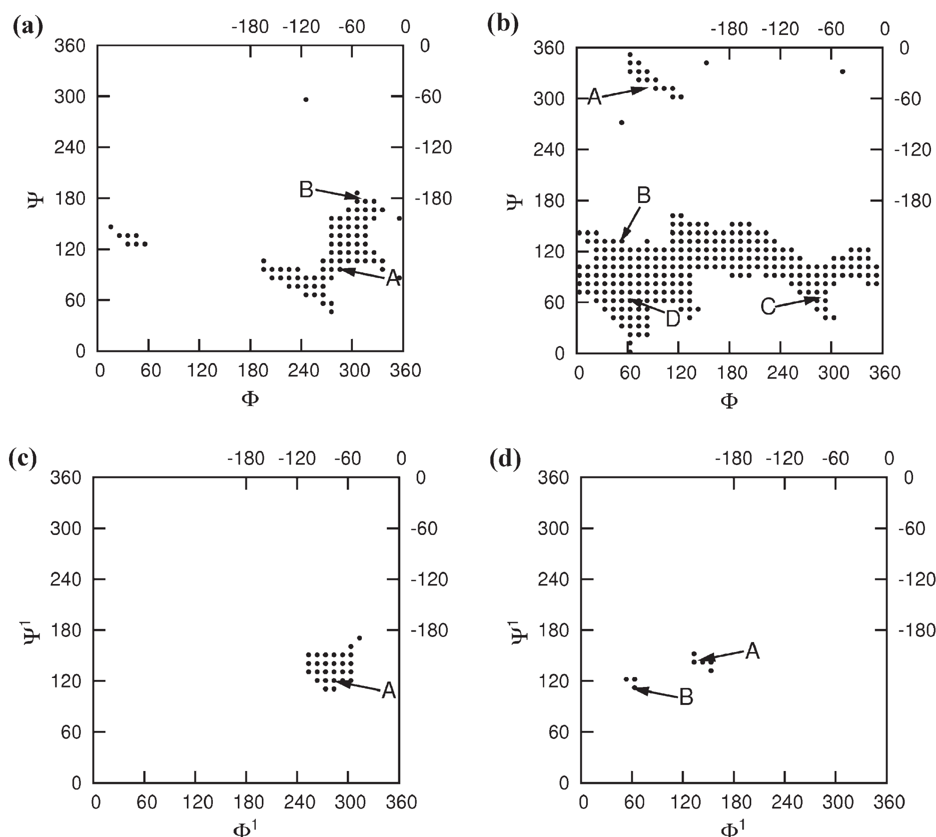


FIGURE 1. Systematic searches Φ/Ψ maps for the 1→3 glycosidic bonds: (a) *β*-D-Gal-(1→3)-GlcNAc in disaccharide **10**; (b) *β*-L-Fuc-(1→3)-GlcNAc in disaccharide **12**; (c) Φ^1/Ψ^1 *β*-D-Gal-(1→3)-GlcNAc in trisaccharide **13**; and (d) Φ/Ψ^1 *β*-L-Fuc-(1→3)-GlcNAc in trisaccharide **15**. Minimized global minima are identified as **A**; local minima found within 3 kcal·mol^{-1} of **A** are labeled **B**−**D** where appropriate.

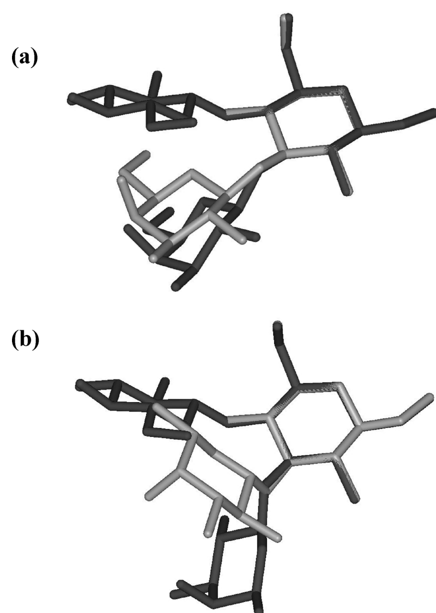


FIGURE 2. Molecular overlays of disaccharide and corresponding trisaccharide global minima: (a) disaccharide acceptor **10** (gray) and trisaccharide **13** (black) and (b) disaccharide acceptor **12** (gray) and trisaccharide **15** (black).

Φ^1/Ψ^1 map obtained for the same bond in trisaccharide **15** (Figure 1d). Indeed, the Φ^1/Ψ^1 map presented in Figure 1d shows that this glycosidic bond in trisaccharide **15** has become very rigid when compared to its flexibility in disaccharide **12** (Figure 1b). Most importantly, we observe that the lowest energy conformation adopted around this glycosidic bond in trisaccharide **15** (Figure 1d, **A**; Table 3, entry 5) is very different from the lowest energy conformation found for disaccharide **12** (Figure 1b, **A**; Table 3, entry 2).

Thus, it implies that either a +190° or a −170° rotation around the Ψ torsion had to occur during the glucosylation at O-4. Indeed such a rotation is required since, as seen in Figure 2b, if the fucosyl residue was to maintain the conformation that is favored in the disaccharide (gray) around the glycosidic bond, its C-6′ would collide with C-5″ of the glucose residue (black) introduced at O-4. Due to this atom overlap, it is reasonable to expect this conformational shift to occur prior to or concurrently to the glycosylation and as such to affect the observed rate of the reaction. We subsequently assessed the energy difference between the global minimum adopted by disaccharide **12** and the conformation that it needs to assume to give trisaccharide **15**. Thus, acceptor **12** was minimized around the side chains and protecting groups while the Φ/Ψ torsions of the *β*-L-Fuc-(1→3)-GlcNAc bond was set and maintained at the values found for this glycosidic bond in the global minimum identified for trisaccharide **15** (Table 3 entry 5, Φ^1/Ψ^1). Indeed, the resulting minimized conformation was found to be over 12 kcal·mol^{-1} higher in energy than the global minimum found for acceptor **12**.

