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PAPER

Regioselectivity of the glycosylation of *N*-dimethylmaleoyl-protected hexosamine acceptors. An experimental and DFT approach†

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Both anomers of the methyl glycoside of 6-*O*-benzyl-*N*-dimethylmaleoyl-D-allosamine (**6** and **7**) are glycosylated exclusively on O3 when reacting with the trichloroacetimidate of peracetylated α-D-galactopyranose (**5**). This regioselectivity is expected for **6**, the α-anomer, as a strong hydrogen bond of its H(O)3 with the carbonyl group of the dimethylmaleoyl group occurs, as shown by NMR temperature dependence. However, this hydrogen bond was not encountered experimentally for **7**, the β-anomer. A DFT study of the energies implied in an analog of the glycosylation reaction charged intermediate has explained neatly this behavior, in terms of strong hydrogen bonds occurring at these charged intermediates. This approach explains both the experimental regioselectivities found for **6** and **7**, but furthermore the calculations have shown a marked agreement with the regioselectivities found for other related compounds in the literature.

Introduction

The increasing importance of the roles that oligosaccharides and glycoconjugates play in biological processes has led to a demand for reliable new procedures for their synthesis. A great deal of attention has been devoted to glycosylation reactions and, consequently, the chemical synthesis of most glycosidic linkages can now be readily achieved.[1] However, the stereo- and regiochemical outcome of glycosylation reactions is often difficult to predict. In fact, small changes in the structure of the glycosyl donor and acceptor, such as protecting groups or stereochemistry, often cause dramatic changes in the outcome of the reaction.[2–4]

The control of the regioselectivity in the synthesis of oligosaccharides usually requires the use of protecting groups in order to select reactions at a specific secondary hydroxyl group, as they have often similar reactivities. This makes synthetic schemes lengthy, laborious and time-consuming. However, sometimes the reactivity of some of those hydroxyl groups is much larger than that of their neighbors, and thus regioselective reactions can be carried out in the presence of limiting amounts of glycosyl donor. Consequently, a better comprehension of the regioselectivity would allow glycosylations to be carried out without the need (or with minimal need) of protections. Several interesting studies directed to understand the factors that affect the regioselectivity of glycosylation reactions have been reported. In a series of publications, Vasella and co-workers pointed out the importance of hydrogen bonds in diol acceptors mainly in reactions with diazirine glycosyl donors.[5,6] The group of Fraser-Reid and López have shown that the nature (armed or disarmed) of the glycosyl donor has a great influence on the regioselectivity of glycosylation reactions. These observations were the basis for the development of the new concept for "matching" donors with acceptors called "reciprocal donor acceptor selectivity" (RDAS).[7] More recently, the group of Martín-Lomas established, using experimental and theoretical studies,[8] that the regioselectivity for the glycosylation of a given hydroxyl group can be considerably activated by increasing its nucleophilicity, for the presence of groups able to form a hydrogen bond with that hydroxyl group.

Given the biological significance of hexosamines,[9] we have previously analyzed the regioselectivity of the 1,2-*trans*-diequatorial diols of α- and β-anomers of 6-*O*-substituted *N*-dimethylmaleoyl-protected D-glucosamine acceptors, using two disarmed donors and also made theoretical calculations justifying the experimental trends.[10,11] More recently, we reported on the assessment of the reactivity of isomeric *N*-dimethylmaleoyl (DMM) 4,6-*O*-benzylidene protected D-glucosamine and D-allosamine acceptors (**1**, **2**, **3** and **4**) through competition experiments with the disarmed donor **5**. The order of reactivity **3** ≫ **1** > **4** > **2** suggested that the strong hydrogen bond between the H(O)3 with a carbonyl of the DMM, determined by ^{1}H NMR and confirmed by modelling experiments, activated O3 by increasing its nucleophilicity, and compensating the steric hindrance expected for the most reactive (axial) acceptor.[12] In the formation of this critical hydrogen bond

