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Glycosylation of *N*-Acetylglucosamine: Imidate Formation and Unexpected Conformation

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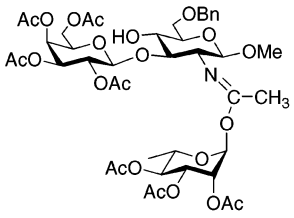
ABSTRACT



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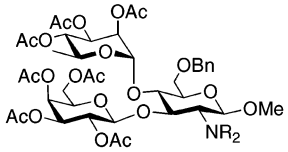

7 R = H, Ac
11 R = Ac, Ac

Rhamnosylation in mild conditions of a disaccharide containing *N*-acetylglucosamine afforded the imidate 6 while at higher temperature and concentration of promoter trisaccharide 7 was isolated. The kinetic imidate 6 was independently rearranged in 50% yield to the thermodynamic trisaccharide 7. Comparative NMR studies of 7 in $CDCl_3$ and DMSO-d_6 suggest the formation of a nonchair conformation in $CDCl_3$. The structure of 7 was confirmed through the independent synthesis of the *N*-acetylacetamido trisaccharide 11.

The overexpression of carbohydrate structures at the surface of tumor cells has been extensively documented.[1] Since carbohydrates can be involved in specific immune responses directed against the cells or bacteria that display them, a number of these tumor-associated carbohydrate antigens are currently being investigated as potential vaccines against cancer.[2] The Lewis blood group antigens (Figure 1) Le^a (**1**) and Le^aLe^x (**3**) are known to be overexpressed on tumor tissues and cells.[1] However, since Le^a is also largely expressed on normal cells, anti-cancer vaccines based on these antigens are likely to trigger an immune reaction that will lead to the destruction of these normal cells.[3] To avoid such autoimmune reactions, we are studying analogues of

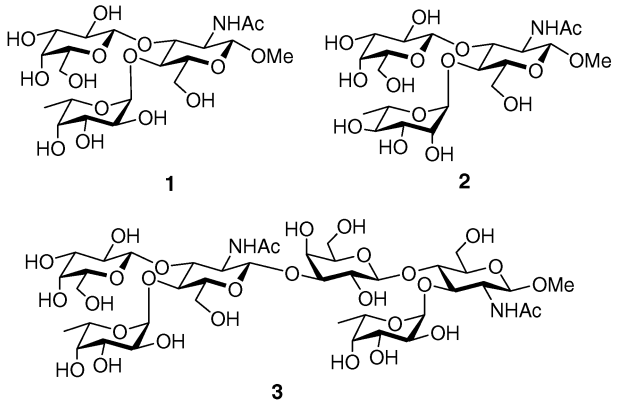

Figure 1. Blood group antigens and analogue: Le^a (**1**), Le^a analogue (**2**), and Le^aLe^x (**3**).

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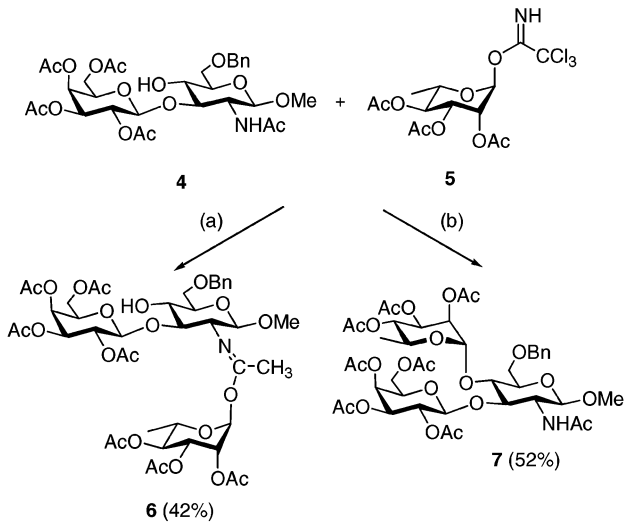
Le^aLe^x that no longer display the Le^a antigen but might retain common antigenic determinants with Le^aLe^x. To assess its cross reactivity with Le^a, we have embarked on the preparation of analogue **2** in which the fucosyl residue is replaced by a rhamnosyl unit. Thus, our synthetic scheme involved the rhamnosylation of the known[4] disaccharide **4** which, along with other similar glycoside derivatives, were successfully fucosylated to prepare Le^a trisaccharide analogues.[5−7]

