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Application and limitations of the methyl imidate protection strategy of *N*-acetylglucosamine for glycosylations at O-4: synthesis of Lewis A and Lewis X trisaccharide analogues

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ABSTRACT

We describe here the synthesis of the allyl Le^a trisaccharide antigen as well as that of an analogue of the Le^x trisaccharide antigen, in which the galactose residue has been replaced by a glucose unit. Although successful fucosylations at O-4 of *N*-acetylglucosamine acceptors have been reported using perbenzylated thioethyl fucosyl donors under MeOTf activation, such conditions led in our case to the conversion of our acceptor to the corresponding alkyl imidates. Indeed, in this synthesis of the Le^a analogue, we demonstrate that the temporary protection of the *N*-acetyl group as a methyl imidate is advantageous to fucosylate at O-4. In contrast, we report here that glucosylation at O-4 of an *N*-acetylglucosamine monosaccharide acceptor using the α-trichloroacetimidate of peracetylated glucopyranose as a donor proceeded in better yields under activation with excess $BF_3{\cdot}OEt_2$ than that of the corresponding methyl imidate. Therefore, we conclude that activation of thioglycoside donors by MeOTf to glycosylate at O-4 of a glucosamine acceptor is best accomplished following the temporary protection of the *N*-acetyl group as a methyl imidate, especially when the donors are highly reactive and prone to degradation. In contrast, if donor and acceptor can withstand multiple equivalents of $BF_3{\cdot}OEt_2$, glycosylations at O-4 of a glucosamine acceptor with a trichloroacetimidate donor does not benefit from the temporary protection of the *N*-acetyl group as a methyl imidate.

1. Introduction

Our group is involved in the design of new anti-cancer vaccines based on the Tumor Associated Carbohydrate Antigens (TACAs) Le^{a}Le^x and dimeric Le^x (dimLe^x).[1,2] These tumor specific antigens consist of hexasaccharides that display either the Le^a or Le^x trisaccharide antigen linked to O-3″ of the galactose residue of another Le^x trisaccharide (Chart 1). Thus, employing these hexasaccharides to raise immune responses against cancer cells is also likely to trigger immune responses against these non-reducing end trisaccharide antigens and will eventually lead to the auto-immune destruction of the non-cancerous cells that display the natural Le^a or Le^x antigens.[3]

We have therefore embarked on the quest to discover analogues of these TACAs that would no longer display the Le^a or Le^x antigens at their reducing end but that would still be able to trigger the desired anti-Le^{a}Le^x or anti-dimLe^x immune responses.[1a,2a,2f] In that context, we have reported the synthesis of Le^a analogues in which either one or both the galactosyl and fucosyl residues have been replaced by glucose and rhamnose, respectively.[4] We describe here the synthesis of the allyl Le^a trisaccharide **1** that will be subsequently conjugated to carrier proteins and used in binding studies.[5] In addition, we have also reported the chemical synthesis of Le^x analogues[6] in which either one or both the *N*-acetylglucosaminyl and fucosyl residues have been replaced by glucose and rhamnose, respectively. We report here the synthesis of an additional Le^x analogue (**2**), in which the galactose residue is replaced by a glucose unit.

Although our syntheses of the Le^x analogues[6] have relied on the glycosylation of lactosyl[6a] or 2-azido lactosaminyl acceptors,[6b] and thus did not entail glycosylation at O-4 of *N*-acetylglucosamine, our work on the Le^a analogues involved either a rhamosylation or fucosylation at this position.[4] However, glycosylations at O-4 of *N*-acetylglucosamine are notoriously difficult to carry out successfully.[4,7,8] The difficulties encountered in such reactions have been attributed to steric hindrance,[7] hydrogen bond network formation involving the amide group and reducing the nucleophilicity at O-4,[8] or to the formation of unwanted stable glycosyl imidates.[4a,b,9] More recently we have reported[4b] that when thioethyl rhamnoside donors were activated with methyl triflate to promote glycosylation at O-4 of an *N*-acetylglucosamine acceptor, the formation of the acceptor methyl imidate could be observed prior or instead of its glycosylation. Turning this result to our advantage

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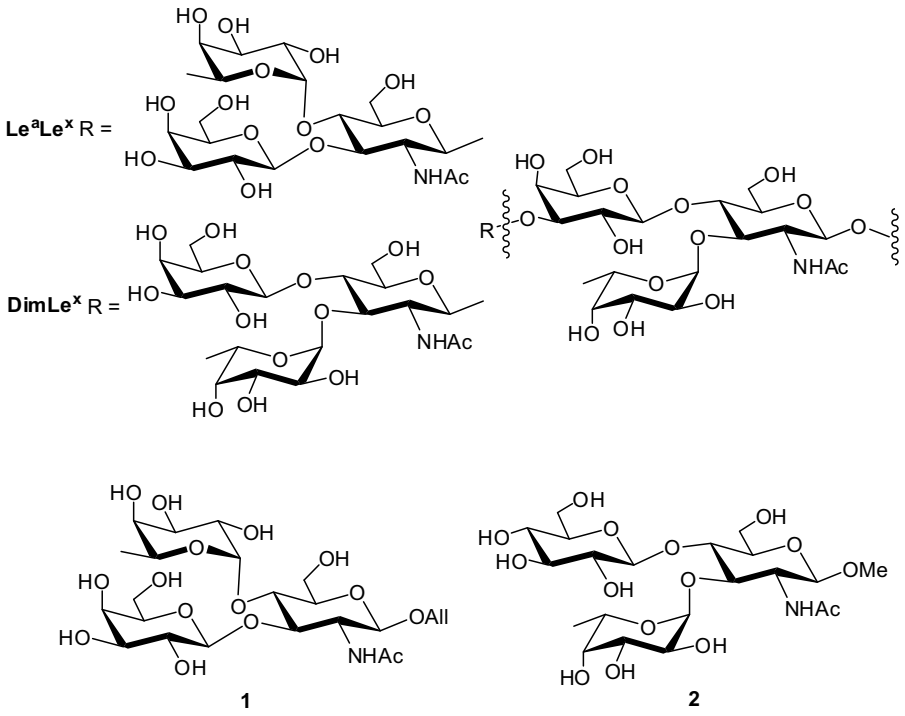


Chart 1.

we have developed[10] a new method for temporarily protecting the amide group of an *N*-acetylglucosamine acceptor immediately prior to its glycosylation at O-4. We showed that this temporary protecting group strategy was compatible with thioglycoside and trichloroacetimidate glycosyl donors as well as with NIS–TMSOTf (or TfOH), MeOTf, or TMSOTf activation conditions.[10] Although successful fucosylations at O-4 of *N*-acetylglucosamine acceptors have been reported,[4b,11] we demonstrate here that in some cases employing the temporary protection of the *N*-acetyl group as a methyl imidate is advantageous even when attempting to introduce a simple fucosyl unit at O-4. In contrast and while preparing analogue **2**, we describe here that glucosylation at O-4 of an *N*-acetylglucosamine acceptor can proceed in good yields if using an excess of $BF_3{\cdot}OEt_2$ as Lewis acid to activate a trichloroacetimidate glucosyl donor.

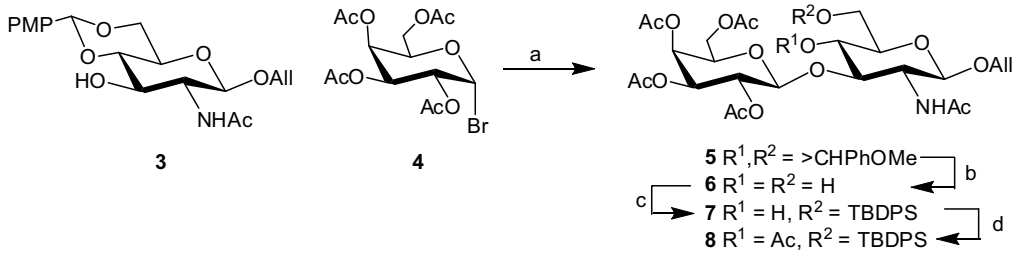
2. Results and discussion

Applying the synthetic strategy originally established by Lemieux et al.[11a,b] and that we have followed to prepare other Le^a analogues,[4] we decided to prepare the allyl Le^a trisaccharide **1** via the galactosylation at O-3 followed by the fucosylation at O-4 of a suitably protected *N*-acetylglucosamine allyl glycoside derivative. Thus, the known[12] *p*-methoxybenzylidene acceptor **3** was galactosylated with the peracetylated bromide[13] **4** under Helferich activation and gave disaccharide **5** in 82% yield (Scheme 1).

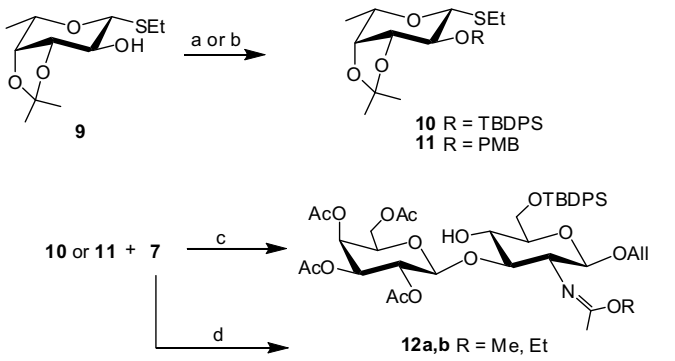
The *p*-methoxybenzylidene protecting group was hydrolyzed off using mild acidic conditions and the resulting diol (**6**) was submitted to selective silylation at O-6 using *t*-butyldiphenyl silyl chloride (TBDPSCl) and imidazole in acetonitrile to give the monosilylated disaccharide **7** in 67% yield. The regioselectivity of the silylation at O-6 of diol **6** was confirmed via the acetylation of the remaining free hydroxyl group in compound **7** to give disaccharide **8**. ^{1}H NMR spectroscopy of disaccharide **8** showed a signal for H-4 at 4.86 ppm, while the same signal in the alcohol **7** was found upfield at approximately 3.8 ppm, thus supporting that O-4 was free in disaccharide **7** because it became acetylated in disaccharide **8**. Having in hand the acceptor **7**, we prepared fucosyl donors **10** and **11** to attempt glycosylation at O-4 (Scheme 2).

Silylation of the known[14,15] thioglycoside **9** with TBDPSCl and imidazole gave the thiofucoside donor **10** while its *p*-methoxybenzylation with PMBCl and NaH following literature procedures gave the known[15] donor **11**.

It has been established[11a-c] in previous syntheses of Le^a analogues that fucosyl donors could successfully glycosylate at O-4 of the *N*-acetylglucosamine residue of a disaccharide such as



Scheme 1. Reagents and conditions: (a) 2 equiv **4**, 2 equiv $Hg(CN)_2$, toluene, CH_3NO_2, MS 4 Å, 50 °C, 3 h, 82%; (b) 50% aq AcOH, 60 °C, 1 h, 67%; (c) TBDPSCl 1 equiv, imidazole 6 equiv, CH_3CN, rt, 15 min, 67%; (d) Ac_2O, pyridine, DMAP, 50 °C, 18 h, 78%.