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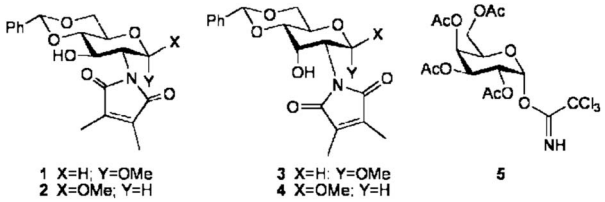
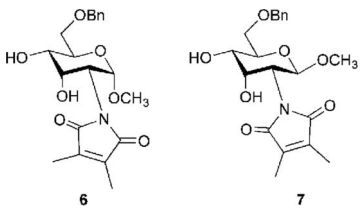
the carbonyl of DMM plays a fundamental role, increasing the benefits of using this protecting group.[13]



Based on these results, we decided to study the differential O3/O4 regioselectivity of the 1,2-*cis*-diols of the anomers of methyl glycosides of *N*-DMM-D-allosamines **6** and **7**, in an attempt to advance our understanding of the electronic factors governing the regioselectivity in the glycosylations of *N*-protected hexosamine derived acceptors.

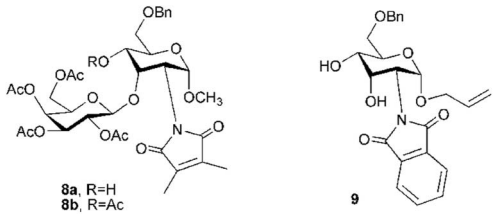


Furthermore, we have attempted to rationalize these observations using modelling tools which analyze the key step for the glycosidation reaction. This analysis showed that the experimental trends were matched by the theory, and it was also used with several literature cases in order to show its ability to predict the trends in other competitive reactions.

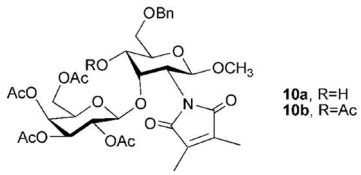
Results and discussion

The anomeric D-allosamine acceptors **6** and **7** were readily obtained by reductive opening of the benzylidene protecting groups of **3** and **4**. With these diols in hand we first carried out an analysis of their ^{1}H NMR spectra in DMSO-d_6 to establish if their hydroxyl groups showed different abilities to form intramolecular H-bonds in solution. The $\Delta\delta/\Delta T$ values obtained for **6** clearly indicated a strong intramolecular H-bond for H(O)3, whereas a weak one was detected for the H(O)4 (−3.5 and −7.1 ppb K^{-1}, respectively).[5,6,14,15] For **7**, the values obtained suggested at best weak intramolecular H-bonds (H(O)3, −6.2 and H(O)4, −5.6 ppb K^{-1}, respectively). On the basis of these $\Delta\delta/\Delta T$ values and on the reactivity observed with acceptors **1–4**, we expected that acceptor **6** would yield mainly the 1→3-linked disaccharide when coupled with the disarmed donor **5**, whereas a mixture of 1→3- and 1→4-linked disaccharides should be expected for acceptor **7**.

When the α-anomer of diol (**6**) was coupled with 1.1 equiv. of donor **5**, under activation with trimethylsilyl triflate (TMSOTf) at −25 °C, the only compound, obtained in 87% yield, was the 1→3-linked disaccharide **8a**. The corresponding 1→4-linked compound was neither detected by TLC nor by ^{1}H NMR spectroscopy. The assignment of the regioisomeric structure of **8a** was readily accomplished by the presence of H1′→C3 and H3→C1′ correlations in its HMBC spectrum and was confirmed by acetylating the disaccharide and determining the chemical shift of the newly downfield-shifted proton (δ_H 5.05, H4 for the acetate **8b**). This result is in complete agreement with our observation that in the α acceptor, the strong hydrogen bond of the axial H(O)3 group with the C=O group of the DMM moiety, by increasing its nucleophilicity, compensated the expected steric hindrance. Furthermore, this result is also in agreement with the report of Maloisel and Vasella regarding the regioselective glycosidation at O3 of the related *N*-phthaloylallosamine-derived acceptor (**9**) with a trichloroacetimidate donor.[16]



In the reaction mixture of the glycosidation of diol **7** only one disaccharide was also detected (TLC, ^{1}H NMR). This was shown to be the 1→3-linked disaccharide **10a**, isolated in 94% yield. The regiochemistry of **10a** was established by using the same methodology described above for **8a** (δ_H 4.98, H4 for the acetate **10b**). The clear preference for the glycosidation at the axial O3 instead of occurring at the equatorial O4 of **7**, showing both weak hydrogen bonds, was surprising at first.