The vicinal coupling constants measured for the sugar units in compounds **10**, **12**, **13**, and **15** supported an average

SCHEME 3. Glucosylation of Acceptors Bearing an α-Linked Sugar at O-3

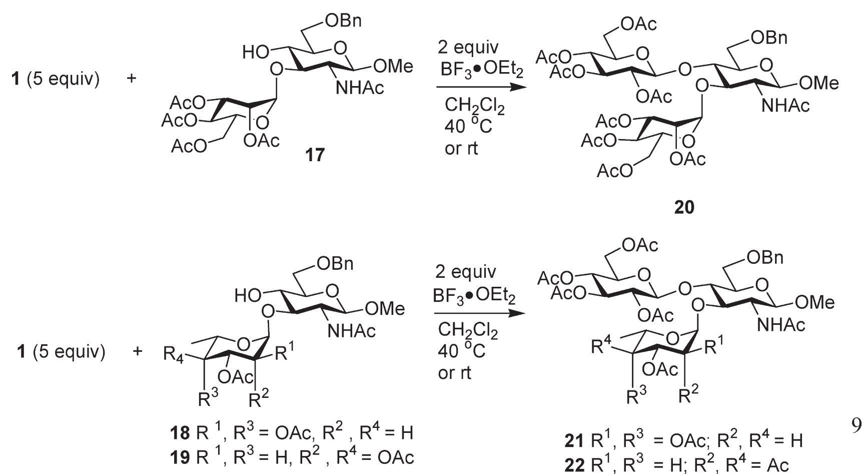


TABLE 4. Glucosylation of Acceptors Bearing an α-Linked Sugar at O-3[a]

					yield (%)	
entry	acceptor	temp (°C)	time (h)	product	product	acceptor
1	**17**	40	1	**20**	55[b]	
2	**17**	40	2	**20**	41[b]	
3	**17**	40	18	**20**	–[c]	
4	**17**	rt	2	**20**	54[b]	
5	**17**	rt	18	**21**	39[b]	
6	**18**	40	1	**21**	26[d]	34[d]
7	**18**	40	3	**21**	37[d]	38[d]
8	**18**	40	8	**21**	–[c]	–[c]
9	**19**	40	1	**22**	27[d]	43[d]
10	**19**	40	2	**22**	34[d]	40[d]
11	**19**	40	18	**22**	–[c]	–[c]

[a]Reagents and conditions: $BF_3 \cdot OEt_2$ (2 equiv), **1** (5 equiv), CH_2Cl_2. [b]Contaminated with some degraded donor, yield estimated by NMR. [c]Trace amounts observed by TLC. [d]Mixture of donor and acceptor, yields estimated by NMR.

$^4C_1$ conformation for the glucose, galactose, and *N*-acetylglucosamine rings and a $^1C_4$ conformation of the fucose ring. Unfortunately, signal overlap in 2D NOESY experiments at 300 K in deuterated chloroform prevented the acquisition of any other usable experimental data to support the modeling results described above. A more detailed study involving the modeling of the reaction trajectory and transition states would be required to fully understand these behaviors. However, the results presented above suggest that the degree of conformational reorientation necessary around the fucosidic linkage when going from acceptor **12** to trisaccharide **15**, as well as the reduction in conformational space accessible around this bond, contributes to the slower glycosylation of the *β*-L-fucosylated acceptor **12** when compared to that of the *β*-D-galactosylated acceptor **10**.

Glycosylation of Disaccharide Acceptors Bearing an α-Linked Residue at O-3 (Scheme 3, Table 4). In turn, we focused our attention on the coupling of the glucosyl α-trichloroacetimidate **1** with O-4 of *N*-acetylglucosamine acceptors that carried peracetylated α-linked D or L sugar residues at O-3 (**17**−**19**, Table 4).

Analytical samples of trisaccharides **20**−**22** were obtained after multiple chromatographies and their structures were confirmed by NMR and HR-ESI mass spectrometry. However, these trisaccharides comigrated on silica gel with either the acceptor or degraded glucosyl donor and the reactions could not be followed by TLC.

The yields reported in Table 4 were therefore estimated by ^{1}H NMR on the mixtures obtained after chromatography. Starting with the conditions established above (5 equiv of **1**, 2 equiv of $BF_3 \cdot OEt_2$, 1 h at 40 °C), glycosylation of the D mannosylated acceptor **17** gave trisaccharide **20** (Table 4, entry 1) in 55% yield while no acceptor was recovered. Extending the reaction time to 2 h (entry 2) gave a decreased yield (41%) of the trisaccharide **20** while after 18 h (entry 3), TLC analysis of the reaction mixture showed only trace amounts of trisaccharide product **20**. Since no acceptor **17** was recovered in any of these reactions, we concluded that it was either degraded before being glycosylated or promptly glucosylated to give the trisaccharide **20** that, in turn, underwent fast degradation. Running the same glycosylation at room temperature (entry 4 and 5) appeared to slow the degradation of the trisaccharide **20**, which was obtained in 54% or 39% yield after 2 or 18 h, respectively. Thus, it appears that similarly to acceptors **10** and **11** that also carry D sugar residues at O-3, the mannosylated acceptor **17** was promptly glucosylated either at 40 °C or at room temperature. However, while the acceptors (**10**, **11**) and trisaccharides (**13**, **14**) that carry *β*-D residues at O-3 were fairly stable in these glycosylation conditions (Table 2), the starting acceptor **17** or trisaccharide **20** that carry an α-D-mannosyl residue at O-3 underwent fast degradation.

Glucosylations of acceptors **18** and **19** that carry respectively an α-L-fucosyl and α-L-rhamnosyl residue at O-3 were then investigated (Table 4, entries 6−11). After 1 h at 40 °C only 26% and 27% of fucosylated trisaccharide **21** or rhamnosylated trisaccharide **22** were obtained, respectively, while unreacted acceptors were recovered in 34% yield for acceptor **18** and 43% for acceptor **19** (entries 6 and 9). Allowing the glycosylations to proceed for longer reaction times at 40 °C increased the yield of the trisaccharides **21** and **22** to 37% and 34%, respectively, but large amounts of unreacted acceptors were still present (entries 7 and 10). Finally, allowing the glucosylation of the acceptors **18** and **19** to run for 8 or 18 h, respectively, resulted in only trace quantities of both of the acceptors and products observed by TLC (entries 8 and 11). These results suggest that the O-4