Scheme 2. Reagents and conditions: (a) TBDPSCl 2 equiv, imidazole 5 equiv, CH_3CN, rt, 18 h, **10** 64%; (b) Literature procedure[15] PMBCl, NaH, DMF, **11** 76%; (c) **10** or **11** (5 equiv), MeOTf up to 15 equiv, Et_2O, MS 4 Å, with **10** and **7**: **12a** 26%, **12b** 16%, with **11** and **7**: formation of **12a**, **12b** observed by TLC; (d) MeOTf 25 equiv 0.5 M, Et_2O, MS 4 Å, **12a**/**12b** 7:3 98%.

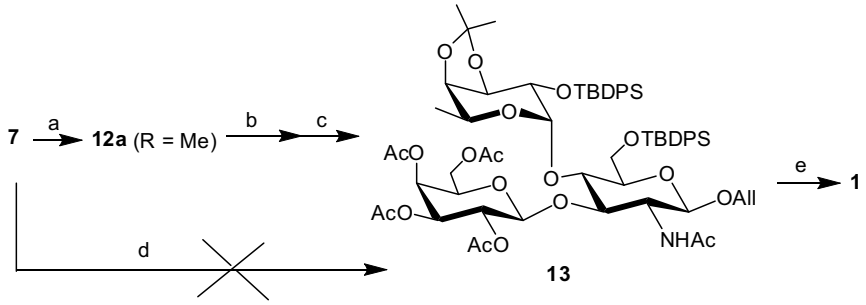
acceptor **7**. Thus, using the conditions that were successful in our previous synthesis of the methyl Le^a trisaccharide,[4b] we attempted the coupling of alcohol **7** with thioglycoside **10** under activation with 5 equiv of MeOTf (0.12 M) at room temperature in Et_2O (Scheme 2). In these conditions, the acceptor remained unreacted and it is only after adding up to 15 equiv (0.36 M) of MeOTf portionwise that we observed the disappearance of alcohol **7** and the formation of two less polar products by TLC. Although no trisaccharide was isolated, the two new products formed in this reaction were separated by chromatography and identified by NMR to be the methyl and ethyl imidates, **12a** and **12b** that were isolated in 26% and 16% yield, respectively.

We have already described the formation of a mixture of methyl and ethyl imidates when treating *N*-acetylglucosamine O-4 acceptors with 0.5 M MeOTf in diethyl ether[10] and their characteristic NMR signals have been well documented in our previous papers.[4b,10] Thus, **12a** gave, as expected, no N*H* signal in the ^{1}H NMR spectrum but an additional OCH_3 singlet around 3.65 ppm and a signal shifted up field from the acetates methyl group and corresponding to the acetimidate methyl group. In addition, ^{13}C NMR spectroscopy for methyl imidate **12a** gave signals at 164.8, 16.4, and 52.4 ppm, corresponding, respectively, to the *C*=N as well as *C*H$_3$ signals for the acetimido group and to the additional *O*-methyl group. NMR spectroscopy of compound **12b** also showed no N*H* signal but a quaternary *C*=N at 164.4 ppm as well as a methyl signal for an acetimido group at 16.1 and 1.90 ppm in the ^{1}H and ^{13}C NMR spectra, respectively. In addition, ^{13}C NMR spectroscopy for ethyl imidate **12b** showed signals corresponding to an *O*-ethyl group at 14.2 and 60.6 ppm and that correlated, respectively, in the HSQC, with a C*H*$_3$ triplet at 1.25 ppm and a multiplet at 4.03 ppm. Finally, ESI-HRMS confirmed the structure of the imidates giving *m*/*z* peaks at $[M+H]^+$ 844.3576 and 858.3402 for **12a** (calcd 844.3591) and **12b** (calcd 858.3732), respectively.

Although we have reported the formation of methyl and ethyl imidates when treating *N*-acetylglucosamine O-4 acceptors with MeOTf in Et_2O, we were surprised that no fucosylation took place during the attempted coupling of acceptor **7** and fucosyl donor **10**. We hypothesized that this glycosylation did not give the desired trisaccharide because of steric hindrance resulting from the presence of the two TBDPS groups on O-2 of fucose and O-6 of *N*-acetylglucosamine. However, glycosylation of acceptor **7** with the less sterically demanding 2-PMB fucosyl donor **11** had a similar outcome than that using the TBDPS donor **10**, that is, no reaction was observed when 5 equiv of MeOTf was added, and upon further addition of up to 15 equiv of MeOTf, TLC showed the formation of imidates **12a** and **12b**, which, in this case, were not isolated. Thus, we concluded that fucosylation of acceptor **7** with either thioglycoside donors **10** or **11** under MeOTf activation could not occur at the low MeOTf concentration usually needed to activate such reactive donors (0.1 M), and that extended reaction times with increased concentrations of MeOTf (up to 0.4 M) was leading to the formation of the alkyl imidates and concurrent degradation of the fucosyl donors. Indeed, treatment of acceptor **7** with MeOTf (0.5 M) in Et_2O according to the conditions that we have previously established[10] gave a 7:3 mixture of methyl and ethyl imidates **12a**,**b** in excellent 98% yield (Scheme 2). Given this excellent yield and the fact that further attempts at coupling donor **11** and O-4 acceptor **7** using other promoters (NIS/TfOH) also failed, we decided to investigate the reactivity of methyl imidate **12a** in fucosylation reactions.

Indeed, we have established[10] that such alkyl imidates are compatible with various donors and glycosylation methods for glycosylation at O-4 of *N*-acetylglucosamine acceptors. Although we have demonstrated previously that both methyl and ethyl imidates could be successfully glycosylated,[10] using mixtures such as **12a**,**b** as acceptors in glycosylation reactions leads to complex TLC, and we thus decided to prepare methyl imidate **12a** as a pure acceptor. Furthermore, because imidates are unstable and difficult to purify without degradation the intermediate trisaccharide imidate was not purified but directly treated with AcOH/Ac_2O to convert the acetimidate to the acetamide as we have described previously.[4b] Thus, treatment of acceptor **7** with 0.5 M MeOTf in CH_2Cl_2 gave the methyl imidate **12a**, which was isolated pure in 92% yield following silica gel chromatography using $CHCl_3$ and MeOH as the solvent system. Glycosylation of methyl imidate **12a** with fucosyl donor **10** under activation with 0.2 M MeOTf in Et_2O at room temperature for 24 h was followed by regeneration of acetamide (Ac_2O, AcOH, 55 °C) and gave the desired trisaccharide **13** in 67% yield over the two steps (Scheme 3).

It is important to point out that quenching the glycosylation reaction with NEt_3 had to be performed slowly and at 0 °C to avoid the unwanted degradation of the trisaccharide. Although we



Scheme 3. Reagents and conditions: (a) MeOTf 25 equiv 0.5 M, CH_2Cl_2, MS 4 Å, **12a** 92%; (b) **10** (5 equiv), MeOTf 10 equiv, 0.2 M, Et_2O, MS 4 Å; (c) Ac_2O, AcOH, 55 °C, 18 h, **13** 67% over two steps; (d) (1) MeOTf 25 equiv, 0.5 M, CH_2Cl_2, MS 4 Å, 18 h rt then in situ **10** (5 equiv) in Et_2O rt up to 24 h, (2) Ac_2O, AcOH, 55 °C, 18 h, **13** 8% over three steps (e) (1) TBAF 7 equiv, THF, (2) Ac_2O, pyridine, (3) 90% aq AcOH, 80 °C, (4) MeONa, MeOH, **1** 77% over four steps.

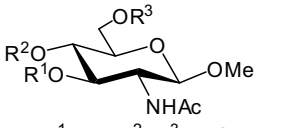
postulated earlier that the high concentration of MeOTf (0.4 M) used when attempting to couple the acetamido **7** and fucosyl donors **10** or **11** led to degradation of the donors prior to their reaction with the acceptors **7** or **12a**,**b**, coupling of the purified methyl imidate **12a** with donor **10** was successful using a fairly high (0.2 M) concentration of MeOTf in Et_2O. Thus, we wondered if addition of the donor after formation of the alkyl imidates **12a**,**b** in situ would allow the two steps: imidate formation and glycosylation, to be carried out successively in one pot. Thus, the acetamide acceptor **7** was converted to the imidates **12a**,**b** with 0.5 M MeOTf in Et_2O and when TLC showed that the starting material had been consumed, a solution of thioglycoside **10** (5 equiv) in Et_2O was added to the reaction mixture (Scheme 3). Unfortunately, TLC showed that the glycosylation of the alkyl imidates **12a**,**b** to the corresponding trisaccharide imidates did not proceed to completion and after an extended reaction time and following the conversion of the acetimide to the acetamide, trisaccharide **13** was only isolated in 8% yield. Thus, we concluded that in 0.5 M MeOTf, the donor **10** was too unstable and degraded faster than it reacted with the imidate acceptors thus not allowing this one pot strategy to be successful.

As we had observed for other protected Le[a] analogs,[16] the *N*-acetylglucosamine ring in trisaccharide **13** assumed a conformational equilibrium between the usual $^4C_1$ chair and a $^1S_5$ or $^3S_5$ skewed conformations in $CDCl_3$. This conformational behavior was characterized by a $J_{H\text{-}1,H\text{-}2}$ of 4.8 Hz in the ^1H NMR spectrum that was smaller than that usually observed for a $^4C_1$ conformation (~8.5 Hz). As expected based on our previous study,[16] ^1H NMR for trisaccharide **13** in CD_3CN gave a larger $J_{H\text{-}1,H\text{-}2}$ coupling constant (7.9 Hz) suggesting that this hydrogen bond-accepting solvent the *N*-acetylglucosamine ring assumed mostly the usual $^4C_1$ conformation.

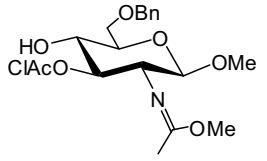
Trisaccharide **13** was, in turn, deprotected in four steps to give the desired allyl Le[a] trisaccharide **1** (Scheme 3). Because the fucosidic bond is known to be acid labile when it is not stabilized at O-2 by an electron-withdrawing substituent,[6a,6b,17] the silyl groups were first removed with TBAF, and the resulting diol was acetylated. Subsequent acid hydrolysis of the isopropylidene group followed by Zemplèn deacetylation gave the desired deprotected allyl Le[a] (**1**) in 77% yield over four steps and after Biogel P2 gel exclusion chromatography. The synthesis of the Le[a] analogue **1** that we have described above constitutes a good application of the imidate synthetic strategy that we have reported previsouly.[10] Indeed, it illustrates that the temporary conversion of *N*-acetyl groups to alkyl imidates when attempting to glycosylate at O-4 of *N*-acetylglucosamine is particularly useful when using a reactive thioglycoside donor such as fucoside **10** under mild activation with MeOTf.