These results indicated that the predictive capability of the regioselectivity outcome for acceptors exhibiting only weak hydrogen bonds is much more complex than for those, such as diol **6**, showing a strong intramolecular hydrogen bond. Consequently, in an attempt to rationalize these observations we turned our attention to a more refined approach, focused on the mechanism of the glycosidation reaction.

As nowadays is generally accepted, we assumed that the glycosidation reaction proceeds through the classical three (or four) steps of an S_N1-like mechanism.[3,17] However, we were not interested in the usually irreversible ionization step, the rate-determining step in classical S_N1 mechanistic discussions as this step would be the same for every spot in the acceptor. Rather, inspired by the report of Whitfield *et al.* we have paid specific attention to the stabilities of the charged intermediates formed in the second step, that is, in the nucleophilic attack of the acceptors on the intermediate formed as a result of the leaving group departure in the donor.[2,18] We have also taken into consideration in the present analysis that Cid *et al.* pointed out that the observed weak hydrogen bonds of the acceptors might not explain the stabilities of the transition states leading to the intermediates and, finally, to the disaccharides.[8] Recently, these authors, in their study on the regioselective glycosylation of inositol-derived diols and other isomeric diol acceptors, found, by DFT modelling of the acceptors and the corresponding complexes prior to transition states, that the weaker hydrogen bonds in the ground state of the acceptors became much more important in these complexes, close in geometry and energy to the transition states.[8]

Moreover, Whitfield *et al.* made a theoretical study directed to rationalize the stereochemical outcome of glycosidation reactions by analyzing the conformations of the electrophilic species

generated from the donor, and decided to replace the acceptor by a simple methanol molecule in order to simplify the calculations.[2,18] By joining the approaches of the groups of Whitfield and Martín-Lomas, and the accepted mechanism of the reaction[3,17] we decided to study the stability (energetics) of the charged intermediates using a methyl group as the donor. Such a simple approach assumes the attack of either of both hydroxyl groups to a methyl carbocation, in an S_N1-like fashion. The intermediate has a (+1) charge, thus giving rise to very strong hydrogen bonds not present in the free acceptor. The balance of hydrogen bonds should show a better stabilization of the intermediate with the methoxyl group on either oxygen, and this result could be then compared with the experimental regioselectivities. This approach assumes that the energy barriers from the intermediates to the disaccharides are very similar, *i.e.* that the relative energies of the intermediates and of their corresponding transition states are similar. One way of testing this approach is to analyze the agreement between the relative stabilities of the intermediates and the observed experimental regioselectivities.

Analogs of **6** and **7** carrying a methyl group on O6 instead of a benzyl group (**6m** and **7m**, respectively) were used as the basis acceptors to which methyl groups were added on O3 and O4. These were analyzed by DFT at the B3LYP/6-31+G(d,p) level. Several different conformers were found for each adduct, depending on the orientation of the exocyclic groups. For **6m**, the two more stable for the substitution on O3 and O4 are shown in Fig. 1.

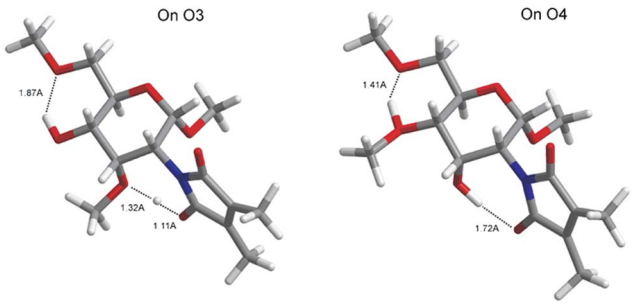


Fig. 1 Molecular representations of the most stable conformers of the compound **6m** methylated on O3 and O4, calculated at the B3LYP/6-31+G** level. The H–O distances for the strong hydrogen bonds are indicated.

The structure methylated on O3 is more stable than the second one by 8.64 kcal mol^{-1}. In the former, a very strong hydrogen bond is formed between the proton on O3 and the carbonyl of the DMM protecting group. This is the same bond occurring in the uncharged ground state acceptor, but much stronger because of the charge. The proton is actually in-between both oxygen atoms, and closer to the oxygen of the carbonyl group (Fig. 1). The hydrogen bond occurring between H(O)4 and O6 in the O4-methylated adduct is strong, but weaker than that occurring in the O3-methylated adduct. This result matches the clear preference for the glycosidation at O3 found experimentally for compound **6**.