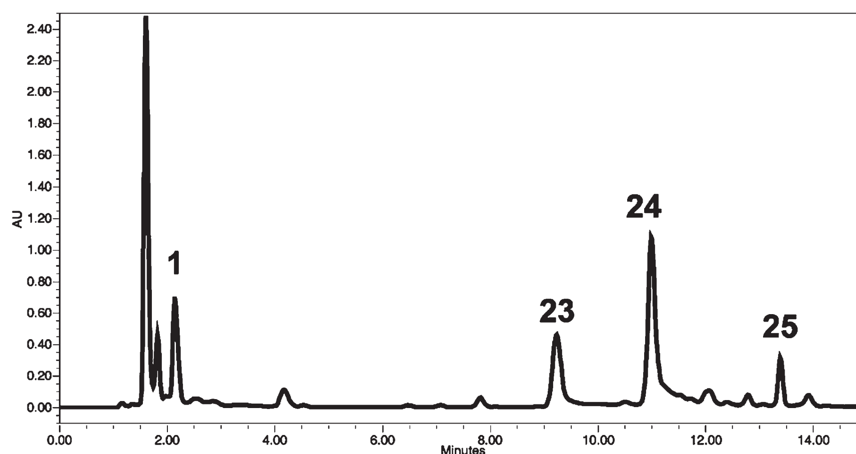


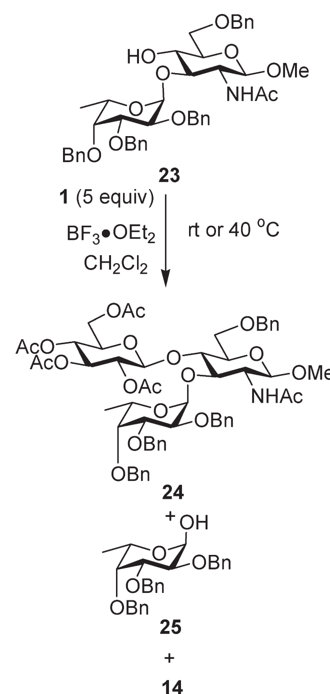
FIGURE 3. HPLC trace at 50 min reaction time for the glucosylation of acceptor **23** (2 equiv of $BF_3 \cdot OEt_2$ at 40 °C).

GlcNAc acceptors **18** and **19** carrying an α-L- sugar residue at O-3 were less reactive than acceptor **17** that carries an α-D-mannosyl residue at the same position. However, it is clear from those reactions left to proceed at 40 °C for extended reaction times that compounds (acceptors or trisaccharides) carrying an α-linked sugar residue at O-3 of the GlcNAc unit were prone to degradation (Table 4, entries 3, 8, and 11). In contrast, O-4 glucosylation of the analogues bearing *β*-linked sugar residue at O-3 led to considerably more stable trisaccharides with, for example, 61% of the 3-*O*-*β*-D-galactosylated trisaccharide **13** still isolated when the reaction was left at 40 °C for 18 h (Table 2, entry 5).

Our conclusion that acceptors and trisaccharides bearing an α-linked residue at O-3 of the GlcNAc unit could undergo degradation was further supported by our study on the glycosylation of the disaccharide acceptor **23** that contains an α-linked perbenzylated fucose residue at O-3 of the *N*-acetylglucosamine unit (Scheme 4). In contrast to the glycosylations studied above, this reaction could be monitored by HPLC on a C18 reverse phase column, using a gradient solvent system starting at 65:35 CH_3CN:H_2O and increasing to 90:10 CH_3CN:H_2O. The disappearance of the acceptor and the formation of the desired trisaccharide as well as the formation of side products was monitored with use of UV detection at 220 nm. As can be seen in Figure 3, the HPLC trace gave well-separated absorbance peaks for the acceptor **23** and two newly formed compounds **24** and **25**. In contrast, the unreacted glycosyl donor and its derivatives were observed close to the solvent front and with a low absorbance since they do not carry aromatic substituents.

A semipreparative RP-HPLC column allowed the separation of compounds **23**, **24**, and **25**. Compound **24** was identified as the desired known[6] trisaccharide while **25** was identified as the known[16] 2,3,4-*O*-benzyl-α-L-fucopyranose. Since acceptor **23**, trisaccharide **24**, and fucose **25** have similar absorbance at 220 nm, the areas measured for these three peaks in the HPLC trace could be correlated to the relative amounts of the three products present at a given time in the reaction mixture. Indeed, glycosylation of acceptor **23** with donor **1** could be followed directly by analytical RP-HPLC: at each time point a 5 *μ*L aliquot of the reaction mixture was diluted in CH_3CN (100 *μ*L) containing NEt_3 and 10 *μ*L of this solution was injected in the HPLC. Three reactions were followed by HPLC and the relative abundance of **23**, **24**, and **25** as a function of time for each reaction is shown in Figure 4.

SCHEME 4



The first reaction was carried out in the standard conditions used above (5 equiv of donor **1**, 2 equiv of $BF_3 \cdot Et_2O$ at 40 °C, Figure 4a). In these conditions, after 5 min of reaction it was observed that already 61% of trisaccharide **24** had been formed while 39% of the acceptor remained. After 50 min of reaction the amount of trisaccharide **24** had slightly increased to 64% while only 30% of the acceptor **23** remained and 10% of the tribenzylated fucose **25** had formed. After 100 min of reaction, HPLC analysis of the reaction showed complete disappearance of the formed trisaccharide **24**, 10% of residual starting acceptor **23**, and 90% of the tribenzylated fucose **25**. Interestingly, column chromatography of the reaction mixture led to isolation of

(16) Dejter-Juszynski, M.; Flowers, H. M. *Carbohydr. Res.* **1971**, *18*, 219–226.

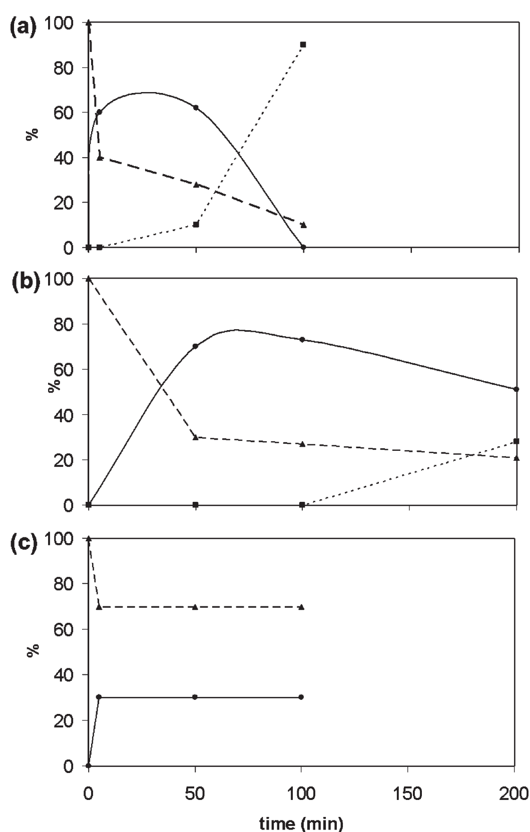


FIGURE 4. Glycosylation of acceptor **23** and degradation of trisaccharide **24**: (a) 2 equiv of $BF_3 \cdot Et_2O$, 40 °C; (b) 2 equiv of $BF_3 \cdot Et_2O$, 20 °C; (c) 1 equiv of $BF_3 \cdot Et_2O$, 20 °C; **23** (▲), **24** (●), and **25** (■).

