Convenient Temporary Methyl Imidate Protection of *N*-Acetylglucosamine and Glycosylation at O-4

Anderson Cheng,[†] Jenifer L. Hendel,[†] Kimberley Colangelo, Michael Bonin, and France-Isabelle Auzanneau*

Department of Chemistry, University of Guelph, Guelph, Ontario, N1G 2W1, Canada

fauzanne@uoguelph.ca

Received May 22, 2008

This paper expands on the scope and utility of the temporary conversion of *N*-acetyl groups to alkyl imidates when attempting to glycosylate at O-4 of *N*-acetylglucosamine acceptors. The optimized synthesis of alkyl imidate protected glucosamine acceptors at position 4 and carrying various protecting groups at O-3 is described. These imidates were prepared immediately prior to glycosylation by treating the 4-OH acceptors with 0.5 M MeOTf to obtain the corresponding methyl imidates still carrying a free 4-OH group. When preparing these imidates in diethyl ether as the reaction solvent, we observed the unexpected formation of ethyl imidates in addition to the desired methyl imidates. While the 3-*O*-allyl acceptors were too unstable to be useful in glycosylation reactions, the 3-*O*-acylated methyl and ethyl imidates of glucosamine were shown to behave well during the glycosylation of the 4-OH with a variety of reaction conditions and various glycosyl donors. Glycosylation of these acceptors was successfully carried out with perbenzylated β-thioethyl rhamnopyranoside under MeOTf promotion, while activation of this donor under NIS/TMSOTf or NIS/TfOH proved less successful. In contrast, activation of the less reactive perbenzylated α-thioethyl and peracetylated β-thioethyl rhamnopyranosides with NIS/TfOH led to successful glycosylations of the 4-OH. Activation of a peracetylated rhamnosyl trichloroacetimidate by TMSOTf at low temperature also gave a high yield of glycosylation. We also report one-pot glycosylation reactions via alkyl imidate protected acceptor intermediates. In all cases the alkyl imidate products were readily converted to their corresponding *N*-acetyl derivatives under mild conditions.

Introduction

A large number of naturally occurring oligosaccharides and polysaccharides, including various tumor-associated carbohydrate antigens, contain *N*-acetylglucosamine residues glycosylated at O-4.[1] Since these compounds are biologically important, it is imperative that there be efficient methods for their chemical preparation. It has been established that the hydroxyl group at the C-4 position of the *N*-acetylglucosamine residue is poorly nucleophilic.[2] Although fucosylation at this position has been reported in high yields when using highly reactive fucosyl donors,[3] glycosylation at this position with less reactive glycosyl donors often leads to poor yields. The low reactivity of the 4-OH has generally been attributed to a combination of steric hindrance[2] and hydrogen bonding involving the *N*-acetylglucosamine amide group.[4] Recently, we demonstrated that the acetamide could act as a competitive nucleophile and react with glycosyl donors to form a glycosyl imidate side product.[5] All of these factors may account for the observed difficulties in

[†] A. Cheng and J. L. Hendel contributed equally to this paper.

(1) For reviews, see: (a) Hakomori, S.-I.; Zhang, Y. *Chem. Biol.* **1997**, *4*, 97−104. (b) Hakomori, S.-I. *Annu. Rev. Immunol.* **1984**, *2*, 103–126.

(2) Paulsen, H. *Angew. Chem., Int. Ed. Engl.* **1982**, *21*, 155–173.

(3) For example, see: (a) Lemieux, R. U.; Bundle, D. R.; Baker, D. A. *J. Am. Chem. Soc.* **1975**, *97*, 4076–4083. (b) Spohr, U.; Lemieux, R. U. *Carbohydr. Res.* **1988**, *174*, 211–237.

(4) Crich, D.; Dudkin, V. *J. Am. Chem. Soc.* **2001**, *123*, 6818–6825.

(5) Liao, L.; Auzanneau, F.-I. *Org. Lett.* **2003**, *5*, 2607–2610.

10.1021/jo801117y CCC: $40.75 © 2008 American Chemical Society
Published on Web 09/04/2008

SCHEME 1

MeOTf 0.5 M, rt: **1** R^1, R^2 = H, Ac → **2** R^1, R^2 = $CH_3(CH_3O)C=$

3, MeOTf 0.1 M, rt

AcOH/Ac_2O 55 °C: **4** R^1, R^2 = $CH_3(CH_3O)C=$ → **5** R^1, R^2 = H, Ac

forming a glycosidic linkage at O-4 of *N*-acetylglucosamine. To circumvent these problems, analogues of *N*-acetylglucosamine that mask the acetamide NH are commonly employed. Indeed, 2-azido-2-deoxy glucosyl derivatives,[6] as well as phthalimido,[7] *N*,*N*-diacetate,[4,5] *N*-trichlorethoxycarbonyl (*N*-Troc),[8] and *N*-acetyl oxazolidinone[9] protected glucosamine acceptors are commonly used for glycosylation at O-4. Studies have shown that all of these analogues exhibit greater reactivity than the native acetamide.[4] The azido group was found by competitive glycosylation to be more reactive than the phthalimido analogue.[4] However, although the 2-azido acceptors are suitable for glycosylation at O-4, the introduction of this substituent and its conversion back to an acetamido is nontrivial. In comparison, *N*,*N*-diacetates can be converted back to the *N*-acetate by a simple Zemplèn deacetylation, but have to be synthesized at an early stage in the synthetic scheme and may be subjected to premature acid hydrolysis.[4] Most other amino-protecting groups must also be introduced early in the synthetic scheme as, for example, the phthalimido or Troc that are usually introduced as a first step when the glucosamine residue is fully unprotected. In a previously reported[10] synthesis of the Le[a] trisaccharide intermediate **5** (Scheme 1), we discovered that treatment of the *N*-acetylglucosamine disaccharide acceptor **1** with 0.5 M MeOTf (25 equiv) in Et_2O led to the formation of a stable methyl imidate (**2**) with the 4-OH remaining intact. In turn, glycosylation of this methyl imidate acceptor **2** with the thioethyl fucosyl donor **3** gave an 85% yield of the trisaccharide **4**, which was easily converted to the *N*-acetylated trisaccharide **5** by treatment with AcOH in Ac_2O at 55 °C.[10] Thus, we discovered a novel method of temporary protection of the *N*-acetyl group in glucosamine glycosyl acceptors that allows glycosylation at O-4. In comparison to the synthetic strategies with 2-azido-2-deoxy glucosyl derivatives,[6] phthalimido,[7] *N*,*N*-diacetate,[4,5] *N*-trichlorethoxycarbonyl (*N*-Troc),[8] and *N*-acetyl oxazolidinone[9] mentioned above, the main advantage of this new strategy employing an imidate as temporary protection of the *N*-acetyl group is that it can be synthesized selectively at the acceptor stage immediately before glycosylation. In addition, it can be converted back into the corresponding *N*-acetyl derivative under mild conditions. In this paper, we expand on the broad scope and utility of this method; we describe the synthesis of imidate acceptors that carry an allyl, acetyl or benzoyl protecting group at O-3 and our subsequent attempts to further glycosylate them at O-4 using either thioglycosides activated with MeOTf, NIS-TfOH, or NIS-TMSOTf or using a trichloroacetimidate glycosyl donor activated with TMSOTf.