In the work described below, we further studied the reactivity of an imidate versus that of the corresponding acetamido acceptor while preparing the Le[x] analogue **2**. As we have mentioned above, although fucosylation at O-4 of an *N*-acetylglucosamine acceptor is usually straightforward,[11] it is generally known that glycosylations using other donors are often difficult and result in poor yields.[7–9] However, there are a few successful reports of such glycosylation reactions involving mono- or disaccharide acceptors.[18] Thus, our synthetic approach to the Le[x] analogue **2** involved first the glucosylation at O-4 followed by the fucosylation at O-3 of a suitably protected glucosamine acceptor. Chloroacetylation (ClAcCl, pyridine, 0 °C) at O-3 of the known[19] benzylidene **14** gave the chloroacetate **15** (81%), which was reductively opened ($NaCNBH_3$, HCl, Et_2O) to give the acetamido acceptor **16** in 79% yield. The acetamido **16** was subsequently converted to the methyl imidate **17** in 77% yield using 0.5 M MeOTf in CH_2Cl_2 and both acceptors **16** and **17** were submitted to glucosylation at O-4 with either the α- or β-trichloro-

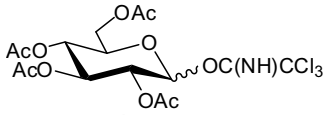
Table 1



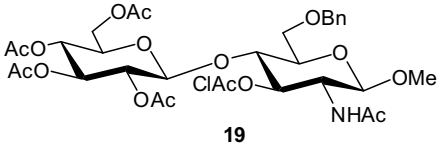
14 R^1 = H; R^2, R^3 = >CHPh
15 R^1 = ClAc; R^2, R^3 = >CHPh
16 R^1 = ClAc, R^2 = H, R^3 = Bn



17



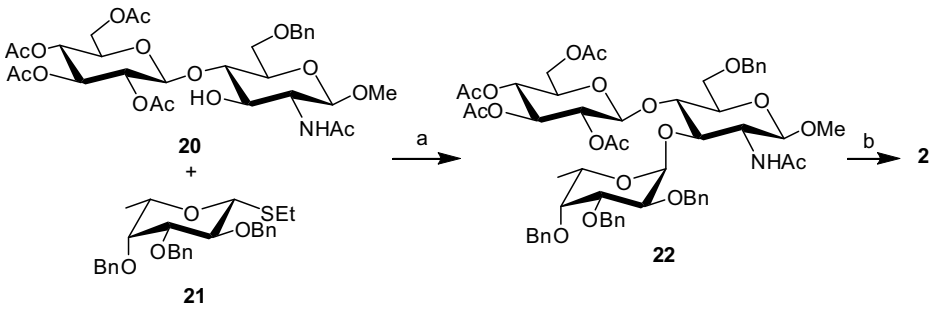
18α
18β



19

Entry	Acceptor	Donor (equiv)	Promoter (equiv)	Temp (°C)	Time	**19** (%)
1	**17**	**18**β (3)	TMSOTf (0.1–1.0)[a]	−78 to rt	18 h	—[b]
2	**17**	**18**β (4)	AgOTf (1.5)[a]	− 40	18 h	11[c]
3	**17**	**18**β (4)	$BF_3{\cdot}OEt_2$ (4–10)[d]	rt	1 h	15[c]
4	**17**	**18**β (5)	$BF_3{\cdot}OEt_2$ (5)[e]	40	5 min	27[c]
5	**17**	**18**α (5)	$BF_3{\cdot}OEt_2$ (4)[d]	40	24 h	50[c]
6	**17**	**18**α (10)	$BF_3{\cdot}OEt_2$ (4)[d]	40	24 h	75[c]
7	**17**	**18**α (5)	$BF_3{\cdot}OEt_2$ (2)[d]	40	24 h	55[c]
8	**16**	**18**α (5)	$BF_3{\cdot}OEt_2$ (2)[f]	40	10 min	88
9	**16**	**18**α (5)	$BF_3{\cdot}OEt_2$ (2)[g]	40	10 min	90
10	**16**	**18**α (5)	$BF_3{\cdot}OEt_2$ (2)[g]	rt	1 h	95
11	**16**	**18**α (1.5)	$BF_3{\cdot}OEt_2$ (2)[g]	40	10 min	71

[a] Reagents and conditions: (i) CH_2Cl_2, MS 4 Å; (ii) AcOH, Ac_2O, 55 °C.
[b] Traces observed by TLC.
[c] Isolated yield over two steps.
[d] Reagents and conditions: (i) CH_2Cl_2; (ii) AcOH, Ac_2O, 55 °C.
[e] Reagents and conditions: (i) CH_2Cl_2, MW 150 W, 200 psi; (ii) AcOH, Ac_2O, 55 °C.
[f] Reagents and conditions: CH_2Cl_2, MW 150 W, 200 psi.
[g] Reagents and conditions: CH_2Cl_2.



Scheme 4. Reagents and conditions: (a) **21** 2 equiv, $CuBr_2$ 2 equiv, Bu_4NBr 2 equiv, CH_2Cl_2, DMF, MS 4 Å, 14 h rt, 70%; (b) 1. MeONa, MeOH, 2. Pd/C, 100 psi H_2, MeOH **2** 83% over two steps.

acetimidate donors **18**α and **18**β (Table 1). To avoid unnecessary degradation of the intermediate disaccharide imidates, glucosylation of the methyl imidate **17** was always followed by the subsequent conversion of the methyl imidate to the acetamido group by treatment with AcOH and Ac_2O at 55 °C.

Entries 1 to 4 in Table 1 summarize the reaction conditions that were attempted for the glycosylation of monosaccharide imidate acceptor **17** with the β-trichloroacetimidate donor[20] **18**β. Applying conditions that we have found[10] to be successful for the glycosylation of a similar methyl imidate acceptor with a rhamnosyl trichloroacetimidate, we first attempted to couple imidate acceptor **17** and donor **18**β at low temperature under activation with 0.1 equiv of TMSOTf (Table 1, entry 1). Under these mild conditions, no reaction was apparent by TLC and thus the amount of promoter was increased to 1.0 equiv, and the temperature allowed to reach ambient temperature overnight. However, after conversion of the imidate to the acetamido, only a trace amount of disaccharide **19** was observed by TLC and the majority of the acceptor was recovered as the acetamido **16**. Using AgOTf (entry 2) as a promoter at −40 °C was moderately more successful as the desired disaccharide **19** was isolated in 11% yield.

Hypothesizing that low temperature reduced the reactivity of the acceptor toward glycosylation, we investigated the use of the milder Lewis acid ($BF_3{\cdot}OEt_2$) at higher temperatures (entries 3–7) to glucosylate imidate **17**. Although activation at rt required up to 10 equiv of $BF_3{\cdot}OEt_2$ to proceed, and only led to 15% yield of disaccharide **19**, activation under microwave irradiation at 40 °C for 5 min in the absence of molecular sieves gave an improved 27% yield of disaccharide **19**. Because these rather low yields were accompanied by fast degradation of the donor **18**β, we investigated the coupling of the more stable α-tricholoracetimidate[20] **18**α with imidate acceptor **17** (entries 5–7). Indeed, unlike the reactions with donor **18**β, TLC analysis of these reactions did not show fast degradation of donor **18**α and a marked yield increase was obtained when the glycosylation was allowed to proceed for 24 h at 40 °C in the presence of 4 equiv of $BF_3{\cdot}OEt_2$. In these conditions, the desired disaccharide **19** was isolated in 50% yield (entry 5); a result, which was further improved to 75% by using 10 equiv of the donor **18**α (entry 6).

We then investigated the coupling of donor **18**α with the acetamido **16** using conditions similar to those that we had just established for the coupling of **18**α with the imidate **17**. In fact, such coupling appeared to be much easier (entries 8–11) than that of **17** with **18**α and only required 2 equiv of $BF_3{\cdot}OEt_2$ to proceed. Indeed, whether the reaction was left to proceed at 40 °C for 10 min, under microwave irradiation or in a conventional oil bath (Table 1, entries 8 and 9), or whether it was left to proceed for 1 h at room temperature (entry 10) the disaccharide **19** was isolated in more than 88% yield when using 2 equiv of activator and 5 equiv of donor **18**α. Finally, as can be seen in Table 1, entry 11, even reducing to 1.5 the number of equiv of donor **18**α still led to an acceptable yield of the desired disaccharide **19** (71%).

The reactions reported in Table 1 clearly indicate that when using an excess of a mild Lewis acid such as $BF_3{\cdot}OEt_2$, the acetamido **16** is more reactive toward glycosylation at O-4 than the methyl imidate **17**. In fact, glucosylation of acceptor **17** using 2 equiv of $BF_3{\cdot}OEt_2$ only gave a 55% yield of disaccharide **19** after 24 h of reaction at 40 °C, although under the same conditions acceptor **16** gave disaccharide **19** in 90% yield after 10 min of reaction (Table 1, entries 7 and 9). These results are not very surprising because the methyl imidate substituent in acceptor **17** is more electron-withdrawing than the acetamido group in acceptor **16** and may therefore further decrease the nucleophilicity of O-4. However, it is interesting to notice that even though the coupling of acceptor **16** with trichloroacetimidate **18**α required relatively high temperatures to proceed, the *N*-acetyl group in acceptor **16** did not seem to impact negatively the glycosylation at O-4 as, for example, we did not observe the formation of glycosyl imidate. Therefore, we propose that the additional equivalent of Lewis acid ($BF_3{\cdot}OEt_2$) present in the reaction mixture effectively allowed glycosylation at O-4 by interacting with the *N*-acetyl group and reducing its nucleophilicity[4b] as well as its ability to form a hydrogen bond network that would impede glycosylation at O-4.[8]

The synthesis of analogue **2** was then easily completed (Scheme 4). The chloroacetate in disaccharide **19** was removed with DABCO in EtOH to give acceptor **20** in 71% yield and fucosylation of acceptor **20** with the known[21] donor **21** under activation with $CuBr_2$ and tetra-*n*-butylammonium bromide gave the protected trisaccharide **22** in 70% yield.

The deprotection of trisaccharide **22** was then accomplished in two steps: Zemplèn deacetylation followed by hydrogenolysis (H_2-Pd/C) gave the Le^x analogue **2** in 83% yield over the two steps.