the diglucosylated trisaccharide **14** synthesized above, and that was obtained in 27% yield. Thus, the data obtained from this first reaction suggest that the desired trisaccharide **24** was formed first and very rapidly in these conditions but then underwent rapid degradation losing the fucosyl residue. The resulting acceptor disaccharide free at O-3 of the glucosamine residue was then glucosylated leading to trisaccharide **14**. In an effort to slow down the kinetics of degradation, a second reaction was carried out at room temperature, using again 5 equiv of donor **1** and 2 equiv of $BF_3 \cdot Et_2O$ (Figure 4b). Once again the trisaccharide **24** was formed rapidly and after 50 min of reaction, 70% of the trisaccharide **24** and 30% of residual acceptor **23** were present in the reaction mixture while no benzylated fucose was observed. At 100 min of reaction time the ratio of product and acceptor was only slightly modified in favor of the desired trisaccharide **24** that was formed at 73% while 27% of acceptor **23** was left in the mixture. Again, no benzylated fucose could be detected suggesting that degradation of the product and starting material was slow. However, at 200 min we observed that the percent abundance of the trisaccharide **24** had decreased by 22% (to 51%), while the percent abundance of the acceptor **23** had decreased by 6% (to 21%). At this time, the hydrolyzed fucosyl peak then accounted for the remaining 28% of the absorbance at 220 nm. Both reactions thus show that glucosylation at O-4 of acceptor **23** took place quite rapidly and much faster than the glucosylation of acceptor **18** that carried an α-L-peracetylated (vs. perbenzylated) fucosyl residue at O-3 of the GlcNAc acceptor (Table 4, entries 6 and 7). It is also clear that the glucosylation of acceptor **23** occurred faster than its degradation but that the formed trisaccharide **24** was unstable in the presence of 2 equiv of $BF_3 \cdot Et_2O$ and lost the fucosyl residue. We also attempted coupling acceptor **23** and donor **1** at room temperature using only 1 equiv of $BF_3 \cdot Et_2O$ (Figure 4c). In these conditions, only 30% the trisaccharide 24 was formed within 5 min while 70% of the acceptor remained unreacted. This ratio remained stable for 100 min and no further glucosylation of **23** nor degradation of **23** and 24 was observed. These results suggest that glucosylation of acceptor **23** by donor **1** requires the use of 2 equiv of $BF_3 \cdot Et_2O$ to proceed, and that in these conditions, it proceeds quickly at either room temperature or 40 °C. However, we also observe that in these conditions, the trisaccharide product **24** undergoes degradation by losing the α-fucosyl residue at O-3 of GlcNAc. Indeed, such degradation has also been observed by Shimizu et al.[17] upon galactosylation of a dichlorophtalimidoglucosamine O-4 acceptor carrying a perbenzylated α-fucosylated unit at O-3.

Conclusion

It is evident from the studies above that the substituent at O-3 of an *N*-acetylglucosamine acceptor impacts the reactivity of the neighboring 4-OH. An acyl or carbonate protecting group at O-3 of the *N*-acetylglucosamine acceptor appears to have little impact on the reactivity of the 4-OH. In contrast, glycosylations of acceptors bearing various sugar residues at O-3 showed that the configuration and nature of the sugar at O-3 had a large impact on the reactivity of the 4-OH toward glycosylation. Two trends seem to be emerging from the results described above for the glycosylations of the acceptors bearing peracetylated *β*-linked (Table 2) and α-linked (Table 4) residues at O-3. First, the acceptors having a D series peracetylated sugar at O-3 were more reactive toward glycosylation than the acceptors carrying L series peracetylated sugars at O-3. However, as could be expected, the acceptor carrying an α-linked per-benzylated L-fucose at O-3 was much more reactive than the acetylated analogue. For the acceptors carrying at O-3 the rather stable *β*-linked D-galactosyl and L-fucosyl residues, molecular mechanics calculations suggest that conformational reorientations around the L-fucosidic linkage and reduction of the conformational space accessible around this bond contribute to its lower reactivity. Second, in the presence of 2 equiv of $BF_3 \cdot Et_2O$, the trisaccharide products and/or disaccharide acceptors that carry α-linked residues at O-3 are prone to degradation through the loss of this residue while those analogues carrying *β*-linked peracetylated sugars at O-3 are stable. While molecular mechanics may suggest an explanation to the lower reactivity of the *β*-fucosylated analogue **12** vs. that of the *β*-galactosylated acceptor **10**, the many stereoelectronic factors leading to these experimental results cannot be rationalized rigorously at this level of computation. A more detailed study involving the modeling of the reaction trajectories and transition states is required to fully understand these behaviors; however, such computational studies are

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not possible at this time due to the size of the molecules involved. However, the results reported here clearly illustrate that the poor yields associated with glycosylation at O-4 of *N*-acetylglucosamine acceptors may be due not only to a lack in the reactivity of the acceptor but also to the instability of the products in some of the harsher reaction conditions required to achieve glycosylation. It has been well recognized that the success of a glycosylation frequently requires that the donor and acceptor be matched[18] based on their respective expected reactivities[19] and configurations (D vs. L and α vs. β).[20] Indeed, it has been well-established that the configurations of donors and acceptors as well as the size and electronic properties of the protecting groups that they carry had a profound impact on the success of a glycosylation reaction.[18−20] This study now illustrates that the configuration (D vs. L and α vs. β) as well as the protecting group pattern (Ac vs. Bn) on a sugar unit that is vicinal to the OH group being glycosylated also has an impact on the success of the glycosylation reaction. Thus, our results further expand the match−mismatch concept from the acceptor structural features to that of the sugar residues that it already carries.

Experimental Section

General Procedure for Glycosylation at O-4 of *N*-Acetylglucosamine Acceptors. $BF_3 \cdot OEt_2$ (2.0 equiv) was added to a solution of the acceptor and the known[7] glucosyl donor **1** (5.0 equiv) in CH_2Cl_2 at 40 °C. The reaction mixture was stirred for 1 h, then the reaction was quenched with Et_3N (2.3 equiv) and diluted with CH_2Cl_2. The mixture was washed with satd aq $NaHCO_3$, the aqueous layer was re-extracted with CH_2Cl_2, and the combined organic layers were dried and concentrated. The residues were purified by flash chromatography to give the glycosylated products.