A similar analysis for compound **7m** leads to the equivalent conformers shown in Fig. 2. Although the uncharged compound **7** does not show experimentally a strong hydrogen bond to the carbonyl group, its reaction intermediate seems to give a very strong one, thus stabilizing again the compound with O3 methylation by 6.93 kcal mol^{-1} against the O4-methylated

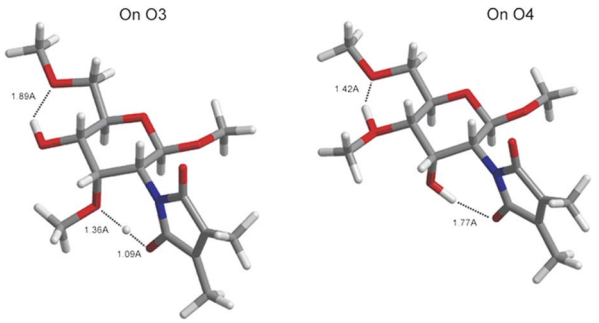
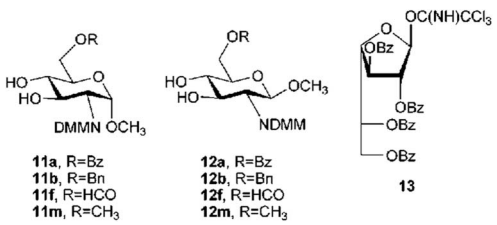


Fig. 2 Molecular representations of the most stable conformers of the compound **7m** methylated on O3 and O4, calculated at the B3LYP/6-31+G** level. The H–O distances for the strong hydrogen bonds are indicated.

compound. The experimental result is once more giving ground to the theoretical approach: compound **7** shows also a marked preference for O3 substitution.

In these examples the H(O)3 and the O═C of the DMM group are in the most favorable position to form a strong hydrogen bond, as O3 is axial. It might thus be arguable if this agreement between the experimental and theoretical data will hold for other *N*-dimethylmaleoyl D-hexosamine acceptors. Thus, we decided to apply the same approach to the D-glucosamine acceptors **11** and **12**. For these acceptors carrying benzoyl and benzyl groups (**11a/11b** and **12a/12b**), we had already determined the differential O3/O4 regioselectivity using two disarmed donors and also made theoretical calculations[11] on their simpler analogs (**11f/11m** and **12f/12m**) justifying the experimental trends.[10] Furthermore, we have currently done the temperature dependence NMR experiments in DMSO-d_6 solution carried out on the acceptors themselves. Their values (see the Experimental section) indicated only weak intramolecular hydrogen bonds for their hydroxyl groups.



11a, R=Bz
11b, R=Bn
11f, R=HCO
11m, R=CH_3

12a, R=Bz
12b, R=Bn
12f, R=HCO
12m, R=CH_3

13

Different conformers of the O3- and O4-methylated adducts of **11f**, **11m**, **12f** and **12m** were analyzed by DFT. For the most stable compounds, the results are shown in Table 1. An astonishing match between the theoretical results and the experimental regioselectivities is observed: **11a/11f** show preference for O3 substitution and show a higher stability for the O3-methylated intermediate, whereas **11b/11m** show the opposite trend both experimentally and theoretically. The other two cases follow the same trend. Furthermore, the energy values are especially adjusted to the reaction with the furanosyl donor **13**: both methylated compounds have about the same energy as **12f**, for which parent compound **12a** no regioselectivity was found experimentally. The other three compounds show the sign and magnitude of the energy difference in agreement with the observed regioselectivity.[10]

We have previously assessed that the strong hydrogen bond between H(O)3 and the O═C of DMM is responsible for the higher

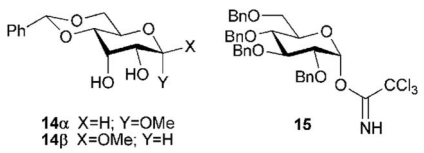
Table 1 Differences in energy for the O3- and O4-methylated adducts of **11m**, **11f**, **12m**, and **12f**, together with the experimental regioselectivities of their parent compounds with the donors **5** and **13**