3. Conclusions

The syntheses described above shed some additional insight on the value and limitation of the methyl imidate strategy that we have developed to assist in difficult glycosylations at O-4 of *N*-acetylglucosamine. Although fucosylations at this position are usually easy to achieve, our synthesis of the Le^a analogue **1** illustrate that it is not always the case. Indeed the glycosylation of disaccharide **7** with either thiofucosyl donors **10** or **11** was unsuccessful. Although the donors were activated with 0.2 M MeOTf they failed to react with the acetamido acceptor, which upon further addition of MeOTf was converted to a mixture of methyl and ethyl imidates. Thus, it appeared that the nucleophilicity of the 4-OH toward the activated fucosyl donors was insufficient to allow glycosylation while that of the *N*-acetyl group toward the MeOTf led to the formation of the alkyl imidates. In contrast, once the acetamido was converted to a methyl imidate the nucleophilicity of the 4-OH toward

the activated donors **10** led to acceptable yields of the desired trisaccharide. Therefore, we conclude that activation of thioglycoside donors by MeOTf to glycosylate at O-4 of a glucosamine acceptor is best accomplished following the temporary protection of the *N*-acetyl group as a methyl imidate, especially when the donors are highly reactive and prone to degradation.

The synthesis of trisaccharide **2** shed light on the relative reactivity of methyl imidate and acetamido glucosamine monosaccharide acceptors when coupled to a relatively stable trichloroacetimidate glycosyl donor such as **18**α under activation with an excess of $BF_3{\cdot}OEt_2$. Indeed, in these conditions, the acetamido acceptor proved to react faster and in better yields than the corresponding methyl imidate, even though such reactions required at least 2 equiv of promoter at room temperature to proceed. We propose that in these conditions 1 equiv of $BF_3{\cdot}OEt_2$ interacts noncovalently with the nucleophilic *N*-acetyl group and that the second one promotes glycosylation. Therefore, as long as donor and acceptor can 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 do not seem to benefit from the temporary protection of the *N*-acetyl group as a methyl imidate. In fact, the presence of a more electron withdrawing methyl imidate at C-2 of the acceptor further decreases its reactivity.

4. Experimental

4.1. General methods

^1H (600.13, 400.13 or 300.13 MHz) and ^{13}C NMR (150, 100 or 75 MHz) spectra were recorded at 300 K for solution in $CDCl_3$ (internal standard, for ^1H residual $CHCl_3$ δ 7.24; for ^{13}C $CDCl_3$ δ 77.0), DMF-d_7 (internal standard, for ^1H residue DMF δ 2.75; for ^{13}C DMF-d_7 δ 29.8), CD_3CN (internal standard, for ^1H residual CH_3CN δ 1.93) or D_2O [external standard 3-(trimethylsilyl)-propionic acid-d_4, sodium salt (TSP) for ^1H δ 0.00, for ^{13}C δ 0.00]. ^1H NMR and ^{13}C NMR chemical shifts are reported in parts per million (ppm). Coupling constants (*J*) are reported in Hertz (Hz). Chemical shifts and coupling constants were obtained from a first-order analysis of one-dimensional spectra. Assignments of proton and carbon resonances were based on two dimensional ^1H–^1H and ^{13}C –^1H correlation experiments. Multiplicities are abbreviated as follows: singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m), and broadened (b). Analytical thin-layer chromatography (TLC) was performed using Silica Gel 60 F254 precoated plates (250 μm) with a fluorescent indicator, visualized under UV and charred with 10% sulfuric acid in ethanol. Compounds were purified by flash chromatography with Silica Gel 60 (230–400 mesh) unless otherwise stated. Solvents were distilled and dried according to standard procedures,[22] and organic solutions were dried over Na_2SO_4 and concentrated below 40 °C, under reduced pressure. Molecular sieves were activated by heating at high temperature over P_2O_5 under vacuum. IR experiments were conducted using BOMEN MB-100 FT-IR. High-resolution electrospray ionization mass spectra (HR-ESI-MS) were recorded by the analytical services of the McMaster Regional Center for Mass Spectrometry, Hamilton, Ontario.

4.2. Allyl 2-acetamido-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-2-deoxy-4,6-*O*-*p*-methoxybenzylidene-β-D-glucopyranoside (5)

The known[12] acceptor **3** (549 mg, 1.43 mmol) was dissolved in a 1:1 mixture of toluene and nitromethane (10 mL). Activated powdered molecular sieves 4 Å (1.6 g), $Hg(CN)_2$ (722 mg, 2.86 mmol, 2 equiv) and the known[13] peracetylated galactosyl bromide **4** (1.17 g, 2.85 mmol, 2 equiv) were added and the reaction mixture was stirred under N_2 for 3 h at 50 °C. The reaction mixture was then filtered on Celite and the solids were washed with CH_2Cl_2 (200 mL). The combined filtrate and washing was washed sequentially with satd aq $NaHCO_3$ (200 mL) and brine (200 mL), and the aq phases were re-extracted with CH_2Cl_2 (100 mL). The organic solutions were combined, dried, and concentrated. Flash chromatography of the residue (8:1 EtOAc–hexanes) gave the pure disaccharide **5** as a colorless glass (0.831 g, 82%). ^1H NMR (400 MHz, $CDCl_3$): δ 7.36, 6.87 (2d, 2 × 2H, *J* = 8.7 Hz, Ar); 5.84 (m, 1H, –C*H*=CH_2); 5.74 (br d, 1H, *J* = 6.9 Hz, NH); 5.45 (s, 1H, C*H*Ph); 5.32–5.20 (m, 4H, H-1, H-4′, –CH=C*H*$_2$); 5.12 (dd, 1H, *J* = 8.0, 10.3 Hz, H-2′); 4.88 (dd, 1H, *J* = 3.4, 10.4 Hz, H-3′); 4.76 (d, 1H, *J* = 8.0 Hz, H-1′); 4.72 (t, 1H, *J* = 9.3 Hz, H-3); 4.33–4.25 (m, 2H, H-6a, OC*H*H allyl); 4.12–3.97 (m, 2H, H-6a′, OCH*H* allyl); 3.90 (dd, 1H, *J* = 5.9, 11.0 Hz, H-6b′); 3.80 (s, 3H, OCH_3); 3.75 (t, 1H, *J* = 10.2 Hz, H-6b); 3.62 (t, 1H, *J* = 8.8 Hz, H-4); 3.52 (m, 2H, H-5, H-5′); 3.01 (m, 1H, H-2); 2.09, 1.97, 1.96, 1.94, 1.93 (5s, 5 × 3H, 5 × CH_3CO). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.8, 170.7, 170.2, 170.1, 169.5 (C=O); 133.5 (–CH=); 127.4, 113.7 (Ar); 118.1 (=CH_2); 101.5 (*C*HPMP); 100.1 (C-1′); 98.4 (C-1); 80.8 (C-4); 76.5 (C-3); 71.0 (C-3′); 70.6 (OCH_2CH=); 69.3, 65.8 (C-5, C-5′); 68.7 (C-6); 66.8 (C-4′); 60.9 (C-6′); 58.5 (C-2); 55.2 (OCH_3); 23.7, 20.7, 20.6, 20.5 (*C*H_3CO). HRMS calcd for $C_{33}H_{43}NO_{16}$ $[M+H]^+$ 710.2660. Found: 710.2659.

4.3. Allyl 2-acetamido-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-2-deoxy-β-D-glucopyranoside (6)

The benzylidene **5** (590 mg, 0.832 mmol) was dissolved in 90% aq AcOH (50 mL) and the solution was stirred for 1 h at 60 °C. The mixture was then co-concentrated with toluene (3 × 250 mL), and chromatography (20:1 $CHCl_3$–MeOH) of the resulting residue gave pure diol **6** (331 mg, 67%) as a colorless glass. ^1H NMR (400 MHz, $CDCl_3$): δ 6.29 (d, 1H, *J* = 8.2 Hz, NH); 5.87 (m, 1H, –CH=); 5.35 (br d, 1H, *J* = 3.2 Hz, H-4′); 5.32–5.17 (m, 3H, H-2′, –CH=C*H*$_2$); 5.03 (dd, 1H, *J* = 3.4, 6.5 Hz, H-3′); 4.94 (d, 1H, *J* = 8.3 Hz, H-1); 4.59 (d, 1H, *J* = 8.1 Hz, H-1′); 4.41 (dd, 1H, *J* = 8.1, 10.0 Hz, H-3); 4.34 (dd, 1H, *J* = 10.1, 15.6 Hz, OC*H*H allyl); 4.18-3.96 (m, 3H, OCH*H* allyl, H-6a, H-6b); 3.91 (dd, 1H, *J* = 3.3, 12.1 Hz, H-6a′); 3.80 (dd, 1H, *J* = 4.0, 11.8 Hz, H-6b′); 3.51 (t, 1H, *J* = 9.3 Hz, H-5); 3.45–3.39 (m, 1H, H-5′); 3.11 (dd, 1H, *J* = 8.2, 14.7 Hz, H-2); 2.17, 2.10, 2.07, 2.00, 1.99 (5s, 5 × 3H, 5 × CH_3CO). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.9, 170.5, 170.0, 169.2 (C=O); 133.7 (–*C*H=CH_2); 117.9 (–CH=*C*H$_2$); 101.3 (C-1′); 98.5 (C-1); 83.2 (C-3); 75.2, 70.0 (C-5, C-5′); 71.0 (C-4); 70.7 (C-3); 70.3 (OCH_2CH=); 68.8 (C-2′); 66.9 (C-4′); 62.6 (C-6′); 61.5 (C-6); 57.3 (C-2); 23.6, 20.7, 20.6, 20.5 (*C*H_3CO). HRMS calcd for $C_{25}H_{37}NO_{15}$ $[M+H]^+$ 592.2241. Found: 592.2253.