TABLE 1. Synthesis of Alkyl Imidate Protected Acceptors

6 R = All
7 R = Ac
8 R = Bz

MeOTf 0.5 M

9a, 9b R^1 = Me, Et; R^2 = All
10a, 10b R^1 = Me, Et; R^2 = Ac
11a, 11b R^1 = Me, Et; R^2 = Bz

entry	substrate	solvent	products	R^1	yield (%)
1	**6**	Et_2O	**9a**	Me	65[a]
			9b	Et	–[b]
2	**6**	CH_2Cl_2	**9a**	Me	69
3	**7**	Et_2O	**10a**	Me	64[a]
			10b	Et	13[a]
4	**7**	CH_2Cl_2	**10a**	Me	74
5	**8**	Et_2O	**11a**	Me	57[a]
			11b	Et	29[a]
6	**8**	CH_2Cl_2	**11a**	Me	81

[a] Estimated from ^{1}H NMR. [b] Traces.

Results and Discussion

We first attempted to prepare the three methyl imidate acceptors **9a**, **10a**, and **11a** that carry respectively an allyl, acetyl, or benzoyl protecting group at O-3. Thus, the known glycosyl acceptors **6**,[3b] **7**,[11] and **8**[12] were treated with 0.5 M MeOTf in Et_2O according to the conditions that we have previously established.[10] Interestingly, in these conditions, we isolated in all three cases the desired methyl imidates (Table 1) **9a**–**11a** which were contaminated with small amounts of what we identified later as the corresponding ethyl imidates **9b**–**11b**. The structures of the major component in each mixture were confirmed to be the desired methyl imidates by NMR and infrared spectroscopy. The characteristic NMR signals of methyl imidates have been well documented in our previous communication.[10] Thus, the major products **9a**–**11a** gave, as expected, ^{13}C NMR signals at 164.0, 164.4, and 164.3 ppm, respectively, corresponding to the *C*=N as well as *C*H_3 signals for the acetimido groups at 15.9, 15.5, and 15.4 ppm, respectively. As expected,[10] these signals were upfield from typical *C*=O (~170 ppm) and *C*H_3C=O (~20 ppm) signals. The ^{13}C NMR spectra for the methyl imidates **9a**–**11a** also showed peaks at 52.4, 52.6, and 52.6 ppm, respectively, corresponding

(6) Toepfer, A.; Schmidt, R. R. *J. Carbohydr. Chem.* **1993**, *12*, 809–822.
(7) For example, see: Nifant'ev, N. E.; Backinowsky, L. V.; Kochetkov, N. K. *Carbohydr. Res.* **1998**, *174*, 61–72.
(8) For example, see: Gege, C.; Oscarson, S.; Schmidt, R. R. *Tetrahedron Lett.* **2001**, *42*, 377–380.
(9) Crich, D.; Vinod, A. U. *Org. Lett.* **2003**, *5*, 1297–1300.
(10) Liao, L.; Auzanneau, F.-I. *J. Org. Chem.* **2005**, *70*, 6265–6273.
(11) DeNinno, M. P. *Tetrahedron Lett.* **1995**, *36*, 669–672.
(12) Zhang, P.; Appleton, J.; Ling, C.-C.; Bundle, D. R. *Can. J. Chem.* **2002**, *80*, 1141–1161.