Compound	Ratio O3/O4 with donor **5**[a]	Ratio O3/O4 with donor **13**[a]	$E_{MeO3} - E_{MeO4}$/ kcal mol^{-1}
11a/11f	2 : 1	1 : 0	–4.60
11b/11m	1 : 1	3.2 : 1	–1.85
12a/12f	1 : 13	1 : 1	–0.03
12b/12m	0 : 1	1 : 2.9	+2.15

[a] From ref. 10

stability of O3-methylated adduct. This is true for both α-anomers **11f** and **11m**, for which a torsion of the DMM group is needed for the hydrogen bond to occur, and where the α-methoxyl group promotes this torsion. For the β-anomers **12f** and **12m**, the torsion of the DMM group is not straightforward: it requires energy to occur.[11] For the main O3-methylated conformers the DMM group leans in order to generate a hydrogen bond, but this is not as favorable as occurred for α-anomers, thus destabilizing partially these structures. The O4-methylated compounds gain ponderation for these anomers, especially when a methyl group is present on O6 (**12m**), as this oxygen atom has more electron availability for a H(O)4 bond than that occurring on a formylated/benzoylated derivative (**12f**).

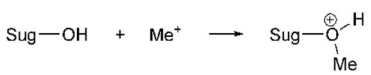
A further example of the application of this approach can be given using a non-nitrogenated acceptor like Vasella's **14**α and **14**β, 4,6-*O*-benzylidene-D-allose methyl glycosides having free O2 (equatorial) and O3 (axial).[6] The present approach (using a ethylidene group instead of a benzylidene group) shows a higher stability for the O3-methylated adduct of 4.39 kcal mol^{-1} (**14**α) and 2.25 kcal mol^{-1} (**14**β). The experimental reactivities against donor **15** favored O3 by 2.6 : 1 (**14**α) and 1.2 : 1 (**14**β), showing once again that the experimental and theoretical trends match.



14α X=H; Y=OMe
14β X=OMe; Y=H

15

These results show that the current approach is useful to compare the relative reactivities of the hydroxyl groups present in diols but we have wondered if it can be used to compare the relative reactivities of two hydroxyl groups present in different compounds. As explained above, the experimental relative reactivities of the monosaccharide derivatives **1** and **2** carrying a single hydroxyl group each are known (**1** ≫ **2**). Using analogs of **1** and **2** where the phenyl group was replaced by a methyl group (**1e** and **2e**), we have analyzed both the ground states (reactants) and the O-methylated derivatives, in order to determine the energy difference for the reaction:



where the lower-energy conformers of Sug–OH and its methoxylated counterpart were used for the calculation. For **1e**, the calculation leads to a Δ*E* of –107.6 kcal mol^{-1}, whereas for **2e** the Δ*E* is –99.7 kcal mol^{-1}. This large difference is clearly indicating that the O3 in **1e** has higher reactivity towards donors, as it has been shown experimentally to occur when comparing the reactivities of **1** and **2**.[12]

We are aware that in the current theoretical approach an overestimation of the hydrogen bonds is feasible, as other factors not considered (solvent, additives, counterion, *etc.*) are present. However, we understand that this approach is useful to identify the trends which might be useful to help in the prediction of regioselectivity in glycosylation reactions.

Experimental

General

Melting points were determined on an Electrothermal 9100 apparatus and are uncorrected. The ^{1}H and ^{13}C NMR spectra were recorded on Bruker Avance 300 spectrometer. For the 2D experiments, Bruker standard software was employed. High resolution mass spectrometry (HRMS-ESI) was performed in a Bruker microTOF-Q II instrument. Optical rotations were measured with a Jasco DIP-1000 polarimeter. Column chromatography was performed on Silica Gel 60 H, slurry-packed, run under low pressure of nitrogen and employing increasing amounts of EtOAc in hexane as solvent. Analytical TLC was carried out using Kieselgel GF254 (E. Merck) with a thickness of 0.20 mm. The homogeneity of all compounds prior to the high-resolution mass spectral determination was carefully verified by TLC. Reactions were routinely run under a dry nitrogen atmosphere with magnetic stirring. All chemicals were used as purchased or purified according to standard procedures.