4.4. Allyl 2-acetamido-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-6-*O*-*tert*-butyldiphenylsilyl-2-deoxy-β-D-glucopyranoside (7)

Diol **6** (1.02 g, 1.69 mmol) and imidazole (702 mg, 10.3 mmol, 6.1 equiv) were dissolved in CH_3CN (20 mL). *t*-Butyldiphenylsilyl chloride (TBDPSCl, 441 μL, 1.75 mmol, 1.0 equiv) was added into the solution and the reaction mixture was stirred for 15 min at rt. The reaction mixture was then concentrated and the residue was dissolved in CH_2Cl_2 (250 mL) and washed with brine (50 mL). The aq phase was re-extracted with CH_2Cl_2 (250 mL) and the combined organic solutions were dried and concentrated. Flash chromatography (3:2 EtOAc–hexanes) of the residue afforded the pure silyl ether **7** (950 mg, 67%) as a colorless glass. The regioselectivity of the silylation was confirmed through the acetylation

of an analytical sample of alcohol **7** as described in Section 4.4.1. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.72–7.64 (m, 4H, Ar); 7.43–7.32 (m, 6H, Ar); 5.89 (m, 1H, –CH=); 5.62 (d, 1H, J = 6.0 Hz, NH); 5.36 (br d, 1H, J = 2.7 Hz, H-4′); 5.29–5.14 (m, 4H, H-1, H-2′, =CH_2); 4.97 (t, 1H, J = 8.8 Hz, H-3′); 4.55 (d, 1H, J = 8.1 Hz, H-1); 4.41 (t, 1H, J = 6.7 Hz, H-3); 4.32 (dd, 1H, J = 3.5, 11.3 Hz, OC*H*H allyl); 4.13–3.95 (m, 3H, H-6a, H-6b, OCH*H* allyl); 3.90–3.72 (m, 3H, H-6a′, H-6b′, H-4); 3.51–3.38 (m, 2H, H-5, H-5′); 3.04 (dd, 1H, J = 4.8, 7.9 Hz, H-2); 2.13, 2.06, 1.99, 1.96, 1.96 (5s, 3H, 5 CH_3CO); 1.03 (s, 9H, (CH_3)$_3$C). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.7, 170.4, 170.1, 170.0, 169.1 (C=O); 135.7, 135.6, 129.5, 127.6 (Ar); 133.9 (–CH=); 117.8 (=CH_2); 101.3 (C-1′); 97.8 (C-1); 83.6 (C-3); 76.2, 69.5 (C-5, C-5′); 71.0 (C-4); 70.8 (C-3′); 69.7 (OCH_2CH=); 68.9 (C-2′); 66.9 (C-4′); 63.6 (C-6′); 61.5 (C-6); 57.8 (C-2); 26.7 ((CH_3)$_3$C); 23.8, 20.8, 20.6, 20.5 (CH_3CO); 19.3 ((CH_3)$_3$*C*). IR (NaCl): 1753 cm^{-1} (4 × C=O acetates), 1655 cm^{-1} (C=O acetamide). HRMS calcd for $C_{41}H_{55}NO_{15}Si$ [M+H]$^+$: 830.3419. Found: 830.3402.

4.4.1. Acetylation of an analytical sample of alcohol 7

An analytical sample of alcohol **7** (20 mg, 24 μmol) was dissolved in Ac_2O (1 mL) and pyridine (1 mL), and DMAP (5 mg, 41 μmol, 1.7 equiv) was added. The reaction solution was stirred at 50 °C for 18 h, diluted in CH_2Cl_2 (30 mL), and washed with 2 M HCl (2 × 30 mL) and satd aq $NaHCO_3$ (2 × 30 mL). The organic layer was dried and concentrated, and flash chromatography (3:2 EtOAc–hexanes) of the residue gave the pure acetylated disaccharide **8** (16 mg, 78% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.67–7.62 (m, 4H, Ar); 7.43–7.33 (m, 6H, Ar); 5.89 (m, 1H, –CH=); 5.65 (d, 1H, J = 7.4 Hz, NH); 5.32 (d, 1H, J = 2.7 Hz, H-4′); 5.25 (dd, 1H, J = 1.5, 17.2 Hz, CH=C*H*H); 5.18 (dd, 1H, J = 1.2, 10.3 Hz, CH=CH*H*); 5.05 (dd, 1H, J = 7.9, 10.4 Hz, H-2′); 4.97 (d, 1H, J = 8.0 Hz, H-1); 4.93 (dd, 1H, J = 3.4, 7.7 Hz, H-3′); 4.86 (t, 1H, J = 9.2 Hz, H-4); 4.53 (d, 1H, J = 7.9 Hz, H-1′); 4.49 (t, 1H, J = 9.1 Hz, H-3); 4.29 (dd, 1H, J = 5.3, 12.7 Hz, OC*H*H allyl); 4.12–4.01 (m, 3H, H-6a′, H-6b′, OCH*H* allyl); 3.84 (t, 1H, J = 6.3 Hz, H-5′); 3.76–3.64 (m, 2H, H-6a, H-6b); 3.55 (m, 1H, H-5); 3.17 (m, 1H, H-2); 2.11, 2.04, 2.03, 2.00, 1.94, 1.88 (6s, 6 × 3H, 6 CH_3CO); 1.02 (s, 9H, (CH_3)$_3$C). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.7, 170.2, 170.2, 169.2, 168.9 (C=O); 135.6, 135.6, 129.6, 127.6 (Ar); 133.7 (CH=); 133.3 (Ar); 117.9 (=CH_2); 100.6 (C-1′); 97.8 (C-1); 77.1 (C-3); 74.8 (C-5); 71.1 (C-3′); 70.5 (C-5); 69.8 (OCH_2C=); 69.3 (C-4, C-2′); 66.9 (C-4); 63.2 (C-6); 61.1 (C-6′); 58.2 (C-2); 26.7 ((CH_3)$_3$*C*); 23.7, 20.8, 20.8 20.6, 20.6, 20.5 (CH_3CO); 19.2 ((CH_3)$_3$*C*).

4.5. Ethyl 2-*O*-*tert*-butyldiphenylsilyl-3,4-*O*-isopropylidene-β-L-thiofucopyranoside (10)

The known[14] alcohol **9** (1.05 g, 4.2 mmol) and imidazole (1.48 g, 21.8 mmol, 5.2 equiv) were dissolved in CH_3CN (35 mL). TBDPSCl (2.16 mL, 8.4 mmol, 2.0 equiv) was added to the solution and the reaction mixture was stirred for 18 h at rt. The solution was diluted with $CHCl_3$ (150 mL), and washed with brine (150 mL) and 1 M aqueous NaOH (150 mL). The organic layer was dried and concentrated, and flash chromatography of the residue (3:100 EtOAc–hexanes) gave the pure silyl ether **10** (1.31 g, 64%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.78–7.65 (m, 4H, Ar); 7.44–7.30 (m, 6H, Ar); 4.44 (d, 1H, J = 8.0 Hz, H-1); 4.17 (t, 1H, J = 5.9 Hz, H-3); 4.02 (dd, 1H, J = 1.9, 6.0 Hz, H-4); 3.91–3.82 (m, 1H, H-5); 3.72 (dd, 1H, J = 5.7, 8.0 Hz, H-2); 2.59–2.33 (m, 2H, SCH_2CH_3); 1.31 (d, 3H, J = 6.3 Hz, H-6); 1.22, 1.13 (2s, 2 × 3H, (CH_3)$_2$C); 1.07 (s, 9H, (CH_3)$_3$C). ^{13}C NMR (75 MHz, $CDCl_3$): δ 136.4, 136.4, 129.6, 129.4, 127.4, 127.2 (Ar); 109.5 (Ar quaternary); 85.3 (C-1); 79.2 (C-3); 76.0 (C-4); 73.7 (C-2); 71.6 (C-5); 27.2, 26.5 ((CH_3)$_3$C and (CH_3)$_2$C); 24.6 (SCH_2CH_3); 19.7 ((CH_3)$_3$*C*); 16.8 (SCH_2CH_3). 13.5 (C-6); HRMS calcd for $C_{27}H_{38}O_4SSi$ [M+Na]$^+$ 509.2158. Found: 509.2140.

4.6. Allyl 3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-6-*O*-*tert*-butyldiphenylsilyl-2-deoxy-2-methylacetimido-β-D-glucopyranoside (12a) and allyl 3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-6-*O*-*tert*-butyldiphenylsilyl-2-deoxy-2-ethylacetimido-β-D-glucopyranoside (12b)

4.6.1. Glycosylation of acceptor 7 with donor 10

A mixture of acceptor **7** (45 mg, 0.054 mmol), thioglycoside **10** (122 mg, 0.250 mmol, 4.6 equiv), and activated powdered 4 Å molecular sieves (150 mg) in Et_2O (2 mL) was stirred for 1 h at rt under N_2. MeOTf (29 μL, 0.256 mmol, 4.6 equiv) was added and the reaction mixture was stirred at rt for 5 h. Up to 15 equiv of MeOTf was added to the reaction mixture over the following 24 h while the reaction was kept at rt under N_2. When TLC (7:3 EtOAc–hexanes) showed no remaining starting acceptor, the reaction mixture was quenched with Et_3N (147 μL). The reaction mixture was filtered, the solids were washed with CH_2Cl_2, and the combined organic solutions were washed with aq satd $NaHCO_3$ and brine. The aq phases were re-extracted with CH_2Cl_2 and the combined organic layers were dried and concentrated. Flash chromatography (3:7 EtOAc–hexanes) of the residue gave first the pure ethyl imidate **12b** as a colorless glass (7.3 mg, 16%) then the pure methyl imidate **12a** also as a colorless glass (12.1 mg, 26%).

4.6.2. Reaction of acceptor 7 with MeOTf in Et_2O gave a mixture 12a,b

Acceptor **7** (25 mg, 0.030 mmol) was dissolved in Et_2O (1.5 mL); activated molecular sieves 4 Å (150 mg) were added and the mixture was stirred under N_2 at rt for 1 h. MeOTf (85 μL, 0.75 mmol, 25 equiv, 0.5 M) was then added and the reaction mixture was stirred at rt under N_2 for 18 h. The reaction mixture was quenched slowly with Et_3N (106 μL, 0.76 mmol, 25 equiv), the solids were filtered off and washed with CH_2Cl_2 (40 mL). The organic solution was washed with satd aq $NaHCO_3$ (40 mL), dried, and concentrated. Chromatography (50:1 $CHCl_3$–MeOH) gave 7:3 mixture of the methyl and ethyl imidates **12a** and **12b**, respectively (25 mg, 98%).

4.6.3. Reaction of acceptor 7 with MeOTf in CH_2Cl_2 gave methyl imidate 12a

The acceptor **7** (100 mg, 0.12 mmol) was dissolved in CH_2Cl_2 (6 mL) containing activated molecular sieves 4 Å (340 mg) and after stirring under N_2 at rt for 1 h, MeOTf (341 μL, 3.0 mmol, 25 equiv, 0.5 M) was added. The reaction was allowed to proceed at rt under N_2 for 18 h and worked up as described above in Section 4.6.2. Chromatography (50:1 $CHCl_3$–MeOH) gave the pure methyl imidate **12a** (95 mg, 92%) as a colorless glass.

4.6.4. Analytical data for methyl imidate 12a

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.73–7.68 (m, 4H, Ar); 7.41–7.29 (m, 6H, Ar); 5.86 (m, 1H, –CH=); 5.35 (br d, 1H, J = 3.0 Hz, H-4′); 5.29–5.12 (m, 3H, H-2, =CH_2); 4.95 (dd, 1H, J = 3.7, 10.4 Hz, H-3′); 4.57 (d, 1H, J = 8.0 Hz, H-1′); 4.39–4.27 (m, 2H, H-1, OC*H*H allyl); 4.14–3.85 (m, 6H, H-6a, H-6b, H-5′, H-6a′, H-6b′, OCH*H* allyl); 3.65–3.50 (m, 5H, H-3, H-4, OCH_3); 3.42 (m, 1H, H-5); 3.30 (t, 1H, J = 8.2 Hz; H-2); 2.14, 2.00, 1.98, 1.97 (4s, 4 × 3H, 4 × CH_3CO); 1.91 (s, 3H, CH_3CN imidate); 1.06 (s, 9H, (CH_3)$_3$C). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.4, 170.1, 170.1, 169.4 (C=O); 164.8 (C=N); 135.7, 135.6, 129.5, 127.6 (Ar); 134.2 (–*C*H=CH_2); 133.8 (Ar quaternary); 116.7 (–CH=*C*H_2); 102.0 (C-1′); 101.7 (C-1); 89.4 (C-3); 77.3 (C-5); 71.1 (C-3′); 70.8 (C-5′); 69.5 (OCH_2 allyl); 68.7 (C-4); 68.4 (C-2′); 66.8 (C-4′); 63.8 (C-6′); 63.7 (C-2); 61.5 (C-6); 52.4 (OCH_3); 26.8 ((CH_3)$_3$*C*); 20.6, 20.5, 20.3, 20.2 (CH_3CO); 19.3 ((CH_3)$_3$*C*); 16.4 (CH_3CN). IR (NaCl): 1754 cm^{-1} (C=O), 1686 cm^{-1} (C=N). HRMS calcd for $C_{42}H_{57}NO_{15}Si$ [M+H]$^+$ 844.3576. Found: 844.3591.