to the additional *O*-methyl group. Finally, infrared spectroscopy confirmed the presence of the *C*=N bonds of the imidate (absorbance peak at ~1687 cm^{-1}) and the absence of the C=O bond of the acetamide (absorbance peak typically at ~1640 cm^{-1}). NMR of the mixtures **9a**,**b**−**11a**,**b** showed complicated systems which seem to be overlaps of two nearly identical spectra. The absence of the amide proton was observed for both products present in each mixture. The imidates are rather unstable when submitted to silica gel chromatography and separation of the products observed by NMR was possible for neither **9a**,**b** nor **11a**,**b**. However, careful chromatography of a large scale reaction leading to **10a**,**b** permitted the isolation of an analytical sample of **10b**, which could be fully characterized by NMR spectroscopy and HRMS. NMR spectroscopy of compound **10b** showed no N*H* signal but a quaternary *C*=N at 163.9 ppm as well as a CH_3 signal for an acetimido group at 15.8 ppm. The HMBC spectrum showed long-range correlations between the *C*=N signal and both a CH_2 quartet found at 3.97 ppm and H-2 found at 3.27 ppm. This new OCH_2 signal correlated in the COSY spectrum with a methyl triplet found at 1.17 ppm supporting that this compound was ethyl imidate **10b**. *N*-Ethylation was excluded based on the absence of long-range correlations in the HMBC spectrum between the glucosamine C-2 signal at 62.9 ppm and the new CH_2 mentioned above as well as between the new carbon CH_2 found at 60.7 ppm and H-2 of the glucosamine residue at 3.27 ppm. Finally, HR-ESI mass spectrometry confirmed that **10b** was indeed an ethyl imidate that gave a *m/z* $[M + H]^+$ peak 14 mass units larger (found 396.2018) than that given by **10a** (found 382.1862). While no pure analytical samples of **9b** and **11b** could be obtained, we confirmed that these products were also ethyl imidates by HRMS. Indeed in both cases HRMS gave two smaller signals 14 mass units higher (found 394.2230 and 458.2169 for **9b** and **11b**, respectively) than the peaks corresponding to the $[M + H]^+$ of the major products **9a** and **11a** (found 380.2063 and 444.2026 for **9a** and **11a**, respectively). Thus, treating **6**, **7**, and **8** with 0.5 M MeOTf in Et_2O gave mixtures of the methyl and ethyl imidates **9a**,**b** (68% yield containing traces of the ethyl imidate), **10a**,**b** (77%, 5:1) and **11a**,**b** (86%, 2:1), respectively (Table 1). Since there was no reagent containing an ethyl group in these reaction mixtures, we hypothesized that the ethyl imidates were formed due to the participation of the solvent: Et_2O in the reaction. To test this hypothesis, compounds **6**−**8** were treated with 0.5 M MeOTf while replacing Et_2O with dichloromethane as the reaction solvent. Indeed, under these conditions only the methyl imidates **9a**−**11a** were isolated in yields that were comparable to the combined yields of the methyl and ethyl imidates obtained when the reactions were carried out in Et_2O (Table 1, entries 2, 4, and 6). Although the mixtures of methyl and ethyl imidates make characterization more difficult, we showed, as described below, that these mixtures can be used directly for glycosylation of 4-OH. It is important to point out that imidates are acid and water sensitive and that they must be purified quickly as well as stored and handled under careful exclusion of water. In fact, purification of the imidates and silica gel chromatography by using a mixture of EtOAc and hexanes as the solvent system led to extensive degradation resulting in low isolated yields, and addition of Et_3N to the solvent systems did not prevent degradation. However, while the 3-OAll imidate **9a** did tend to degrade and was not obtained in excellent purity, the imidates **10a** and **11a** could be purified in good isolated yields with silica gel chromatography with use of a mixture of $CHCl_3$ and MeOH as the solvent system. Thus, we attempted to glycosylate these imidate acceptors using various types of glycosyl donors and various promoters and reaction conditions.

TABLE 2. Glycosylations with Thioglycoside Donors

12 R^1 = SEt, R^2 = H, R^3 = Bn
13 R^1 = H, R^2 = SEt, R^3 = Bn
14 R^1 = SEt, R^2 = H, R^3 = Ac

15 R^1 = Ac, R^2 = Bn
16 R^1 = Bz, R^2 = Bn
17 R^1 = Bz, R^2 = Ac

entry	acceptor	donor (equiv)	MeOTf (M)	NIS (equiv)	TMSOTf (equiv)	TfOH (equiv)	product	yield (%)
1	**10a**	**12** (3)	0.1[a]	–[b]	–[b]	–[b]	**15**	62[c]
2	**11a**,**b**	**12** (3)	0.2[a]	–[b]	–[b]	–[b]	**16**	69[c]
3	**7** to **10a**,**b**	**12** (3)	0.5[d]	–[b]	–[b]	–[b]	**15**	56[c]
4	**8** to **11a**,**b**	**12** (3)	0.5[d]	–[b]	–[b]	–[b]	**16**	64[c]
5	**11a**	**12** (3)	–[b]	4	0.1[e]	–[b]	**16**	<20[f]
6	**11a**	**12** (4)	–[b]	4	–[b]	0.1–0.2[g]	**16**	25[c]
7	**11a**	**13** (3)	–[b]	4	0.05–0.1[e]	–[b]	**16**	63[c]
8	**11a**	**13** (5)	–[b]	5	–[b]	0.5[g]	**16**	67[c]
9	**11a**	**14** (5)	–[b]	5	–[b]	0.5[h]	**17**	50[c]

[a] Reagents and conditions: (i) MeOTf (5−10 equiv), CH_2Cl_2, MS 4 Å, rt; (ii) AcOH, Ac_2O, 55 °C. [b] Not applicable. [c] Isolated yield over multiple steps. [d] One-pot formation of the methyl/ethyl imidates followed by glycosylation with **12**. Reagents and conditions: (i) **7** or **8**, MeOTf (25 equiv), Et_2O, MS 4 Å, rt; (ii) **12** in Et_2O, rt; (iii) AcOH, Ac_2O, 55 °C. [e] Reagents and conditions: (i) Et_2O, MS 4 Å, entry 5 −60 °C, entry 7 −70 to −50 °C; (ii) AcOH, Ac_2O, 55 °C. [f] As evaluated from NMR, the product could not be purified. [g] Reagents and conditions: (i) Et_2O, MS 4 Å, −60 °C; (ii) AcOH, Ac_2O, 55 °C. [h] Reagents and conditions: (i) $Cl(CH_2)_2Cl$, MS 4 Å, 40 to 60 °C; (ii) AcOH, Ac_2O, 55 °C.

The first set of conditions that we tested were based on our previous report[10] and employed the known[13] perbenzylated β-thioglycoside rhamnosyl donor **12** under activation with 0.1 to 0.2 M MeOTf. In these mild conditions we observed total degradation of the 3-*O*-allyl acceptor **9a** prior to its glycosylation and we thus concluded that this acceptor was not suited for use in glycosylations at O-4 of glucosamine. In contrast, the methyl imidate **10a** as well as the mixture of methyl and ethyl imidates **11a**,**b** were efficiently glycosylated with donor **12** by using 0.1−0.2 M MeOTf at room temperature (Table 2, entries 1 and 2). Since, as mentioned above, the imidates are difficult to purify without degradation, the resulting disaccharide imidates were not purified but were directly subjected to treatment with AcOH/Ac_2O to convert the acetimidate to the acetamide. Thus, from the acceptor **10a** and from the mixture of methyl and ethyl imidates **11a**,**b**, disaccharides **15** and **16** were obtained pure in 62% and 69% yield, respectively (Table 2, entries 1 and 2). Measurements of the $J_{C\text{-}1',H\text{-}1'}$ coupling constants for each of **15** ($^1J_{C\text{-}1',H\text{-}1'}$ = 168 Hz) and **16** ($^1J_{C\text{-}1',H\text{-}1'}$ = 170 Hz) confirmed[14] that the newly formed rhamnosidic bonds were indeed in the α configuration. The comparable yields obtained for the formation of disaccharide **15** and **16** from pure **10a** and from the mixture **11a**,**b**, respectively, suggest that the glycosylation occurred equally well on a pure methyl imidate acceptor as on a mixture of methyl and ethyl imidate acceptors.