Methyl 6-*O*-benzyl-2-deoxy-2-dimethylmaleimido-α-D-allopyranoside (6). To a solution of the 4,6-*O*-benzylidene acetal **3** (630 mg, 1.61 mmol) and $BH_3{\cdot}N(CH_3)_3$ (230 mg, 3.15 mmol) in CH_3CN (16.5 ml) in an ice-water bath was added dropwise $BF_3{\cdot}OEt$ (0.407 ml, 3.73 mmol). After 1 h at this temperature, the solution was stirred at room temperature for an additional 0.5 h (TLC), and $NaHCO_3$ (243 mg) was then added, and the solution was evaporated to dryness. The crude product in Cl_2CH_2 was washed with brine, dried (Na_2SO_4), and evaporated. The residue was chromatographed to yield **6** (426 mg, 67% yield), as a foamy solid $[\alpha]_D^{27}$ +125.5 (*c* 0.3, $CHCl_3$); R_f 0.18 (1 : 1 hexane–EtOAc). ^{1}H NMR (300 MHz; $CDCl_3$; Me_4Si): δ 7.40–7.26 (5H, m, ArH), 5.77 (1H, d, J = 1.6 Hz, H(O)3), 4.69 (1H, d, $J_{1,2}$ = 3.6 Hz, H1), 4.64 (2H, s, CH_2Ph), 4.29 (1H, dd with appearance of t, $J_{2,3}$ = 3.4 Hz, H2), 4.26–4.22 (1H, m, H3), 4.08 (1H, ddd, $J_{5,6a}$ = 2.4, $J_{5,6b}$ = 4.6, $J_{5,4}$ = 9.9 Hz, H5), 3.85 (1H, dd, $J_{6a,6b}$ = 10.7 Hz, H6a), 3.79 (1H, dd, H6b), 3.74 (1H, ddd with appearance of dt, $J_{4,3}$ = 2.8, $J_{4,OH}$ = 10.4 Hz, H4), 3.34 (3H, s, OCH_3), 2.76 (1H, d, H(O)4), 2.00 (6H, s, CCH_3 × 2). ^{13}C NMR (75 MHz; $CDCl_3$; Me_4Si): δ 172.50 (CO × 2), 138.30 (C-Ar), 137.77 (C × 2) 128.31–127.50 (C-Ar), 98.39 (C1), 73.49 (CH_2Ph), 69.38 (C6), 68.49 (C3), 68.10 (C4), 67.53 (C5), 55.86 (OCH_3), 54.57 (C2), 8.93 (CCH_3 × 2). ESI-HRMS: calcd for $[C_{20}H_{25}NO_7 + Na]^+$ 414.1523. Found, *m*/*z* 414.1515.

Methyl 6-*O*-benzyl-2-deoxy-2-dimethylmaleimido-β-D-allopyranoside (7). Following the same procedure described for the preparation of **6**, starting with **4** (348 mg, 0.89 mmol), **7** was obtained (250 mg, 72% yield), as a foam; $[\alpha]_D^{29}$ –42.5 (*c* 0.6, $CHCl_3$); R_f 0.15 (1 : 1 hexane–EtOAc). ^{1}H NMR (300 MHz;

$CDCl_3$; Me_4Si): δ 7.38–7.27 (5H, m, ArH), 5.39 (1H, d, $J_{1,2}$ = 8.7 Hz, H1), 4.62 (2H, s, CH_2Ph), 4.15 (1H, br t, H3), 4.02 (2H, dd, $J_{2,3}$ = 2.4 Hz, H2), 3.96 (1H, dd, $J_{5,6}$ = 5.0, $J_{5,4}$ = 9.9 Hz, H5), 3.79 (1H, dd, H6), 3.78 (1H, br s, H(O)3), 3.77–3.68 (1H, m, H4), 3.43 (3H, s, OCH_3), 3.02 (d, 1H, J = 6.5 Hz, H(O)4), 1.98 (6H, s, CH_3 × 2). ^{13}C NMR (75 MHz; $CDCl_3$; Me_4Si): δ 172.50 (CO), 137.76 (C-Ar), 137.55 (C × 2), 128.48–127.76 (C-Ar), 96.87 (C1), 73.72 (CH_2Ph), 72.43 (C5), 70.93 (C3), 70.76 (C6), 69.87 (C4), 56.78 (OCH_3), 55.62 (C2), 8.86 (CCH_3, × 2). ESI-HRMS: calcd for $[C_{20}H_{25}NO_7 + Na]^+$ 414.1523. Found, m/z 414.1520.