4.6.5. Analytical data for ethyl imidate 12b

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.73–7.68 (m, 4H, Ar); 7.41–7.29 (m, 6H, Ar); 5.86 (m, 1H, –CH=); 5.35 (br d, 1H, J = 3.0 Hz, H-4′); 5.29–5.12 (m, 3H, H-2, =CH_2); 4.95 (dd, 1H, J = 3.7, 10.4 Hz, H-3′); 4.53 (d, 1H, J = 8.0 Hz, H-1′); 4.39–4.27 (m, 2H, H-1, OC*H*H allyl); 4.14–3.85 (m, 8H, H-6a, H-6b, H-5′, H-6a′, H-6b′, OCH*H* allyl, O*CH*$_2$CH$_3$ imidate); 3.60–3.50 (m, 2H, H-3, H-4); 3.42 (m, 1H, H-5); 3.30 (t, 1H, J = 8.2 Hz; H-2); 2.14, 2.00, 1.98, 1.97 (4s, 4 × 3H, 4 × CH_3CO); 1.90 (s, 3H, CH_3CN imidate); 1.25 (t, 3H, J = 7.1 Hz, OCH$_2$*CH*$_3$ imidate); 1.06 (s, 9H, $(CH_3)_3$C). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.4, 170.1, 170.1, 169.4 (C=O); 164.4 (C=N); 135.7, 135.6, 129.5, 127.6 (Ar); 134.2 (–*CH*=CH$_2$); 133.8 (Ar quaternary); 116.7 (–CH=*CH*$_2$); 102.1 (C-1′); 101.7 (C-1); 89.4 (C-3); 77.3 (C-5); 71.1 (C-3′); 70.8 (C-5′); 69.5 (OCH$_2$ allyl); 68.7 (C-4); 68.4 (C-2′); 66.8 (C-4′); 63.8 (C-6′); 63.7 (C-2); 61.5 (C-6); 60.6 (OCH$_2$ imidate); 26.8 ((*CH*$_3$)$_3$C); 20.6, 20.5, 20.3, 20.2 (*CH*$_3$CO); 19.3 ((CH$_3$)$_3$*C*); 16.1 (*CH*$_3$CO); 14.2 (*CH*$_3$CH$_2$ imidate). HRMS calcd for $C_{43}H_{59}NO_{15}Si$ $[M+H]^+$ 858.3732. Found: 858.3714.

4.7. Allyl 2-acetamido-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-6-*O*-*tert*-butyldiphenylsilyl-4-*O*-(2-*O*-*tert*-butyldiphenylsilyl-3,4-*O*-isopropylidene-α-L-fucopyranosyl)-2-deoxy-β-D-glucopyranoside (13)

A solution of the acceptor **12a** (23 mg, 0.027 mmol) and thioglycoside **10** (68 mg, 0.140 mmol, 5.2 equiv) in anhyd Et_2O (1.4 mL) containing activated molecular sieves 4 Å (100 mg) was stirred under N_2 for 1 h at rt. MeOTf (32 μL, 0.28 mmol, 10 equiv, 0.2 M) was added to the reaction mixture that was then stirred for 24 h at rt under N_2. The reaction mixture was cooled to 0 °C, diluted with anhyd Et_2O (10 mL), and the reaction was quenched by adding Et_3N (40 μL) slowly to the mixture. It was then filtered, solids were washed with CH_2Cl_2, and the combined filtrate and washings were concentrated to dryness. The residue was dissolved in Ac_2O (2.0 mL) and AcOH (0.7 mL) and the solution was stirred at 55 °C for 18 h. The mixture was co-concentrated with toluene (3 × 20 mL), and the residue was dissolved in CH_2Cl_2 (40 mL), and washed with satd aq $NaHCO_3$. The aq phases were extracted with CH_2Cl_2 (40 mL), and the combined organic solutions were dried and concentrated. Flash chromatography (3:2 EtOAc–hexanes) of the residue gave pure trisaccharide **13** as a colorless glass (23 mg, 67%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.73–7.57 (m, 8 H, Ar); 7.41–7.26 (m, 12H, Ar); 6.46 (d, 1H, J = 9.4 Hz, NH); 5.67 (m, 1H, –CH=); 5.33 (br d, 1H, J = 3.2 Hz, H-4′); 5.24–4.99 (m, 4H, H-2′, H-3′, =CH_2); 4.72 (d, 1H, J = 8.0 Hz, H-1′); 4.68 (d, 1H, J = 2.9 Hz, H-1); 4.65 (d, 1H, J = 3.3 Hz, H-1″); 4.31–4.00 (m, 9H, H-2, H-3, H-4, H-6a, H-6b, H-6a′, H-3″, H-4″, OC*H*H allyl); 3.95–3.65 (m, 6H, H-5, H-5′, H-6b′, H-2″, H-5″, OCH*H* allyl); 2.01 (s, 3H, CH_3CO); 2.00 (s, 9H, 3 × CH_3CO); 1.98 (s, 3H, CH_3CO); 1.26–1.21 (m, 9H, H-6, $(CH_3)_2$C); 1.06, 1.01 (2s, 2 × 9H, 2 $(CH_3)_3$C). ^{1}H NMR (400 MHz, CD_3CN): δ 7.78–7.23 (m, 20H, Ar); 6.47 (d, 1H, J = 9.5 Hz, NH); 5.87 (m, 1H, –CH=); 5.39 (br d, 1H, J = 3.1 Hz, H-4′); 5.28–5.02 (m, 4H, H-2′, H-3′, = CH_2); 5.01 (br d, 1H, J = 3.6 Hz, H-1″); 4.93 (m, 2H, H-1′, H-5″); 4.45 (d, 1H, J = 7.9 Hz, H-1); 4.38–3.69 (m, 13H, H-2, H-3, H-4, H-6a, H-6b, H-5′, H-6a′, H-6b′, H-2″, H-3″, H-4″, OC*H*$_2$ allyl); 3.44 (m, 1H, H-5); 2.09, 2.07, 2.01, 1.98, 1.93 (5s, 5 × 3H, 5 × CH_3CO); 1.29 (d, 3H, J = 6.6 Hz, H-6″); 1.20 (s, 3H, one of $(CH_3)_2$C); 1.02, 1.01 (2s, 2 × 9H, 2 of $(CH_3)_3$C); 0.93 (s, 3H, one of $(CH_3)_2$C). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.2, 170.1, 170.0, 169.4, 169.2 (C=O); 136.4, 135.9, 135.6, 129.8, 120.5, 127.6, 127.5 (Ar); 133.9 (–CH=); 133.8 (Ar quaternary); 117.1 (=CH_2); 100.7 (C-1′); 97.2 (C-1); 93.8 (C-1″); 76.3, 67.9, 67.3 (C-3, C-4, C-3″); 75.4 (OCH$_2$ allyl); 72.0, 71.2 (C-5, C-5′); 70.5 (C-3′); 69.9 (C-2″); 67.9 (C-4, C-5″); 67.6 (C-2′); 66.3 (C-4′); 62.7 (C-6′), 60.0 (C-6); 48.9 (C-2); 27.1, 26.9 ((*CH*$_3$)$_3$C); 26.3 ((*CH*$_3$)$_2$C); 23.7, 20.8, 20.6 (*CH*$_3$CO); 19.3, 19.2 ((CH$_3$)$_3$*C*); 16.0 (C-6″). HRMS calcd for $C_{66}H_{87}NO_{19}Si_2$ $[M+H]^+$ 1254.5489. Found: 1254.5430.

4.8. Allyl 2-acetamido-2-deoxy-3-*O*-(α-L-fucopyranosyl)-4-*O*-(β-D-galactopyranosyl)-β-D-glucopyranoside (1)

Trisaccharide **13** (29 mg, 0.023 mmol) was dissolved in THF (3 mL) and a 1 M solution of TBAF in THF (169 μL, 0.169 mmol, 7.3 equiv) was added. The reaction mixture was stirred at rt for 1.5 h and concentrated. Ac_2O (1 mL) and pyridine (1 mL) were added to the dry residue and acetylation was allowed to proceed for 2.5 h. The mixture was co-concentrated with toluene (3 × 10 mL), and the residue was submitted to flash chromatography (8:2 EtOAc–hexanes). The fractions containing the diol were pooled, concentrated, and the residue was dissolved in 90% aq AcOH (2 mL). The solution was stirred for 0.5 h at rt, the temperature was raised to 80 °C in 1 h and stirring at 80 °C was continued for 6 h. The solution was co-concentrated with toluene (2 × 10 mL), and the residue was dissolved in anhyd MeOH (2 mL). A 1 M solution of NaOMe in MeOH (40 μL) was added to the solution and the reaction mixture was stirred for 18 h at rt. The solution was deionized with DOWEX® H^+ resin, the resin was filtered and rinsed with MeOH (10 mL). The pooled filtrate and washings were concentrated, and the dry residue was purified by gel permeation chromatography on a Biogel P2 (100 × 1 cm) column eluted with water. The pure allyl Le^a **1** (10.3 mg, 77%) was obtained as a white amorphous powder after freeze-drying. $[\alpha]_D$ −55.6 (*c* 0.5, H_2O). ^{1}H NMR (300 MHz, D_2O): δ 5.80 (m, 1H, CH=); 5.18 (m, 2H, =CH_2); 4.92 (d, 1H, J = 3.7 Hz, H-1″); 4.75 (m, 1H, H-5″); 4.46 (d, 1H, J = 8.4 Hz, H-1); 4.38 (d, 1H, J = 7.6 Hz, H-1′); 4.23 (m, 1H, OC*H*H allyl); 4.05 (m, 1H, OCH*H* allyl); 4.00–3.3 (m, 15H, H-2, H-3, H-4, H-5, H-6a, H-6b, H-2′, H-3′, H-4′, H-5′, H-6a′, H-6b′, H-2″, H-3″, H-4″); 1.92 (s, 3H, CH_3CO); 1.07 (d, 3H, J = 6.5 Hz, H-6″). ^{13}C NMR (75 MHz, D_2O): δ 174.5 (C=O); 133.3 (–CH=); 118.2 (=CH_2); 102.9 (C-1′); 99.5 (C-1); 98.1 (C-1″); 76.1, 75.4, 74.8, 72.4, 72.3, 71.9, 70.5, 69.1, 68.4, 67.8 (C-3, C-4, C-5, C-2′, C-3′, C-4′, C-5′, C-2″, C-3″, C-4″); 70.5 (CH_2 allyl); 66.8 (C-5″); 61.6, 59.7 (C-6, C-6′); 55.7 (C-2′); 22.3 (*CH*$_3$CO); 15.4 (C-6″). HRMS calcd for $C_{23}H_{39}NO_{15}$ $[M+H]^+$ 570.2398. Found: 570.2401.