(13) Yu, H.; Yu, B.; Wu, X.; Hui, Y.; Han, X. *J. Chem. Soc., Perkin Trans. 1* **2000**, *9*, 1445–1453.
(14) Bock, K.; Pedersen, C. *J. Chem. Soc., Perkin Trans. 2* **1974**, 293–297.

Since we could glycosylate the imidate acceptors in adequate yields, we investigated a one-pot synthetic strategy using the imidate methodology. We postulated that the 0.5 M MeOTf used to form the alkyl imidates **10a**,**b** or **11a**,**b** should also allow for the subsequent activation of thioglycoside donor **12**. The advantage of this one-pot strategy is that it allows for the formation of the imidate in situ, thereby eliminating the isolation and purification of the imidate acceptor. The acetamide acceptors **7** and **8** were converted to the corresponding imidates **10a**,**b** and **11a**,**b**, respectively, and when TLC showed that the starting materials had been consumed, a solution of thioglycoside **12** in Et_2O was added to the reaction mixture. Subsequent treatment of the reaction mixtures with $AcOH/Ac_2O$ gave disaccharides **15** and **16** in 56% and 64% yield respectively over the 3 steps (Table 2, entries 3 and 4). Thus, this one-pot synthetic strategy gave higher yields than those overall yields obtained following the purification of the imidate donors. Indeed with intermediate purification of the imidates, the disaccharide **15** and **16** were obtained in lower yields of 46% and 59% from **7** and **8**, respectively.

We also investigated the applicability of the methyl imidate protection with thioglycoside donors activated with *N*-iodosuccinimide (NIS) and catalytic trimethylsilyl triflate (TMSOTf) or trifluoromethane sulfonic acid (TfOH). In these reactions, we once again decided to convert the imidates back to the desired *N*-acetates immediately after glycosylation without attempting to purify the disaccharide imidates. Thus, starting with the perbenzylated *β*-thioglycoside donor **12**, which, as described above, had been used successfully under MeOTf activation, we attempted glycosylation of the 3-OBz acceptor **11a** using NIS and TMSOTf catalysis at low temperature. However, under these conditions the desired disaccharide **16** could not be isolated pure and NMR evaluation of the crude material indicated that it had been formed at best in 20% yield (Table 2, entry 5). Using a catalytic amount of TfOH instead of TMSOTf did not significantly increase the yield of the reaction but in these conditions the disaccharide **16** was isolated pure in 25% yield (Table 2, entry 6). While the results obtained with the *β*-thioglycoside **12** activated with NIS/TMSOTf (or TfOH) were disappointing, a significant improvement was observed when we employed the α-thioglycoside donor **13**.[13] Indeed, coupling donor **13** with acceptor **11a** by using NIS and TfOH at low temperature gave the disaccharide **16** in 63% yield (Table 2, entry 7). Similarly, a 67% yield of **16** was obtained when using NIS and TMSOTf for activation (Table 2, entry 8). In contrast, glycosylation with the peracetylated *β*-thioglycoside donor **14** proved to be fairly challenging as it was difficult to activate this donor. Indeed, excess amounts of MeOTf at room temperature or excess NIS with catalytic amounts of TfOH (or TMSOTf) at low temperature did not lead to significant reaction. However, using 1,2-dichloroethane as solvent and heating the reaction mixture to 60 °C while activating donor **14** with NIS and 0.5 equiv of TfOH gave disaccharide **17** in 50% yield (Table 2, entry 9). Much to our surprise, TLC analysis of the reaction mixture indicated that the imidate was relatively stable under these rather harsh glycosylation conditions. The α configuration of the newly formed rhamnosidic bond in disaccharide **17** was confirmed by NMR ($^1J_{C-1',H-1'} = 171$ Hz) as described above for disaccharides **15** and **16**. From the experimental results described here, it appears that glycosylation of an imidate acceptor with a less reactive thioglycoside donor (**13** or **14**) requires activation under NIS-catalytic TfOH (or TMSOTf) conditions, while glycosylations with more reactive donors such as the perbenzylated *β*-thioglycoside **12** give acceptable results under activation with excess MeOTf at room temperature. We finally investigated the reaction of the known α-rhamnosyl trichloroacetimidate glycosyl donor **18**[15] with methyl imidate **11a** under TMSOTf catalysis. Once again the disaccharide imidate was not isolated but converted directly to the corresponding *N*-acetylated product. Thus, coupling of the acceptor **11a** with donor **18** at low temperature with 0.1 equiv of TMSOTf gave the desired disaccharide **17** in 80% yield (Scheme 2). This result is particularly interesting, as it was while using similar conditions that we had previously observed[5] the formation of a rhamnosyl imidate when attempting to glycosylate at O-4 of an *N*-acetylated glucosamine disaccharide acceptor.