Methyl 2-Acetamido-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-3-*O*-benzoyl-6-*O*-benzyl-2-deoxy-β-D-glucopyranoside (7). Alcohol **3** (15 mg, 0.036 mmol) was coupled to the donor **1** (92 mg, 0.180 mmol, 5.0 equiv) in CH_2Cl_2 (1.3 mL) according to the general protocol. Column chromatography (8:2 EtOAc−hexanes) of the residue gave pure disaccharide **7** (19 mg, 76%) as a colorless glass. $[\alpha]_D$ −13 (*c* 0.6, CH_2Cl_2); ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.01 (d, 2H, *J* = 7.1 Hz, Ar), 7.60−7.31 (m, 8H, Ar), 5.59 (d, 1H, *J* = 9.4 Hz, NH), 5.33 (t, 1H, *J* = 9.1 Hz, H-3), 4.98 (t, 1H, *J* = 9.5 Hz, H-3), 4.90−4.70 (m, 3H, H-2′, H-4′, C*H*HPh), 4.58−4.47 (m, 2H, H-1′, C*H*HPh), 4.42 (d, 1H, *J* = 8.0 Hz, H-1), 4.17 (t, 1H, *J* = 8.1 Hz, H-2), 4.12 (t, 1H, *J* = 8.8 Hz, H-4), 3.89−3.73 (m, 3H, H-6a, H-6b, H-6a′), 3.64 (dd, 1H, *J* = 12.2, 2.1 Hz, H-6b′), 3.59−3.45 (m, 4H, H-5, OCH_3), 3.31−3.22 (m, 1H, H-5′), 1.93, 1.89, 1.84 (3s, 15H, 5 × $COCH_3$); ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.5, 170.2, 169.3, 169.1, 166.2 (C=O), 137.9, 133.3, 129.8, 129.4, 128.6, 128.4, 128.0, 127.9 (Ar), 102.1 (C-1), 100.0 (C-1′), 74.9 (C-4) 74.9 (C-5), 73.8 (CH_2Ph), 73.1 (C-3), 72.9 (C-3′), 71.7 (C-5′), 71.5 (C-2′), 67.9 (C-4′), 67.6 (C-6), 61.5 (C-6′), 56.6 (OCH_3), 53.7 (C-2), 23.3, 20.7, 20.6, 20.5 (COCH_3); HRESIMS calcd for $C_{37}H_{45}NO_{16}$ $[M + H]^+$ 760.2817, found 760.2791.

Methyl 2-Acetamido-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-3-*O*-allyloxycarbonyl-6-*O*-benzyl-2-deoxy-β-D-glucopyranoside (8). Acceptor **4** (30 mg, 0.073 mmol) was coupled to the donor **1** (180 mg, 0.368 mmol, 5.0 equiv) in CH_2Cl_2 (2.0 mL) according to the general protocol. Column chromatography (8:2 EtOAc−hexanes) of the residue gave pure disaccharide **8** (48 mg, 87%) as a colorless glass. $[\alpha]_D$ −31 (*c* 1.0, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42−7.28 (m, 5H, Ar), 5.95−5.77 (m, 1H, CH_2CH=CH_2), 5.75 (d, 1H, *J* = 8.8 Hz, NH), 5.38−5.18 (m, 2H, CH_2CH=CH_2), 5.10−4.92 (m, 3H, H-3, H-3′, H-4'), 4.87 (t, 1H, *J* = 8.3 Hz, H-2'), 4.77−4.59 (m, 2H, C*H*HPh, C*H*HCH=CH_2), 4.55−4.41 (m, 4H, H-1, H-1′, C*H*HPh, CH*H*CH=CH_2), 4.25 (dd, 1H, *J* = 12.2, 4.7 Hz, H-6a′), 4.00−3.76 (m, 3H, H-2, H-4, H-6b′), 3.72 (d, 2H, *J* = 2.4 Hz, H-6a, H-6b), 3.56−3.41 (m, 5H, H-5, H-5′, OCH_3), 2.04, 1.97, 1.94, 1.90, 1.89 (5s, 15H, 5 × $COCH_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.4, 170.3, 170.2, 169.4, 169.1, 154.8 (C=O), 137.9 (CH_2CH=CH_2), 131.4, 128.6, 128.5, 128.0, 127.7 (Ar), 118.8 (CH_2CH=CH_2), 101.5 (C-1), 100.4 (C-1′), 76.6 (C-3), 75.8 (C-4), 74.4 (C-5), 73.7 (CH_2Ph), 72.9 (C-3′), 71.7 (C-5′), 71.5 (C-2'), 68.6 (CH_2CH=CH_2), 68.2 (C-4′), 67.5 (C-6), 62.0 (C-6′), 56.6 (OCH_3), 54.1 (C-2), 23.3, 20.7, 20.6, 20.5 ($COCH_3$); HRESIMS calcd for $C_{34}H_{45}NO_{17}$ $[M + H]^+$ 740.2766, found 740.2794.

Methyl 2-Acetamido-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-6-*O*-benzyl-3-*O*-trichloroethyloxycarbonyl-2-deoxy-β-D-glucopyranoside (9). Acceptor **5** (21 mg, 0.043 mmol) was coupled to the donor **1** (105 mg, 0.215 mmol, 5.0 equiv) in CH_2Cl_2 (1.5 mL) according to the general protocol. Column chromatography (8:2 EtOAc−hexanes) of the residue gave pure disaccharide **9** (32 mg, 90%) as a colorless glass. $[\alpha]_D$ +9 (*c* 1.0, CH_2Cl_2); ^{1}H NMR (400, $CDCl_3$) δ 7.51−7.31 (m, 5H, Ar), 5.64 (d, 1H, *J* = 8.5 Hz, NH), 5.14 (dd, 1H, *J* = 10.1, 8.7 Hz, H-3), 5.09−4.91 (m, 3H, H-3′, H-4′, C*H*HCCl$_3$), 4.85 (t, 1H, *J* = 8.4 Hz, H-2′), 4.73 (d, 1H, *J* = 12.8 Hz, C*H*HPh), 4.59 (d, 1H, *J* = 8.0 Hz, H-1), 4.57−4.40 (m, 3H, H-1′, C*H*HPh, CH*H*CCl$_3$), 4.34 (dd, 1H, *J* = 12.3, 4.9 Hz, H-6a′), 4.01−3.91 (m, 2H, H-4, H-6b′), 3.80−3.68 (m, 3H, H-2, H-6a, H-6b), 3.52−3.40 (m, 5H, H-5, H-5′, OCH_3), 2.02, 1.94, 1.91, 1.89, 1.87 (5s, 15H, 5 × $COCH_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.6, 170.2, 170.1, 169.4, 169.2, 168.4, 153.8 (C=O), 137.7, 128.6, 128.1, 128.0 (Ar), 101.1 (C-1), 100.2 (C-1′), 94.5 (CCl_3), 77.3 (C-3), 76.7 (CH_2CCl_3), 75.6 (C-4), 74.3 (C-5), 73.7 (CH_2Ph), 72.9 (C-3′), 71.8 (C-5′), 71.4 (C-2′), 68.1 (C-4′), 67.3 (C-6), 62.0 (C-6′), 56.8 (OCH_3), 54.6 (C-2), 23.3, 20.8, 20.6, 20.5 ($COCH_3$); HRESIMS calcd for $C_{33}H_{42}Cl_3NO_{17}$ $[M + H]^+$ 830.1597, found 830.1597.