4.9. Methyl 2-acetamido-4,6-*O*-benzylidene-3-*O*-chloroacetyl-2-deoxy-β-D-glucopyranoside (15)

Pyridine (2.8 mL, 34 mmol, 15 equiv) was added to a solution of the known[19] alcohol **14** (750 mg, 2.31 mmol) in anhyd CH_2Cl_2 (83 mL), and the mixture was cooled down to 0 °C under N_2. Chloroacetyl chloride (430 μL, 5.4 mmol, 2.3 equiv) was then slowly added to the mixture over 15 min. The reaction mixture was allowed to warm up to rt over 2 h and was stirred for 18 h at rt. The solution was co-concentrated with toluene (3 × 15 mL) and the solid obtained was recrystallized from hot ethanol (50 mL). The mixture was filtered and the solid was washed with cold EtOH (10 mL). The pure benzylidene acetal **15** was obtained as a white powder (745 mg, 81%). Mp = 257–259 °C, $[\alpha]_D$ −3.80 (*c* 1.0, DMF). ^{1}H NMR (600 MHz, DMF-d_7): δ 8.07 (d, 1H, J = 9.1 Hz, NH); 7.47–7.39 (m, 5H, Ar); 5.73 (s, 1H, C*H*Ph); 5.36 (dd, 1H, J = 9.8, 9.9 Hz, H-3); 4.75 (d, 1H, J = 8.4 Hz, H-1); 4.45–4.31 (m, 3H, $ClCH_2$–, H-6a); 3.98 (dd, 1H, J = 9.1, 9.6 Hz, H-2); 3.92–3.85 (m, 2H, H-4, H-6b); 3.64 (m, 1H, H-5); 3.45 (s, 3H, OCH_3); 1.86 (s, 3H, $COCH_3$). ^{13}C NMR (150 MHz, DMF-d_7): δ 171.2, 168.3 (C=O); 139.3 (Ar, quat); 130.1, 129.3, 127.5 (Ar); 103.6 (C-1); 102.1 (*CH*Ph); 79.7 (C-4); 75.2 (C-3); 69.0 (C-6); 67.2 (C-5); 57.2 (OCH_3); 55.4 (C-2); 42.1 ($ClCH_2$–); 23.3 ($COCH_3$). HRMS calcd for $C_{18}H_{22}ClNO_7$ $[M+H]^+$ 400.1163. Found: 400.1165.

4.10. Methyl 2-acetamido-6-*O*-benzyl-3-*O*-chloroacetyl-2-deoxy-β-D-glucopyranoside (16)

A solution of benzylidene acetal **15** (80 mg, 0.163 mmol), $NaCNBH_3$ (128 mg, 2.03 mmol, 12.5 equiv), and methyl orange indicator (2 mg) in anhyd THF (3.6 mL) containing activated MS 3 Å (150 mg) was cooled to 0 °C under N_2 and stirred for 0.5 h. M HCl in Et_2O (~1.6 mL) was then added to the reaction mixture at 0 °C until the methyl red indicator turned pink, remained as such for 10 min, and H_2 (g) was no longer generated. The reaction mixture was then stirred for 1 h at room temperature and filtered over Celite®. The solids were washed with THF (3 × 5 mL), and the pooled filtrate and washings were concentrated. Flash chromatography (25:1 $CHCl_3$–MeOH) of the residue gave pure alcohol **16** (69 mg, 79%) as a colorless glass. $[\alpha]_D$ −42.1 (*c* 1.0, $CHCl_3$). ^1H NMR (600 MHz, $CDCl_3$): δ 7.35–7.29 (m, 5H, Ar); 5.69 (d, 1H, *J* = 9.2 Hz, NH); 5.14 (dd, 1H, *J* = 10.6, 9.2 Hz, H-3); 4.60 (d, 1H, *J* = 11.9 Hz, C*H*-Ph); 4.55 (d, 1H, *J* = 11.9 Hz, C*H*–Ph); 4.45 (d, 1H, *J* = 8.3, H-1); 4.10 (m, 2H, $ClCH_2CO$–); 3.94–3.90 (m, 1H, H-2); 3.81–3.73 (m, 3H, H-4, H-6a, H-6b); 3.46 (s, 3H, OCH_3); 1.91 (s, 3H, $COCH_3$). ^{13}C NMR (150 MHz, $CDCl_3$): δ 170.5, 168.3 (C=O); 137.4 (Ar quat); 128.6, 128.0, 127.8 (Ar); 101.63 (C-1); 77.3 (C-3); 73.8 (*C*H_2Ph); 73.6 (C-3); 70.9 (C-4); 70.3 (C-6); 56.7 (OCH_3); 53.9 (C-2); 40.9 ($ClCH_2CO$–); 23.4 ($COCH_3$). HRMS calcd for $C_{18}H_{24}ClNO_7$ $[M+H]^+$ 402.1320. Found: 402.1323.

4.11. Methyl 6-*O*-benzyl-3-*O*-chloroacetyl-2-deoxy-2-methylacetimido-β-D-glucopyranoside (17)

A mixture of the acetamido **16** (450 mg, 1.12 mmol), CH_2Cl_2 (53 mL), and activated powdered MS 4 Å (2.65 g) was stirred at rt for 1 h. MeOTf (3.2 mL, 28 mmol, 25 equiv) was added and the mixture was stirred at rt for 3 h. The reaction mixture was cooled to 0 °C and was quenched slowly with Et_3N (4.6 mL, 34 mmol, 30 equiv). The reaction mixture was then filtered over Celite® and the solids were washed with CH_2Cl_2 (2 × 25 mL). The filtrate and washings were combined and washed with satd aq $NaHCO_3$ (80 mL). The aq layer was re-extracted with CH_2Cl_2 (20 mL), and the combined organic phases were dried and concentrated. Column chromatography (30:1 $CHCl_3$–MeOH) of the residue gave pure methyl imidate **17** (350 mg, 77%) as a colorless glass. $[\alpha]_D$ −14.1 (*c* 1.0, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ 7.32–7.24 (m, 5H, Ar); 5.14 (dd, 1H, *J* = 9.4, 9.5 Hz, H-3); 4.59 (q, 2H, *J* = 10.9 Hz, C*H*$_2$Ph); 4.34 (d, 1H, *J* = 7.48 Hz, H-1); 4.00 (d, 2H, *J* = 2.5 Hz, $ClCH_2CO$–); 3.83–3.73 (m, 3H, H-6a, H-6b, H-4); 3.61–3.53 (m, 4H, =$COCH_3$, H-5); 3.44 (s, 3H, OCH_3); 3.31 (dd, 1H, *J* = 9.7, 7.7 Hz, H-2); 2.91 (br s, 1H, OH); 1.88 (s, 3H, $COCH_3$). ^{13}C NMR (100 MHz, $CDCl_3$): δ 167.3 (C=O); 147.7 (C=N); 137.5 (Ar quat); 128.5, 127.9, 127.8 (Ar); 103.9 (C-1); 79.5 (C-3); 73.8 (*C*H_2Ph); 73.7 (C-5); 70.9 (C-4); 70.5 (C-6); 62.9 (C-2); 57.3, 54.1 (OCH_3); 40.7 ($ClCH_2$–); 15.7 (OCH_3 imidate). HRMS calcd for $C_{19}H_{26}ClNO_7$ $[M+H]^+$ 416.1476. Found: 416.1502.

4.12. Methyl 2-acetamido-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-6-*O*-benzyl-3-*O*-chloroacetyl-2-deoxy-β-D-glucopyranoside (19)

4.12.1. Glycosylation of the methyl imidate 17

$BF_3{\cdot}OEt_2$ (73 μL, 0.58 mmol, 4.0 equiv) was added to a solution of methyl imidate **17** (60 mg, 0.144 mmol) and known[20] glucosyl donor **18**α (711 mg, 1.44 mmol, 10 equiv) in CH_2Cl_2 (4 mL) stirred at 40 °C. The reaction mixture was stirred for 18 h at 40 °C and quenched by addition of Et_3N (93 μL, 0.64 mmol, 4.5 equiv). A mixture of Ac_2O and AcOH (1:1, 3 mL) was added and heated for 18 h at 55 °C. The reaction mixture was diluted with CH_2Cl_2 (20 mL), washed with satd aq $NaHCO_3$ (20 mL), and the aqueous layer was re-extracted with CH_2Cl_2 (20 mL). The combined organic layers were dried and concentrated, and the residue was submitted to flash chromatography (8:2 EtOAc–hexanes) yielding the pure disaccharide **19** (80 mg, 75%) that was obtained as a colorless glass.

4.12.2. Glycosylation of the acetamido 16

$BF_3{\cdot}OEt_2$ (13 μL, 0.080 mmol, 2.0 equiv) was added to a solution of the acceptor **16** (20 mg, 0.049 mmol) and the glucosyl donor **18**α (122 mg, 0.248 mmol, 5.0 equiv) in CH_2Cl_2 (2.5 mL) that was stirred under N_2 at rt. The reaction mixture was stirred for 1 h at rt under N_2 and quenched with Et_3N (15 μL, 0.11 mmol, 2.3 equiv). The mixture was diluted with CH_2Cl_2 (10 mL) and washed with satd aq $NaHCO_3$ (10 mL). The aqueous layer was re-extracted with CH_2Cl_2 (2 × 10 mL), and the combined organic layers were dried and concentrated. Purification, as described above Section 4.12.1, gave the pure disaccharide **19** (35 mg, 95%) as a colorless glass.