SCHEME 2

OC(NH)CCl_3
AcO, AcO, OAc
18 + **11a**
0.1 equiv TMSOTf | CH_2Cl_2 - 78 °C
$AcOH/Ac_2O$ | 55°C
17 (80%)

Conclusion

We have clearly established that the temporary protection of the *N*-acetyl group as a methyl or ethyl imidate allows glycosylation at O-4 of *N*-acetylglucosamine acceptors. On the basis of the experimental observations described here, it is clear that imidate acceptors are compatible with glycosylations with use of either thioglycoside or trichloroacetimidate donors. By using 0.5 M MeOTf alkyl imidate protected glucosamine acceptors can be prepared efficiently and immediately prior to glycosylation at O-4. Interestingly, when using Et_2O as the solvent, one may observe the formation of mixtures of methyl and ethyl imidates, whereas reaction in CH_2Cl_2 only provides methyl imidate acceptors. The alkyl imidate acceptors can be used in glycosylations with thioglycoside donors under activation by either MeOTf or NIS-TMSOTf (or TfOH). Glycosylation of an imidate acceptor with the more reactive *β*-perbenzylated thiorhamnoside was most effective under MeOTf activation, whereas NIS/TfOH or NIS/TMSOTf activation at low temperature gave the best results with the corresponding less reactive perbenzylated α-thiorhamnoside. Interestingly, the relatively harsh conditions (60 °C, 0.5 equiv of TfOH) required for the activation of peracetylated *β*-thioethyl rhamnoside did not lead to degradation of the imidate acceptor but allowed the formation and isolation of the desired disaccharide, albeit in moderate yield. We also report a successful one-pot synthesis in which the imidate acceptor was formed in situ, then glycosylated with a thioglycoside donor. An imidate acceptor was also shown to be reactive in a glycosylation reaction by using a trichloroacetimidate donor that was activated with TMSOTf at low temperature. Together with its ease of introduction and removal in mild conditions, the imidate has proven to be a versatile temporary protecting group that allows glycosylation at O-4 of glucosamine. Indeed, this paper demonstrates that when gly-

(15) Van Steijn, A. M. P.; Kamerling, J. P.; Vliegenthart, J. F. G. *Carbohydr. Res.* **1991**, *211*, 261–277.

cosylation at O-4 of an acetamide-containing acceptor does not proceed as desired, the imidate strategy constitutes a viable alternative that is compatible with various donors and glycosylation methods.

Experimental Section

General Procedure for the Synthesis of the Methyl and Ethyl Imidates. A mixture of the acetamide **6**, **7**, or **8** (20 μmol/mL) in Et_2O (Method A) or CH_2Cl_2 (Method B) containing 4 Å molecular sieves (~25 mg/mL) was stirred at room temperature for 2 h. MeOTf (25 equiv, 0.5 M) was added and the mixture was stirred for 18 h at room temperature. The reaction was quenched with Et_3N (25 equiv), the reaction mixture was filtered, and the solids were washed with CH_2Cl_2. The combined filtrate and washings were diluted with CH_2Cl_2 (50 mL) and washed with saturated aqueous $NaHCO_3$ (50 mL). The organic layer was dried and concentrated and column chromatography ($CHCl_3$−MeOH) of the residue gave the imidates **9**−**11**. When Et_2O was used as the reaction solvent the methyl and ethyl imidates were isolated as a mixture and the ratio of these two compounds was estimated by ^{1}H NMR. When the reaction was performed in CH_2Cl_2, the methyl imidates were isolated as pure compounds.

Methyl 3-*O*-Allyl-6-*O*-benzyl-2-deoxy-2-methylacetimido-β-D-glucopyranoside (9a) and Methyl 3-*O*-Allyl-6-*O*-benzyl-2-deoxy-2-ethylacetimido-β-D-glucopyranoside (9b). Method A. From the known[3b] acetamide **6** (24 mg, 67 μmol) the imidate **9a** containing traces of ethyl imidate **9b** that could not be separated was isolated. The mixture of **9a**,**b** (17 mg, 68%) was obtained as a colorless glass after column chromatography (30:1 $CHCl_3$−MeOH).

Method B. From the known[3b] acetamide **6** (26 mg, 70 μmol) the imidate **9a** (18 mg, 69%) was obtained as a colorless glass after column chromatography (30:1 $CHCl_3$−MeOH).

Methyl Imidate 9a. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36−7.24 (m, 5H, Ar); 5.90−5.79 (m, 1H, allyl C*H*═CH_2); 5.24−5.09 (m, 2H, CH═C*H₂*); 4.64−4.51 (m, 2H, C*H₂*Ph); 4.25 (d, 1H, *J* = 7.7 Hz, H-1); 4.19−4.07 (m, 2H, allyl OCH_2); 3.80−3.70 (m, 2H, H-6a, H-6b); 3.70−3.48 (m, 5H, H-4, H-5, OCH_3); 3.43 (s, 3H, OCH_3); 3.37 (m, 1H, H-3); 3.21 (dd, 1H, *J* = 7.7 Hz, 9.2 Hz, H-2); 2.71 (br s, 1H, OH); 1.91 (s, 3H, acetimidate CH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.0 (*C*═N); 137.9 (Ar quat); 135.1 (allyl *C*H═CH_2); 128.3, 127.8, 127.7 (Ar); 117.0 (allyl CH═*C*H_2); 104.3 (C-1); 84.6 (C-3); 74.1 (C-4); 73.9 (*C*H_2Ph); 73.7 (allyl OCH_2); 71.9 (C-5); 70.7 (C-6); 64.9 (C-2); 57.2, 52.4 (OCH_3); 15.9 (*C*H_3 acetimidate). HRMS calcd for $C_{20}H_{29}NO_6$ $[M + H]^+$ 380.2073, found 380.2063. IR (NaCl) 1687 cm^{-1} (*C*═N acetimidate).

Ethyl Imidate 9b in the Mixture 9a,b. Only traces of ethyl imidate **9b** were formed when methyl imidate **9a** was prepared in Et_2O (Method A). NMR signals could not be specifically assigned to ethyl imidate **9b**. HRMS on the mixture **9a**,**b** showed a minor molecular signal corresponding to ethyl imidate **9b**. HRMS calcd for **9b** $C_{21}H_{31}NO_6$ $[M + H]^+$ 394.2256, found 394.2230.