Methyl 2-Acetamido-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-6-*O*-benzyl-2-deoxy-β-D-glucopyranoside (13). Alcohol **10** (20 mg, 0.031 mmol) was coupled to the donor **1** (75 mg, 0.152 mmol, 5.0 equiv) in CH_2Cl_2 (1.0 mL) according to the general protocol. Column chromatography (9:1 EtOAc:hexanes) of the residue gave the trisaccharide **13** (28 mg, 93%) as a colorless glass. $[\alpha]_D$ −47 (*c* 1.0, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41−7.29 (m, 5H, Ar), 6.46 (d, 1H, *J* = 6.1 Hz, NH), 5.40 (d, 1H, *J* = 2.9 Hz, H-4′), 5.24−5.12 (m, 2H, H-2′, H-3″), 5.09−4.99 (m, 2H, H-3′, H-4″), 4.94 (dd, 1H, *J* = 9.8, 8.1 Hz, H-2″), 4.81 (d, 1H, *J* = 7.9 Hz, H-1′), 4.56 (d, 2H, *J* = 0.9 Hz, CH_2Ph), 4.42 (d, 1H, *J* = 8.0 Hz, H-1″), 4.28 (dd, 2H, *J* = 12.8, 4.1 Hz, H-1, H-6a″), 4.22−4.07 (m, 4H, H-2, H-5, H-6a′, H-6b′), 4.02−3.93 (m, 3H, H-3, H-5′, H-6b″), 3.86−3.79 (m, 1H, H-4), 3.74 (d, 2H, *J* = 6.9 Hz, H-6a, H-6b), 3.51−3.44 (m, 1H, H-5″), 3.35 (s, 3H, OCH_3), 2.11, 2.10, 2.09, 2.08, 2.07, 2.06, 2.05, 2.03, 1.99 (9s, 27H, 9 × $COCH_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.7, 170.5, 170.2, 170.1, 169.9, 169.7, 169.4 (C=O), 138.3, 128.4, 127.9, 127.8 (Ar), 101.4 (C-1), 98.9 (C-1′), 98.5 (C-1″), 74.5 (C-3), 74.4 (C-4), 73.4 (CH_2Ph), 72.6 (C-5), 72.0 (C-5″), 71.8 (C-3″), 71.3 (C-2″), 70.7 (C-3′, C-5′), 69.8 (C-6), 68.2 (C-2′), 68.1 (C-4″), 66.9 (C-4′), 61.3 (C-6″), 60.4 (C-6′), 56.1 (OCH_3), 48.3 (C-2), 23.4, 23.0, 20.9, 20.8, 20.7, 20.6, 20.5 (COCH_3); HRESIMS calcd for $C_{44}H_{59}NO_{24}$ $[M + H]^+$ 986.3505, found 986.3513.

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Methyl 2-Acetamido-3-*O*-(2,3,4,6-tetra-*O*-acetyl-*β*-D-glucopyranosyl)-4-*O*-(2,3,4,6-tetra-*O*-acetyl-*β*-D-glucopyranosyl)-6-*O*-benzyl-2-deoxy-*β*-D-glucopyranoside (14). Alcohol **11** (16 mg, 0.029 mmol) was coupled to the donor **1** (71 mg, 0.145 mmol, 5.0 equiv) in CH_2Cl_2 (1.0 mL) according to the general protocol. Column chromatography (9:1 EtOAc:hexanes) of the residue gave the trisaccharide **14** (21 mg, 88%) as a colorless glass. $[\alpha]_D$ −62 (*c* 1.0, CH_2Cl_2); ^1H NMR (400 MHz, $CDCl_3$) δ 7.42−7.29 (m, 5H, Ar), 6.39 (d, 1H, *J* = 8.0 Hz, NH), 5.26−5.15 (m, 2H, H-3′, H-3″), 5.14−5.01 (m, 2H, H-4′, H-4″), 5.00−4.88 (m, 2H, H-2′, H-2″), 4.79 (d, 1H, *J* = 8.0 Hz, H-1″), 4.54 (s, 2H, CH_2Ph), 4.43 (d, 1H, *J* = 8.0 Hz, H-1′), 4.38−4.29 (m, 3H, H-1, H-6a′, H-6a″), 4.13 (dd, 1H, *J* = 12.5, 2.2 Hz, H-6b″), 4.08−3.92 (m, 4H, H-2, H-3, H-4, H-6b′), 3.82−3.64 (m, 4H, H-5, H-6a, H-6b, H-5′), 3.54−3.48 (m, 1H, H-5″), 3.35 (s, 3H, OCH_3), 2.05, 2.04, 2.03, 2.02, 1.99, 1.99, 1.98, 1.97 (8s, 27H, 9 × $COCH_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.0, 170.6, 170.4, 169.9 (C=O), 148.1, 138.6, 128.9, 128.4, 128.3 (Ar), 101.6 (C-1), 99.2 (C-1′, C-1″), 75.0 (C-5), 73.8 (C-3), 73.2 (CH_2Ph), 72.4 (C-4), 72.3, 71.7 (C-3′, C-3″), 71.6 (C-5′, C-5″), 70.1 (C-2′, C-2″), 68.6 (C-6), 68.5 (C-4′, C-4″), 61.7 (C-6′, C-6″), 56.5 (OCH_3), 50.1 (C-2), 23.5, 21.3, 21.1, 21.0 ($COCH_3$); HRESIMS calcd for $C_{44}H_{59}NO_{24}$ $[M + Na]^+$ 1008.3325, found 1008.3318.