4.12.3. Analytical data for disaccharide 19

$[\alpha]_D$ −7.0 (*c* 1.0, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ 7.45–7.24 (m, 5H, Ar); 5.64 (d, 1H, *J* = 9.3 Hz, NH); 5.09 (dd, 1H, *J* = 10.4, 9.1 Hz, H-3′); 5.97 (m, 2H, H-4′, H-3′); 4.81–4.74 (m, 2H, H-2′, PhC*H*); 4.49–4.36 (m, 4H, PhC*H*, H-6a, H-1, H-1′); 4.19–3.89 (m, 5H, $ClCH_2CO$-, H-6b, H-2, H-4); 3.72 (d, 2H, *J* = 2.31 Hz, H-6a′, H-6b′); 3.50–3.38 (m, 4H, OCH_3, H-5); 3.34 (m, 1H, H-5′); 2.08, 1.98, 1.95, 1.93, 1.91 (4s, 4 × 3H, 4 × CH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.6, 170.1, 169.3, 169.1, 168.3 (C=O); 138.0 (Ar quat); 128.7, 128.2, 128.1 (Ar); 101.8 (C-1′); 100.1 (C-1); 74.7 (C-4); 74.5 (C-3, C-5); 73.7 (*C*H_2Ph); 72.9 (C-3′); 71.8 (C-5′); 71.4 (C-2′); 67.4 (C-4′); 67.1 (C-6); 61.0 (C-6′); 56.7 (OCH_3); 53.6 (C-2); 40.7 ($ClCH_2CO$-); 23.3 ($NCOCH_3$); 20.6, 20.5 ($OCOCH_3$). HRMS calcd for $C_{33}H_{44}ClNO_{16}$ $[M+H]^+$ 732.2319. Found: 732.2270.

4.13. Methyl 2-acetamido-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-6-*O*-benzyl-2-deoxy-β-D-glucopyranoside (20)

A mixture of disaccharide **19** (30 mg, 0.04 mmol) and DABCO (114 mg, 1.02 mmol, 26 equiv) in EtOH (2.6 mL) was stirred for 18 h at 55 °C. The reaction mixture was diluted with MeOH (10 mL) and deionized with Dowex H^+ resin. The resin was filtered off, washed with MeOH (5 mL), and the combined filtrate and washing was concentrated. Chromatography (50:1 $CHCl_3$–MeOH) of the residue gave pure acceptor **20** (18 mg, 71%) as a colorless glass. $[\alpha]_D$ −3.9 (*c* 1.0, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ 7.43–7.29 (m, 5H, Ar); 5.67 (d, 1H, *J* = 8.0 Hz, NH); 5.10 (dd, 1H, *J* = 9.5, 9.6 Hz, H-3′); 4.99 (dd, 1H, *J* = 9.9, 9.5 Hz, H-4′); 4.93 (dd, 1H, *J* = 9.6, 8.1 Hz, H-2′); 4.70 (d, 1H, *J* = 12.1 Hz, C*H*Ph); 4.63 (d, 1H, *J* = 8.2 Hz, H-1); 4.49 (d, 1H, *J* = 9.1 Hz, H-1′); 4.48 (d, 1H, *J* = 12.0 Hz, C*H*Ph); 4.14 (d, 2H, *J* = 3.3 Hz, H-6a′, H-6b′); 3.92 (dd, 1H, *J* = 9.7, 8.6 Hz, H-3); 3.72–3.59 (m, 4H, H-4, H-5′, H-6a, H-6b); 3.51–3.39 (m, 5H, OCH_3, H-2, H-5); 2.08, 2.05, 2.01, 1.97, 1.94 (5s, 5 × 3H, 5 × $COCH_3$). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.6, 170.1, 169.3, 169.1 (C=O); 137.9 (Ar quat); 128.5, 127.9, 127.8 (Ar); 101.1 (C-1); 100.7 (C-1′); 80.9 (C-4); 73.9 (C-5); 73.6 (*C*H_2Ph); 72.6 (C-3′); 71.9 (C-5′); 71.5 (C-3); 71.1 (C-2′); 68.1 (C-4′); 67.9 (C-6); 61.6 (C-6′); 56.7 (OCH_3); 56.7 (C-2); 23.7 ($NCOCH_3$); 20.5 ($OCOCH_3$). HRMS calcd for $C_{30}H_{41}NO_{15}$ $[M+H]^+$ 678.2402. Found: 678.2374.

4.14. Methyl 2-acetamido-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-6-*O*-benzyl-3-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-2-deoxy-β-D-glucopyranoside (22)

Disaccharide acceptor **20** (68 mg, 0.104 mmol) and known[21] fucosyl donor **21** (99 mg, 0.208 mmol, 2.0 equiv) were dissolved in a mixture of anhyd CH_2Cl_2 (1.5 mL) and anhyd DMF (1.5 mL)

containing activated powdered MS 4 Å (150 mg), and the mixture was stirred for 1 h at rt. Cu(II)Br_2 (46 mg, 0.208 mmol, 2.0 equiv) and *n*-Bu_4NBr (64 mg, 0.218 mmol, 2.1 equiv) were then added to the reaction mixture and stirring was continued for 14 h at rt. The reaction mixture was filtered over Celite® and the solids were washed with CH_2Cl_2 (40 mL). The combined filtrate and washings were washed with brine (35 mL) and satd aq $NaHCO_3$ (6 × 30 mL). The aq phases were re-extracted with CH_2Cl_2 (40 mL), and the combined organic solutions were dried and concentrated. Flash chromatography (3:2 EtOAc–hexanes) of the residue gave pure trisaccharide **22** (77 mg, 70%) as a colorless glass. $[\alpha]_D$ −33.3 (*c* 1.0, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ 7.36–7.26 (m, 20H, Ar); 6.05 (d, 1H, *J* = 7.7 Hz, NH); 5.15 (d, 1H, *J* = 3.7 Hz, H-1′); 5.09 (dd, 1H, *J* = 9.6, 9.5 Hz, H-3″); 4.99–4.88 (m, 3H, H-2″, H-4″, C*H*HPh); 4.84 (d, 1 H, *J* = 11.7 Hz, C*H*HPh); 4.80–4.70 (m, 2H, 2 C*H*HPh); 4.69–4.61 (m, 5H, H-1, H-1″, 4 × C*H*HPh); 4.44 (d, 1H, *J* = 12.0 Hz, C*H*HPh); 4.33 (dd, 1H, *J* = 12.4, 4.3 Hz, H-6a″); 4.18 (q, 1H, *J* = 6.5 Hz, H-5′); 4.12 (dd, 1H, *J* = 10.1, 3.7 Hz, H-2′); 4.05 (dd, 1H, *J* = 6.4, 6.3 Hz, H-3); 3.97–3.84 (m, 4H, H-4, H-6a, H-3′, H-6b″); 3.79 (dd, 1H, *J* = 10.3, 4.0 Hz, H-6b); 3.73–3.63 (m, 2H, H-2, H-4′); 3.59 (m, 1H, H-5); 3.40–3.33 (m, 4H, H-5″, OCH_3); 2.02, 2.01, 2.00, 1.93 (4s, 4 × 3H, 4 × $OCOCH_3$); 1.80 ($NCOCH_3$); 1.16 (d, 3 H, *J* = 6.5, H-6′). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.9. 170.6, 169.9 (C═O); 139.2, 139.0, 138.5 (Ar quat); 129.0, 128.9, 128.8, 128.6, 128.3, 128.1, 127.9, 127.6 (Ar); 101.3 (C-1″); 99.6 (C-1); 97.1 (C-1′); 79.9 (C-3″); 78.1 (C-4′); 77.5 (C-2′); 75.1 (*C*H_2Ph, C-2′); 74.7 (C-4);74.6 (C-5); 73.8 (*C*H_2Ph, C-6); 73.7 (C-3); 73.6 (*C*H_2Ph, C-3′); 73.1 (*C*H_2Ph, C-4′); 72.9 (C-3″); 72.3 (C-5″); 71.6 (C-2″); 69.3 (C-6); 68.5 (C-4″); 67.2 (C-5′); 62.1 (C-6″); 57.0 (OCH_3); 53.2 (C-2); 23.6 ($NCOCH_3$); 21.1, 21.0 ($OCOCH_3$); 17.1 (C-6′). HRMS calcd for $C_{57}H_{69}NO_{19}$ $[M+H]^+$ 1072.4581. Found: 1072.4542.

4.15. Methyl 2-acetamido-2-deoxy-3-*O*-(α-L-fucopyranosyl)-4-*O*-(β-D-glucopyranosyl)-β-D-glucopyranoside (2)

Na (11 mg, 0.5 mmol) was reacted with MeOH (4 mL) at 0 °C. The protected trisaccharide **22** (85 mg, 0.079 mmol) was dissolved in 2 mL of anhyd MeOH and added to the solution of NaOMe in MeOH. The reaction mixture was stirred at rt for 1 h, diluted with MeOH (5 mL) and deionized with Dowex H^+ resin. The resin was filtered off and washed with MeOH (5 mL). The combined filtrate and washing was concentrated to dryness, and MeOH (7 mL) and 10% Pd/C (250 mg) were added to the residue. The mixture was stirred under H_2 (100 psi) for 24 h and diluted with MeOH (10 mL). Solids were filtered off, washed with MeOH (5 mL), and the combined filtrate and washings were concentrated. The residue was purified by gel permeation on a Biogel P2 column eluted with water and gave pure trisaccharide **2** (35 mg, 83%), which was obtained as a white amorphous powder after freeze-drying. $[\alpha]_D$ +35.5 (*c* 0.8, H_2O). ^1H NMR (600 MHz, D_2O): δ 5.16 (d, 1H, *J* = 4.0 Hz, H-1′); 4.87 (q, 1H, *J* = 6.6 Hz, H-5′); 4.56 (d, 1H, *J* = 7.9 Hz, H-1″); 4.52 (d, 1H, *J* = 7.9 Hz, H-1); 4.05 (dd, 1H, *J* = 12.3, 2.1 Hz, H-6a); 4.00–3.88 (m, 5H, H-2, H-4, H-3, H-3′, H-6b, H-6a″); 3.81 (d, 1H, *J* = 2.3 Hz, H-4′); 3.75 (dd, 1H, *J* = 10.4, 4.0 Hz, H-2′); 3.76–3.58 (m, 2H, H-5, H-6b″); 3.58–3.52 (m, 4H, H-3″, OCH_3); 3.49–3.55 (m, 1H, H-5″); 3.28–3.22 (m, 2H, H-2″, H-4″); 2.07 (s, 3H, $COCH_3$); 1.22 (d, 3H, *J* = 6.6, H-6′). ^{13}C NMR (150 MHz, D_2O): δ 174.6 (C═O); 101.9 (C-1); 101.5 (C-1″); 98.9 (C-1′); 76.2 (C-5″); 75.7 (C-3″); 75.5 (C-5); 75.4 (C-3); 74.0 (C-4); 73.8 (C-2″); 72.1 (C-4′); 70.6 (C-4″); 69.4 (C-3′); 67.9 (C-2′); 66.6 (C-5′); 61.8 (C-6″); 59.9 (C-6); 57.2 (OCH_3); 55.8 (C-2); 22.4 ($COCH_3$); 15.5 (C-6′). HRESIMS calcd for $C_{21}H_{37}NO_{15}$ $[M+H]^+$ 544.2264. Found: 544.2241.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.carres.2008.08.025.

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