Studies with glycosyl donors less reactive than fucosyl donors have clearly established[8] the poor reactivity of the 4-OH group in *N*-acetylglucosamine derivatives when compared to the 4-OH group in other acceptors. In fact, construction of a glycosidic bond at this position is often achieved after the introduction of an *N*-phathalimido[9] or azido[10] substituent at C-2 of the glucosamine residue. Thus, it is not surprising that glycosylation of **4** with a less reactive rhamnosyl donor was more delicate than its fucosylation. The low reactivity of the 4-OH group in *N*-acetylglucosamine derivatives has long been believed to result from steric hindrance at this position.[8] Recently Crich et al. proposed an alternative explanation based on the formation of a hydrogen bond involving the glucosamine amide group and lowering the reactivity of such acceptors toward glycosylation.[11] In our attempt to synthesize analogue **2**, we have found that, under mild conditions, the NH group in acceptor **4** could act as a competitive nucleophile in the glycosylation reaction with donor **5** and lead to the formation of imidate **6** as the major product (Scheme 1). Although the formation of such

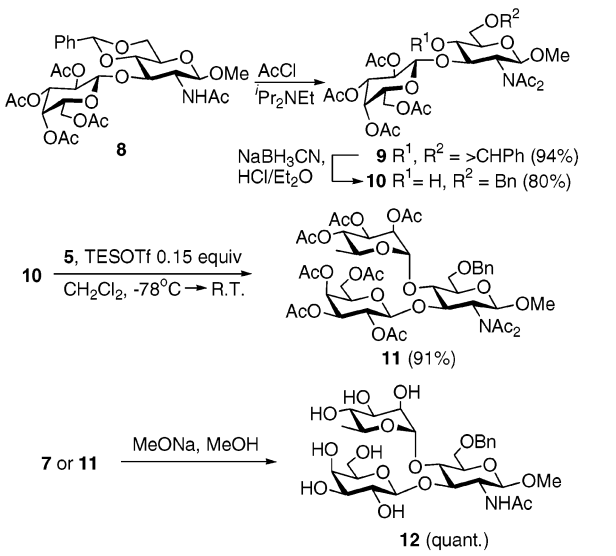
Scheme 1[a]



[a] Reagents and conditions: (a) TESOTf (0.1 equiv), CH_2Cl_2, −78 °C → rt. (b) TESOTf (0.5 equiv), CH_2Cl_2, rt.

imidates has been postulated[12] these had never been isolated. When the glycosylation was conducted at higher temperature and concentration of promoter, the *O*-glycosylated product **7** was obtained in 52% yield. In addition, when the imidate **6** was stirred in these glycosylation conditions it rearranged to the trisaccharide in 50% yield. Therefore, our results provide a third explanation for the low reactivity of the 4-OH groups in *N*-acetylglucosamine glycosyl acceptors. We propose that imidates form first as the kinetic products of the glycosylation reactions and that depending on the structure of the glycosyl donor and the reaction conditions they do or do not rearrange to the thermodynamic glycosides. NMR analysis of trisaccharide **7** in deuterated chloroform showed that the glucosamine ring adopted an unusual conformation that disappeared when it was dissolved and analyzed in deuterated DMSO. To confirm the structure of **7**, we also report here the unequivocal and efficient synthesis of the *N*-acetylacetamido trisaccharide **11** (Scheme 2) via

Scheme 2



the glycosylation of acceptor **10** with rhamnosyl donor **5**. Deacetylation of both **7** and **11** led to the identical trisaccharide **12**.

Coupling of the known[4] disaccharide glycosyl acceptor **4** with α-L-rhamnopyranosyl trichloroacetimidate glycosyl donor[13] **5** was first attempted at low temperature and with 0.1 equiv of triethylsilyl trifluoromethanesulfonate (TESOTf) as promoter (Scheme 1, a). In these mild conditions the

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(9) For example, see: Nilsson, M.; Norberg, T. *Carbohydr. Res.* **1988**, *183*, 71−82.

(10) For example, see: Toepfer, A.; Schmidt, R. R. *J. Carbohydr. Chem.* **1993**, *12*, 809−822.