Methyl 2-Acetamido-3-*O*-(2,3,4-tri-*O*-acetyl-*β*-L-fucopyranosyl)-4-*O*-(2,3,4,6-tetra-*O*-acetyl-*β*-D-glucopyranosyl)-6-*O*-benzyl-2-deoxy-*β*-D-glucopyranoside (15). Alcohol **12** (13 mg, 0.021 mmol) was coupled to the donor **1** (52 mg, 0.106 mmol, 5.0 equiv) in CH_2Cl_2 (0.8 mL) according to the general protocol. Column chromatography (9:1 EtOAc:hexanes) of the residue gave an inseparable mixture of the acceptor **12** and trisaccharide **15** (16 mg). The yields of **12** and **15** in the mixture were determined by NMR to be 24% and 63%, respectively. An analytical sample of trisaccharide **15** was obtained upon additional column chromatography (25:1 $CHCl_3$:MeOH). $[\alpha]_D$ −5 (*c* 0.4, CH_2Cl_2); ^1H NMR (400 MHz, $CDCl_3$) δ 7.44−7.30 (m, 5H, Ar), 5.98 (d, 1H, *J* = 8.1 Hz, NH), 5.29 (d, 1H, *J* = 3.4 Hz, H-4′), 5.19−4.99 (m, 4H, H-2′, H-3′, H-3″, H-4″), 4.93 (d, 1H, *J* = 8.2 Hz, H-2″), 4.82 (d, 1H, *J* = 7.8 Hz, H-1′), 4.71−4.50 (m, 4H, H-1, H-1″, CH_2Ph), 4.24 (dd, 1H, *J* = 12.4, 4.0 Hz, H-6a″), 4.14 (t, 1H, *J* = 6.8 Hz, H-3), 4.03−3.94 (m, 2H, H-4, H-6b″), 3.86 (q, 1H, *J* = 6.8 Hz, H-5′), 3.83−3.70 (m, 3H, H-2, H-6a, H-6b), 3.64−3.55 (m, 1H, H-5), 3.48 (s, 3H, OCH_3), 3.44−3.38 (m, 1H, H-5″), 2.18, 2.08, 2.04, 2.03, 1.98 (5s, 24H, 8 × $COCH_3$), 1.24 (d, 3H, *J* = 6.4 Hz, H-6′); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.0, 170.5, 170.4 (C=O), 129.0, 128.4, 128.3 (Ar), 102.1 (C-1), 100.6 (C-1′), 99.0 (C-1″), 76.8 (C-3), 75.3 (C-5), 75.0 (C-4), 73.9 (CH_2Ph), 72.9 (C-3″), 72.4 (C-5″, 72.0 (C-2″), 71.6 (C-3′), 70.8 (C-4′), 70.0 (C-2′), 69.8 (C-5′), 69.4 (C-6), 68.5 (C-4″), 61.8 (C-6″), 57.0 (OCH_3), 54.2 (C-2), 23.8, 21.4, 21.2, 21.1, 21.0 ($COCH_3$), 16.4 (C-6′); HRESIMS calcd for $C_{42}H_{57}NO_{22}$ $[M + H]^+$ 928.3424, found 928.3434.

Methyl 2-Acetamido-4-*O*-(2,3,4,6-tetra-*O*-acetyl-*β*-D-glucopyranosyl)-3-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-6-*O*-benzyl-2-deoxy-*β*-D-glucopyranoside (20). Alcohol **17** (22 mg, 0.034 mmol) was coupled to the donor **1** (83 mg, 0.167 mmol, 5.0 equiv) in CH_2Cl_2 (1.2 mL) according to the general protocol. Column chromatography (10:1 EtOAc:hexanes) of the residue gave a colorless glass (21 mg) containing the trisaccharide **20** contaminated with some degraded donor. The yield of trisaccharide **20** (18 mg, 55%) was calculated from the ^1H NMR spectrum while an analytical sample of trisaccharide **20** was obtained after additional column chromatography (35:1 $CHCl_3$:MeOH). $[\alpha]_D$ +5 (*c* 0.6, CH_2Cl_2); ^1H NMR (600 MHz, $CDCl_3$) δ 7.48−7.30 (m, 5H, Ar), 5.98 (d, 1H, *J* = 8.7 Hz, NH), 5.43 (s, 1H, H-2′), 5.33−5.21 (m, 2H, H-3′, H-4′), 5.12 (d, 1H, *J* = 1.4 Hz, H-1′), 5.09−4.96 (m, 2H, H-3″, H-4″), 4.89 (t, 1H, *J* = 8.1 Hz, H-2″), 4.75 (d, 1H, *J* = 11.9 Hz, CHHPh), 4.60−4.47 (m, 3H, H-1, CHHPh, H-1″), 4.34−4.13 (m, 3H, H-5′, H-6a′, H-6a″), 4.13−3.95 (m, 4H, H-3, H-4, H-6b′, H-6b″), 3.82−3.69 (m, 3H, H-2, H-6a, H-6b), 3.53−3.39 (m, 5H, H-5, OCH_3, H-5″), 2.16, 2.13, 2.07, 2.03, 2.01, 2.00, 1.99, 1.98, 1.97 (9s, 27H, 9 × $COCH_3$); ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.9, 170.6, 170.1, 170.0, 169.7, 169.3 (C=O), 137.9, 128.6, 128.0 (Ar), 102.5 (C-1), 101.1 (C-1′), 98.9 (C-1″), 77.0 (C-3), 74.7 (C-4), 74.1 (C-5), 73.7 (CH_2Ph), 72.7 (C-4″), 72.1 (C-5″), 71.3 (C-2″), 69.5 (C-2′), 68.7 (C-3′, C-5′, C-3″), 68.1 (C-6), 66.6 (C-4′), 62.8 (C-6″), 62.1 (C-6′), 56.7 (OCH_3), 54.1 (C-2), 23.5, 21.0, 20.8, 20.7, 20.6 ($COCH_3$); HRESIMS calcd for $C_{44}H_{59}NO_{24}$ $[M + H]^+$ 986.3505, found 986.3533.