Methyl 3-*O*-Acetyl-6-*O*-benzyl-2-deoxy-2-methylacetimido-β-D-glucopyranoside (10a), and methyl 3-*O*-Acetyl-6-*O*-benzyl-2-deoxy-2-ethylacetimido-β-D-glucopyranoside (10b). Method A. From the known[11] acetamide **7** (20 mg, 56 μmol) a mixture (5:1) of the imidates **10a**,**b** (19 mg, 86%) was obtained as a colorless glass after column chromatography (25:1 $CHCl_3$−MeOH). Repeated chromatographies on a large scale synthesis provided analytical samples of purified **10a** and **10b**, which could be fully and independently characterized by NMR and HRMS.

Method B. From the known[11] acetamide **7** (20 mg, 56 μmol) the imidate **10a** (16 mg, 74%) was obtained as a colorless glass after column chromatography (30:1 $CHCl_3$−MeOH).

Methyl Imidate 10a. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35−7.23 (m, 5H, Ar); 5.00 (t, 1H, *J* = 9.4 Hz, H-3); 4.63−4.54 (m, 2H, OC*H₂*Ph); 4.32 (d, 1H, *J* = 7.7 Hz, H-1); 3.79 (m, 2H, H-6a, H-6b); 3.74−3.63 (m, 1H, H-4); 3.63−3.52 (m, 4H, H-5, OCH_3); 3.45 (s, 3H, OCH_3); 3.27 (dd, 1H, *J* = 7.7, 9.6 Hz, H-2); 2.99 (br s, 1H, OH); 2.02 (s, 3H, *O*-acetyl); 1.88 (s, 3H, acetimidate). ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.5 (C═O); 164.4 (*C*═N); 137.8 (Ar quat); 128.4, 127.8, 127.7 (Ar); 104.1 (C-1); 78.2 (C-3); 74.4 (C-5); 73.7 (*C*H_2Ph); 70.8 (C-4); 70.3 (C-6); 65.4 (C-2); 57.2, 52.6 (*C*H_3O); 20.9 (*C*H_3CO); 15.5 (acetimidate CH_3). HRMS calcd for $C_{19}H_{27}NO_7$ $[M + H]^+$ 382.1866, found 382.1862. IR (NaCl) 1749 cm^{-1} (C═O benzoate), 1685 cm^{-1} (*C*═N acetimidate).

Ethyl Imidate 10b. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32 (m, 5H, Ar); 4.99 (t, 1H, *J* = 9.5 Hz, H-3); 4.63−4.54 (m, 2H, OC*H₂*Ph); 4.30 (d, 1H, *J* = 7.6 Hz, H-1); 3.97 (q, 2H, *J* = 7.1 Hz, OC*H₂*CH_3); 3.78 (m, 2H, H-6a, H-6b); 3.68 (bt, 1H, *J* = 9.4 Hz, H-4); 3.59−3.52 (m, 1H, H-5); 3.45 (s, 3H, OCH_3); 3.27 (dd, 1H, *J* = 7.7, 9.6 Hz, H-2); 2.97 (br s, 1H, OH); 2.02 (s, 3H, *O*-acetyl); 1.87 (s, 3H, acetimidate); 1.17 (t, 3H, *J* = 7.1 Hz, OCH_2C*H₃*). ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.5 (C═O); 163.8 (*C*═N); 137.7 (Ar quat); 128.4, 127.8, 127.7 (Ar); 104.2 (C-1); 78.2 (C-3); 74.3 (C-5); 73.7 (*C*H_2Ph); 70.8 (C-4); 70.4 (C-6); 62.9 (C-2); 60.7 (O*C*H_2CH_3); 57.3 (*C*H_3O); 20.9 (*C*H_3CO); 15.8 (acetimidate CH_3); 14.1 (OCH_2*C*H_3). HRMS calcd for $C_{20}H_{29}NO_7$ $[M + H]^+$ 396.2022, found 396.2018.

Methyl 3-*O*-Benzoyl-6-*O*-benzyl-2-deoxy-2-methylacetimido-β-D-glucopyranoside (11a) and Methyl 3-*O*-Benzoyl-6-*O*-benzyl-2-deoxy-2-ethylacetimido-β-D-glucopyranoside (11b). Method A. From the known[12] acetamide **8** (41 mg, 97 μmol) a mixture (2:1) of the imidates **11a**,**b** (34 mg, 77%) was obtained as colorless glass after column chromatography (30:1 $CHCl_3$−MeOH). The imidates could not be separated by chromatography.

Method B. From the known[12] acetamide **8** (30 mg, 70 μmol) the imidate **11a** (25 mg, 81%) was obtained as a colorless glass after column chromatography (25:1 $CHCl_3$−MeOH).

Methyl Imidate 11a. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.02−7.95 (m, 2H, Ar); 7.60−7.51 (m, 1H, Ar); 7.48−7.41 (m, 2H, Ar); 7.39−7.24 (m, 5H, Ar); 5.30 (t, 1H, *J* = 9.4 Hz, H-3); 4.76−4.53 (m, 2H, C*H₂*Ph); 4.42 (d, 1H, *J* = 7.62 Hz, H-1); 3.87−3.82 (m, 3H, H-4, H-6a, H-6b); 3.71−3.65 (m, 1H, H-5); 3.52−3.45 (m, 7H, H-2, 2 × OCH_3); 3.09 (br s, 1H, OH), 1.90 (s, 3H, acetimidate CH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.9 (C═O); 164.3 (*C*═N); 137.8, 129.6 (Ar quat); 133.2, 129.7, 128.4, 127.9 (Ar); 104.1 (C-1); 78.9 (C-3); 74.5 (C-5); 73.7 (*C*H_2Ph); 70.7 (C-4); 70.2 (C-6); 63.2 (C-2); 57.3, 52.6 (OCH_3); 15.4 (acetimidate CH_3). HRMS calcd for $C_{24}H_{29}NO_7$ $[M + H]^+$ 444.2022, found 444.2026. IR (NaCl) 1724 cm^{-1} (C═O benzoate), 1685 cm^{-1} (*C*═N acetimidate).