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imidate **6** was isolated in 42% yield. The imidate structure for **6** was first assigned on the basis of its characteristics measured by NMR. While ^{1}H NMR at 600 MHz showed no signal corresponding to the 2-NH expected in the wanted trisaccharide, an exchangeable signal coupling to H-4 of the glucosamine unit was identified at 3.90 ppm supporting the presence of the free 4-OH group. In accordance with the few examples of *N*-methyl glycosyl acetimidates reported by Sinaÿ et al.[7] a signal assigned to the rhamnosyl H-1″ was found at 6.08 ppm. In addition, an HMBC experiment showed a long-range correlation between this signal and a quaternary carbon at 162 ppm, which was assigned to the imidate carbon C═N. In the ^{13}C NMR spectrum the rhamnosyl anomeric carbon gave a signal at lower chemical shift (92 ppm) than that expected for an *O*-glycoside (∼100 ppm). The $^1J_{C,H}$ coupling constant (177 Hz) measured for this anomeric carbon was in perfect agreement with that of an α-rhamnosidic linkage.[14] Finally, the *N*-acetimidate methyl group gave a signal at 16 ppm while the *N*-acetyl methyl group in acceptor **4** was found at 24 ppm. The structure of **6** was further supported by HRCI-MS giving an observed molecular ion [M + H] at 928.3427 for a calculated value of 928.3450. The formation of imidates during the glycosylation of *N*-acetylglucosamine glycosyl acceptors has long been hypothesized by Hindsgaul et al.[12] However, because they are highly susceptible to hydrolysis and did not survive chromatography on silica gel, no structural evidence could be obtained. Although we did see degradation during workup and purification steps, the imidate **6** was stable enough to undergo a quick column chromatography and be obtained in sufficient amounts and purity to permit its full characterization. When the amount of TESOTf was increased to 0.5 equiv and the reaction conducted overnight at room temperature, only traces of **6** were seen by TLC. A new major product was formed and isolated pure in 52% yield (Scheme 1, b). The NMR characteristics in $CDCl_3$ for this new compound showed the presence of an α-rhamnosyl residue (H-1″ 4.87 ppm, $^1J_{C-1'',H-1''} = 172$ Hz) and the disappearance of the glucosamine 4-OH signal while the NH signal was found at 5.87 ppm. These assignments together with the HRCI-MS data obtained for the molecular ion (found [M + H] 928.3513, calcd 928.3450) led us to identify this compound as being the wanted trisaccharide **7**. Thus it appears that higher temperature and concentration of TESOTf favored the formation of the trisaccharide while the imidate was readily formed at low temperature and concentration of TESOTf. An obvious explanation is that imidate **6** formed initially as the kinetic product of the reaction and then rearranged to the thermodynamically more stable trisaccharide **7** when the temperature and catalyst concentration were high enough. To investigate this hypothesis the imidate **6** was allowed to rearrange in the conditions used when **7** was formed. Indeed, the trisaccharide **7** was then isolated in 50% yield while some degradation of the imidate giving the acceptor **4** and the rhamnose hemiacetal was also observed by TLC. Thus the competitive formation of kinetic imidates that, depending on their stability and the reaction conditions, may or may not rearrange to the thermodynamic glycosides constitutes a third explanation to the low reactivity toward glycosylation of the 4-OH group in *N*-acetylglucosamine derivatives.

Careful study of the ^{1}H NMR spectrum measured for **7** dissolved in $CDCl_3$ showed unusual features. The coupling constants $^3J_{H-1,H-2}$ and $^1J_{C-1,H-1}$ measured for the glucosamine ring were respectively 4.2 and 168 Hz and did not support the expected $^4C_1$ conformation for this residue. As observed for Fuc H-5″ in protected[10,15] and deprotected[6] Le^a-containing oligosaccharides, we also expected that, providing they had similar conformations, H-5″ in the rhamnose analogue **7** would be deshielded to ∼4.7 ppm due to its proximity to GlcNAc O-3, Gal O-4′, and Gal O-5′.[6] However, this signal was found at 4.30 ppm, a chemical shift typically measured for H-5 of rhamnosyl residues when they are not submitted to any deshielding effect.[16] Therefore, it appears that trisaccharide **7** has a different conformational behavior than Le^a derivatives. On the basis of the Karplus equation[17] the $^3J_{H-1,H-2}$ coupling constant can result from **7** existing as a major conformer in which the glucosamine residue is flattened leading to a dihedral angle $\Phi_{H-1,H-2} \approx 130-140°$. However, since coupling constants measure averaged spin couplings, **7** could also exist as a mixture of two conformers: the $^4C_1$ chair ($\Phi_{H-1,H-2} \approx 180°$) and a $^1S_3$ skew ($\Phi_{H-1,H-2} \approx 110°$). Low-temperature NMR experiments (down to −55 °C) did not allow a conformational freeze out, therefore if **7** exists as a mixture of conformers in $CDCl_3$ the energy barrier to interconversion between these conformers must be small. So far NMR experiments did not allow a complete and unambiguous analysis of the conformational behavior of **7** in $CDCl_3$.