Methyl 2-Acetamido-3-*O*-(2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl)-4-*O*-(2,3,4,6-tetra-*O*-acetyl-*β*-D-glucopyranosyl)- 6-*O*-benzyl-2-deoxy-*β*-D-glucopyranoside (21). Alcohol **18** (13 mg, 0.021 mmol) was coupled to the donor **1** (60 mg, 0.123 mmol, 5.0 equiv) in CH_2Cl_2 (0.8 mL) according to the general protocol. Column chromatography (10:1 EtOAc:hexanes) of the residue gave an inseparable mixture of the acceptor **18** and trisaccharide **21** (10 mg). The yields of **18** and **21** in the mixture were determined by NMR to be 34% and 26%, respectively. An analytical sample of **21** was obtained after additional column chromatography (8:2 CH_2Cl_2:acetone). $[\alpha]_D$ −59 (*c* 0.4, CH_2Cl_2); ^1H NMR (400 MHz, $CDCl_3$) δ 7.43−7.31 (m, 5H, Ar), 5.56 (d, 1H, *J* = 8.5 Hz, NH), 5.34 (d, 1H, *J* = 3.8 Hz, H-1′), 5.28 (dd, 1H, *J* = 10.8, 3.2 Hz, H-2′), 5.19 (d, 1H, *J* = 2.8 Hz, H-4′), 5.03−4.87 (m, 3H, H-3′, H-4″, H-3″), 4.78 (t, 1H *J* = 8.6 Hz, H-2″), 4.75−4.63 (m, 2H, CHHPh, H-5′), 4.61−4.38 (m, 4H, H-1, H-6a″, CHHPh, H-1″), 4.06−3.89 (m, 2H, H-3, H-4, H-6b″), 3.84−3.69 (m, 2H, H-6a, H-6b), 3.64 (q, 1H, *J* = 7.0 Hz, H-2), 3.49−3.39 (m, 1H, H-5), 3.36 (s, 3H, OCH_3), 3.31−3.21 (m, 1H, H-5″), 2.06, 2.01, 2.00, 1.99, 1.94, 1.93, 1.90, 1.89 (8s, 24H, 8 × $COCH_3$), 1.07 (d, 3H, *J* = 6.5 Hz, H-6′); ^{13}C NMR (150 MHz, $CDCl_3$) δ 171.1, 170.7, 170.5, 170.2, 169.5, 169.4, 169.1(C=O), 137.8, 128.7, 128.5, 128.4, 128.1, 128.0, 127.8 (Ar), 100.9 (C-1), 99.6 (C-1″), 95.1 (C-1′), 74.6 (C-3), 74.5 (C-5), 73.6 (CH_2Ph), 73.3 (C-5″), 72.7 (C-4), 72.2 (C-3′), 71.7 (C-4′), 71.1 (C-2″), 68.6 (C-4″), 68.0 (C-6), 67.7 (C-3″), 67.6 (C-2′), 64.5 (C-5′), 61.0 (C-6″), 56.6 (OCH_3), 56.3 (C-2), 23.4, 21.0, 20.7, 20.6 ($COCH_3$), 15.7 (C-6′); HRESIMS calcd for $C_{42}H_{57}NO_{22}$ $[M + Na]^+$ 950.3270, found 950.3266.

Methyl 2-Acetamido-4-*O*-(2,3,4,6-tetra-*O*-acetyl-*β*-D-glucopyranosyl)-3-*O*-(2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl)-6-*O*-benzyl-2-deoxy-*β*-D-glucopyranoside (22). Alcohol **19** (14 mg, 0.029 mmol) was coupled to the donor **1** (56 mg, 0.114 mmol, 5.0 equiv) in CH_2Cl_2 (0.8 mL) according to the general protocol. Column chromatography (9:1 EtOAc:hexanes) of the residue gave an inseparable mixture of the acceptor **19** and trisaccharide **22** (12 mg). The yields of **19** and **22** in the mixture were determined by NMR to be 43% and 27%, respectively. An analytical sample of **22** was obtained after additional column chromatography (8:2 CH_2Cl_2:acetone). $[\alpha]_D$ −9 (*c* 0.8, CH_2Cl_2); ^1H NMR (400 MHz, $CDCl_3$) δ 7.51−7.31 (m, 5H, Ar), 6.07 (d, 1H, *J* = 8.6 Hz, NH), 5.27−5.19 (m, 2H, H-2′, H-3′), 5.16−5.01 (m, 2H, H-4′, H-3″), 4.98 (s, 1H, H-1′), 4.95−4.77 (m, 4H, H-1, CHHPh, H-2″, H-4″), 4.61 (d, 1H, *J* = 8.2 Hz, H-1″), 4.53 (d, 1H, *J* = 11.9 Hz, CHHPh), 4.46−4.36 (m, 1H, H-5′), 4.32−4.10 (m, 3H, H-3, H-6a″, H-6b″), 3.96 (t, 1H, *J* = 7.6 Hz, H-4), 3.82 (d, 2H, *J* = 3.3 Hz, H-6a, H-6b), 3.61−3.54 (m, 1H, H-5), 3.53−3.40 (m, 5H, H-2, H-5″, OCH_3), 2.10, 2.08, 2.05, 2.00, 1.97, 1.96, 1.95, 1.94 (8s, 24H, 8 × $COCH_3$), 1.24 (d, 3H, *J* = 6.3 Hz, H-6′); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.8, 171.1, 170.7, 170.4, 170.2, 169.9, 169.5, 169.3 (C=O), 128.6, 128.1, 128.0 (Ar), 100.2 (C-1), 98.9 (C-1″), 96.3 (C-1′), 74.2 (C-5), 73.8 (C-4), 73.6 (CH_2Ph), 73.1 (C-3), 72.7 (C-3″), 71.8 (C-5″), 71.4 (C-4″), 71.0 (C-4′), 70.1 (C-3′), 69.3 (C-2″), 69.0 (C-2′), 68.2 (C-6), 66.3 (C-5′), 62.7 (C-6″), 56.8 (C-2), 56.8 (OCH_3), 23.4, 21.0, 20.8, 20.7, 20.6 ($COCH_3$), 17.1 (C-6′); HRESIMS calcd for $C_{42}H_{57}NO_{22}$ $[M + H]^+$ 928.3450, found 928.3492.

Experimental Methods for Analytical and Preparative HPLC during the Glucosylation of Disaccharide Acceptor 23. An aliquot (5 μL) of the reaction mixture was diluted in CH_3CN (100 μL) containing NEt_3 and 10 μL of this solution was injected in the HPLC. Analytical HPLC analysis was carried out on a Novapak C18 reverse phase column (3.9 × 150 mm) eluted with a gradient solvent system of CH_3CN:H_2O (65:35 to 90:10) at a 2 mL/min flow rate. Detection was carried out with a UV detector set at 220 nm. The trisaccharide **24**, unreacted acceptor **23**, and fucose **28** were separated by RP-HPLC on a C18 column (8 × 200 mm) eluted (2 mL/min) with the gradient solvent system mentioned above over 15 min.

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Supporting Information Available: The Results and Discussion and Experimental Section for the Synthesis of the Glycosyl Acceptors **4**, **5**, **11**, **12**, **17**, and **18**, as well as the General Experimental Methods and the Computational Methods are given, along with ^{1}H, COSY, and ^{13}C NMR data for all new compounds **4**, **5**, **7**−**9**, **11**−**15**, **17**, **18**, **20**−**22**, **31**, **32**, **35**, **36**, **38**, 40. This material is available free of charge via the Internet at http://pubs.acs.org.