Ethyl Imidate 11b in the Mixture 11a,b. ^{1}H NMR of the mixture **11a**,**b** showed a 2:1 ratio of methyl and ethyl imidates **11a** and **11b**, respectively (Method A). ^{1}H NMR (400 MHz, $CDCl_3$) of the mixture **11a**,**b** showed a signal at 1.13 (t, 3H for **11b**, *J* = 7.0 Hz, OCH_2C*H₃*); other signals could not be specifically assigned to the ethyl imidate **11b**. HRMS on the mixture **11a**,**b** showed a minor molecular signal corresponding to ethyl imidate **11b**. HRMS calcd for $C_{25}H_{31}NO_7$ $[M + H]^+$ 458.2179, found 458.2169.

Methyl 2-Acetamido-3-*O*-acetyl-6-*O*-benzyl-4-*O*-(2,3,4-tri-*O*-benzyl-α-L-rhamnopyranosyl)-2-deoxy-β-D-glucopyranoside (15). From Isolated Imidate 10a. A solution of imidate **10a** (14 mg, 36 μmol) and thioglycoside **12** (55 mg, 115 mmol, 3 equiv) in Et_2O (2 mL) containing 4 Å molecular sieves (167 mg) was stirred at room temperature for 1 h. MeOTf (20 μL, 179 μmol, 5 equiv, 0.1 M) was added and the mixture was stirred for 18 h at room temperature. The reaction was quenched with Et_3N (29 μL) and the mixture was filtered over Celite. The solids were washed with CH_2Cl_2 (2 × 15 mL) and the combined filtrate and washings were concentrated. The residue was dissolved in Ac_2O (2.0 mL) and AcOH (0.7 mL) and the solution was stirred for 18 h at 55 °C. The mixture was coconcentrated with toluene (3 × 40 mL) and flash chromatography (7:3 EtOAc−hexanes) of the residue gave disaccharide **15** as a colorless glass (17 mg, 62%).

From Imidates 10a,b Generated in Situ. A solution of acetamide **7** (81 mg, 221 μmol) in Et_2O (11 mL) containing 4 Å

molecular sieves (300 mg) was stirred at room temperature for 1.5 h. MeOTf (620 μL, 5.48 mmol, 25 equiv, 0.5 M) was added and the mixture was stirred for 18 h at room temperature. A solution of thioglycoside donor **12** (330 mg, 690 mmol, 3.1 equiv) dissolved in Et_2O (2.0 mL) was added and stirring was continued for 18 h at room temperature. The reaction was quenched and worked up, then the residue was treated with Ac_2O and AcOH and worked up as described immediately above. Flash chromatography (7:3 EtOAc−hexanes) of the residue gave disaccharide **15** as a colorless glass (96 mg, 56%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34−7.20 (m, 20H, Ar); 5.43 (d, 1H, J = 9.4 Hz, NH); 5.01−4.89 (m, 2H, H-3, C*H*HPh); 4.79 (d, 1H, J = 1.9 Hz, H-1′); 4.70−4.39 (m, 7H, C*H*HPh, 3 × C*H*$_2$Ph); 4.26 (d, 1H, J = 8.4 Hz, H-1); 3.96 (t, 1H, J = 7.4 Hz, H-2); 3.75 (t, 1H, J = 9.2 Hz, H-4); 3.71−3.58 (m, 2H, H-3′, H-5′); 3.58−3.50 (m, 3H, H-6a, H-2′, H-4′); 3.50−3.38 (m, 4H, H-6b, OCH_3); 3.38 − 3.29 (m, 1H, H-5); 2.03 (s, 3H, CH_3C=O); 1.92 (s, 3H, CH_3C=O); 1.23 (d, 3H, J = 6.4 Hz, H-6′). ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.6, 170.2 (C=O); 138.6, 138.4, 138.1, 138.0 (Ar quat); 128.3, 128.3, 127.8, 127.7, 127.7, 127.6, 127.5, 127.4 (Ar); 102.0 (C-1′, $^1J_{C-H}$ = 168 Hz); 99.6 (C-1); 80.3, 75.4 (C-2′, C-4′); 79.1, 69.0 (C-3′, C-5′); 75.6 (C-4); 75.2, 73.5, 72.7, 72.2 (*C*H$_2$Ph), 74.8 (C-5); 74.2 (C-3); 68.4 (C-6); 56.4 (OCH_3); 54.0 (C-2); 23.3, 21.1 (*C*H$_3$C=O); 17.8 (C-6′). HRMS calcd for $C_{45}H_{53}NO_{11}$ [M + H]$^+$ 784.3697, found 784.3683.

Methyl 2-Acetamido-3-*O*-benzoyl-6-*O*-benzyl-4-*O*-(2,3,4-tri-*O*-benzyl-α-L-rhamnopyranosyl)-2-deoxy-*β*-D-glucopyranoside (16). From Isolated Imidates 11a,b. A solution of the imidates **11a**,**b** (22 mg, 50 μmol) and thioglycoside **12** (67 mg, 139 mmol, 2.8 equiv) in Et_2O (2 mL) containing 4 Å molecular sieves (50 mg) was stirred at room temperature for 45 min. MeOTf (56 μL, 495 μmol, 10 equiv, 0.2 M) was added and the mixture was stirred for 18 h at room temperature. The reaction was quenched and worked up, then the residue was treated with Ac_2O and AcOH and worked up as described above for the synthesis of disaccharide **15**. Flash chromatography (2:8 EtOAc−hexanes to 100% EtOAc) of the residue gave the disaccharide **16** as a colorless glass (29 mg, 69%).