Upon Zemplén deacetylation trisaccharide **7** gave a compound the NMR and HRCI-MS characteristics of which did not present any conformational or structural abnormalities and supported its identification as the mono-benzylated analogue **12** (Scheme 2). However, since to our knowledge this is the first time that a conformational behavior such as that observed in trisaccharide **7** is being reported, we engaged in an alternative synthesis of trisaccharide **12**. To prevent imidate formation and thus increase the reactivity of the glycosyl acceptor toward glycosylation at O-4 of the glucosamine residue, we investigated the methodology developed by Crich et al.[11] in which the amino group in glucosamine glycosyl acceptors was bis-acetylated. The known[4] disaccharide **8** was *N*-acetylated in the presence of Hünig's base to give **9** that was converted to the wanted glycosyl acceptor **10** by treatment with $NaBH_3CN$/HCl. As expected, the glycosylation of **10** with donor **5** could be conducted at low temperature, using only 0.15 equiv of TESOTf, and gave the trisaccharide **11** in excellent yield (91%). Zemplén deacetylation of **7** and **11** gave in both cases

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(17) Karplus, M. *J. Am. Chem. Soc.* **1963**, *85*, 2870−2871.

the mono-benzylated trisaccharide **12** in quantitative yield, thus confirming that the NMR characteristics measured for **7** in $CDCl_3$ resulted from this trisaccharide adopting an unusual conformation. Despite their similar structures, trisaccharides **11** and **7** did not show the same conformational behavior in $CDCl_3$. Thus, we postulated that the conformational behavior of **7** resulted from the formation of an inter- or intramolecular hydrogen bond involving the glucosamine NH group rather than from steric hindrance around the glycosidic bonds. To support this hypothesis we recorded NMR data for trisaccharide **7** in the hydrogen bonding-solvent DMSO-d_6. Indeed, in this solvent the coupling constants $^3J_{H-1,H-2}$ and $^1J_{C-1,H-1}$ measured for the glucosamine ring were respectively, 8.0 and 161 Hz and thus typical of a $^4C_1$ conformation for this β-glycoside.[14] In addition, the H-5″ signal of the rhamnosyl residue was then downfield shifted to 4.75 ppm as one would expect in such derivatives.[6,10,15] Therefore, these results support the formation of an inter- or intramolecular H-bond in trisaccharide **7** when it is dissolved in non-hydrogen bonding solvents. Further NMR experiments at various temperatures showed a linear negative temperature dependence of the NH chemical shift δ_{NH} for **7** dissolved in $CDCl_3$. Linear regression led to a $\Delta\delta_{NH}/\Delta T$ value of -3.1 ppb/K, which suggests the formation of an intramolecular hydrogen bond.[18,19] However, NMR spectra collected for various concentrations of **7** in $CDCl_3$ showed a positive concentration dependence of δ_{NH}, which is usually indicative of an intermolecular H-bond.[18,19] Therefore we conclude that the NH group in **7** can form either an inter- or an intramolecular hydrogen bond leading to the unusual conformational behavior that we observed by NMR in $CDCl_3$.

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In summary, we have confirmed that imidate formation could be the predominant reaction under glycosylation conditions when *N*-acetylglucosamine derivatives are used as glycosyl acceptors. Such an imidate was isolated for the first time in amounts and purity that allowed its full characterization by NMR and HRCI-MS. Subsequent glycosylation at higher temperature and concentration of catalyst led to the formation of the wanted trisaccharide in 52% yield. When the kinetic imidate was stirred in these conditions, it rearranged to the thermodynamically more stable trisaccharide that was obtained in 50% yield. Thus, the formation of kinetic imidates provides an additional explanation to the exceptionally low reactivity of 4-OH groups toward glycosylation in *N*-acetylglucosamine glycosyl acceptors. We have also established that oligosaccharides containing *N*-acetylglucosamine could adopt unusual conformations induced by inter- or intramolecular hydrogen bonding when dissolved in non-H-bonding solvent. To the best of our knowledge, this is the first time that such conformational behavior is reported for *N*-acetylglucosamine derivatives. Finally, to confirm the chemical structure assigned to this unusual conformation, the Le^a trisaccharide analogue was synthesized unequivocally and in excellent yield employing, as an alternative synthetic strategy, a *N*,*N*-diacetyl protected glucosamine disaccharide derivative as the glycosyl acceptor.

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Supporting Information Available: Spectroscopic data for compounds **6**, **7**, and **9**−**12**. This material is available free of charge via the Internet at http://pubs.acs.org.

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