From Imidates 11a,b Generated in Situ. A solution of acetamide **8** (95 mg, 221 μmol) in Et_2O (11 mL) containing 4 Å molecular sieves (300 mg) was stirred at room temperature for 1.5 h. MeOTf (620 μL, 5.48 mmol, 25 equiv, 0.5 M) was added and the mixture was stirred for 18 h at room temperature. A solution of thioglycoside **12** (332 mg, 695 mmol, 3 equiv) in Et_2O (5.5 mL) was then added to the reaction mixture and stirring was continued for 18 h at room temperature. The reaction was quenched and worked up, then the residue was treated with Ac_2O and AcOH and worked up as described above for the synthesis of disaccharide **15**. Flash chromatography (7:3 EtOAc−hexanes) of the residue gave disaccharide **16** as a colorless glass (120 mg, 64%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.05−7.94 (m, 2H, Ar); 7.60−7.47 (m, 1H, Ar); 7.46−7.08 (m, 22H, Ar); 5.55 (d, 1H, NH); 5.31 (t, 1H, J = 9.1 Hz, H-3); 4.86 (d, 1H, J = 1.6 Hz, H-1′); 4.78 (d, 1H, J = 11.1 Hz, CH*H*Ph); 4.70−4.36 (m, 8H, CH*H*Ph, 3 × C*H*$_2$Ph, H-1); 4.15 − 4.07 (m, 1H, H-2); 3.97 (t, 1H, J = 9.1 Hz, H-4); 3.68−3.34 (m, 10H, H-5, H-6a, H-6b, H-2′, H-3′, H-4′, H-5′, OCH_3); 1.78 (s, 3H, *N*-acetyl); 0.74 (d, 3H, J = 5.9 Hz, H-6′). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.2, 167.0 (C=O); 138.7, 138.5, 138.1, 138.0, 129.4 (Ar quat); 133.3, 129.9, 128.4, 128.3, 128.2, 127.8, 127.7, 127.6, 127.5, 127.5, 127.4 (Ar); 101.9 (C-1); 99.4 (C-1′, $^1J_{C-H}$ = 170 Hz); 80.3, 79.1, 75.4, 75.3 (C-5, C-2′, C-3′, C-4′, C-5′); 75.6 (C-4); 74.8, 73.6, 72.5, 72.3 (*C*H$_2$Ph); 74.0 (C-3); 68.4 (C-6); 56.4 (OCH_3); 54.3 (C-2); 23.2 (*C*H$_3$C=O); 17.4 (C-6′). HRMS calcd for $C_{50}H_{55}NO_{11}$ [M + H]$^+$ 846.3853, found 846.3818.

Methyl 2-Acetamido-4-*O*-(2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl)-3-*O*-benzoyl-6-*O*-benzyl-2-deoxy-*β*-D-glucopyranoside (17). From Isolated Imidate 11a and Thioglycoside 14. A solution of imidate **11a** (10 mg, 22 μmol) and thioglycoside **14** (37 mg, 110 μmol, 5 equiv) in $Cl(CH_2)_2Cl$ (1 mL) containing 4 Å molecular sieves (57 mg) and NIS (25 mg, 111 μmol, 5 equiv) was stirred at room temperature for 1 h. TfOH (1 μL, 11 μmol, 0.5 equiv) was then added to the reaction mixture. The reaction was stirred at room temperature for 1 h and was then stirred at 60 °C for 18 h. The reaction was quenched and worked up, then the residue was treated with Ac_2O and AcOH and worked up as described above for the synthesis of disaccharide **15**. Flash chromatography (8:2 EtOAc−hexanes) of the residue gave the disaccharide **17** as a colorless glass (8 mg, 50%).

From Isolated Imidate 11a and Trichloroacetimidate 18. A solution of imidate **11a** (25 mg, 57 μmol) and the known[15] trichloroacetimidate rhamnosyl donor **18** (74.0 mg, 170 mmol, 3.0 equiv) in CH_2Cl_2 (3 mL) containing 4 Å molecular sieves (150 mg) was stirred at room temperature for 1 h and then cooled to −70 °C. A freshly prepared 0.11 M solution of TMSOTf in anhyd CH_2Cl_2 (50 μL, 5.7 μmol, 0.1 equiv) was added and the mixture was stirred for 1 h at −70 °C. The reaction was quenched with NEt_3 and worked up, then the residue was treated with Ac_2O and AcOH and worked up as described above for the synthesis of disaccharide **15**. Flash chromatography (4:6 EtOAc−hexanes to 100% EtOAc) of the residue gave disaccharide **17** as a colorless glass (32 mg, 80%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.04−7.21 (m, 10 H, Ar); 5.48 (d, 1H, J = 9.4 Hz, NH); 5.42 (t, 1H, J = 9.1 Hz, H-3); 5.18 − 5.02 (m, 2H, H-2′, H-3′); 4.91 (d, 1H, J = 1.5 Hz, H-1′); 4.85 (t, 1H, J = 10.0 Hz, H-4′); 4.61 − 4.50 (m, 2H, C*H*$_2$Ph); 4.45 (d, 1H, J = 8.9 Hz, H-1); 4.21 − 4.05 (m, 2H, H-2, H-4); 3.86−3.74 (m, 2H, H-6a, H-6b); 3.65−3.54 (m, 2H, H-5, H-5′); 3.49 (s, 3H, OCH_3); 2.02, 1.94, 1.90, 1.80 (4 × acetate CH_3); 0.59 (d, 3H, J = 6.2 Hz). ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.2, 170.4, 166.8 (C=O); 133.4, 130.0, 128.5, 128.4, 128.3, 127.9, 127.8, 127.5 (Ar); 131.2, 129.4 (Ar quat); 101.9 (C-1); 98.4 (C-1′, $^1J_{C-H}$ = 171 Hz); 75.8 (C-4); 74.8, 67.2 (C-5, C-5′); 74.0 (C-3); 73.2 (*C*H$_2$Ph); 70.6 (C-4); 69.8 (C-4′); 68.9 (C-3′); 68.2 (C-2′); 68.2 (C-6); 56.5 (OCH_3); 54.4 (C-2); 20.8, 20.7, 20.7 (CH_3 acetates); 16.8 (C-6′). HRMS calcd for $C_{35}H_{43}NO_{14}$ [M + H]$^+$ 702.2762, found: 702.2725.

Acknowledgment. We thank the National Science and Engineering Research Council of Canada, the Canada Foundation for Innovation, and the Ontario Innovation Trust for financial support of this work.

Supporting Information Available: General experimental procedures, ^{1}H and ^{13}C NMR data for compounds **9a**, **10a**, **10b**, **11a**, **15**, **16**, and **17**, ^{1}H NMR for **9a**,**b** and **11a**,**b**, as well as IR data for compounds **9a**, **10a**, and **11a**. This material is available free of charge via the Internet at http://pubs.acs.org.

JO801117Y