Temperature dependence of the chemical shift of the hydroxyl groups of 6, 7, 11 and 12 in ^{1}H NMR. ^{1}H NMR (300 MHz) spectra were recorded for solutions of **6**, **7**, **11a**, **11b**, **12a** and **12b** in DMSO-d_6 (internal standard, for the ^{1}H residual DMSO). Assignments of proton resonances were based on two-dimensional ^{1}H–^{1}H correlation experiments. Four spectra were recorded at different temperatures in the 298–350 K range. The $\Delta\delta/\Delta T$ (ppb K^{-1}) were obtained from a linear fit.

The $\Delta\delta/\Delta T$ values obtained for **11a**: H(O)3 (–5.9 ppb K^{-1}) and H(O)4 (–5.5 ppb K^{-1}); for **11b**: H(O)3 (–5.9 ppb K^{-1}) and H(O)4 (–6.0 ppb K^{-1}); for **12a**: H(O)3 (–5.4 ppb K^{-1}) and H(O)4 (–5.6 ppb K^{-1}) and for **12b**: H(O)3 (–5.5 ppb K^{-1}) and H(O)4 (–6.0 ppb K^{-1}).

General procedure for glycosylation and acetylation reactions

A suspension of the acceptor **6** or **7** (0.1 mmol), donor **5** (0.11 mmol), activated 4 Å molecular sieves (106 mg) in anhydrous CH_2Cl_2 (4.9 ml) and CH_3CN (133 µl) were stirred at room temperature. After 40 min, the mixture was cooled to –25 °C, TMSOTf (0.21 mmol) was slowly added and the stirring continued for 30 min. The mixture was then neutralized by addition of solid $NaHCO_3$ (188 mg) and filtered through a silica gel pad with copious washings with EtOAc. The filtrate was dried (Na_2SO_4) and evaporated. The residue was chromatographed to give the products. The acetylations were carried out under standard conditions: pyridine, DMAP, Ac_2O, room temperature, overnight.

Methyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→3)-6-*O*-benzyl-2-deoxy-2-dimethylmaleimido-α-D-allopyranoside (8a). 86.5%; as a foamy solid: $[\alpha]_D^{31}$ + 26.8 (*c* 0.4, $CHCl_3$); R_f 0.09 (1 : 1 hexane–EtOAc). ^{1}H NMR (300 MHz; $CDCl_3$; Me_4Si): δ 7.36–7.26 (5H, m, ArH), 5.31 (1H, d, $J_{4',3'}$ = 3.1 Hz, H4′), 5.12 (1H, dd, $J_{2',1'}$ = 8.0, $J_{2',3'}$ = 10.3 Hz, H2′), 5.02 (1H, d, $J_{1,2}$ = 4.7 Hz, H1), 4.94 (1H, dd, H3′), 4.63 (1H, dd with appearance of t, $J_{3,2} \approx J_{3,4}$ = 4.3 Hz, H3), 4.61 (1H, d, $J_{2',1'}$ = 8.0 Hz, H1′), 4.60 (1H, d, J = 12.0 Hz, CH_2Ph), 4.54 (1H, d, CH_2Ph), 4.34 (1H, dd with appearance of t, H2), 4.07–4.00 (1H, m, H5), 3.95 (1H, dd, $J_{6'a,5'}$ = 7.6, $J_{6'a,\ 6'b}$ = 10.7 Hz, H6′a), 3.90–3.73 (4H, m, H4, H5′, H6′b, H(O)4), 3.70 (2H, d, $J_{6,5}$ = 4.1 Hz, H6), 3.37 (3H, s, OCH_3), 2.12 (3H, s, $COCH_3$), 1.99 (3H, s, $COCH_3$), 1.95 (3H, s, $COCH_3$), 1.94 (6H, s, CCH_3 × 2), 1.92 (3H, s, $COCH_3$). ^{13}C NMR (75 MHz; $CDCl_3$; Me_4Si): δ 172.17–169.15 (CO), 137.72 (C-Ar), 137.18 (C × 2), 128.46–127.71 (C-Ar), 100.49 (C1′), 96.85 (C1), 73.64 (CH_2Ph), 73.10 (C3), 70.89 (C3′), 70.57 (C6), 70.37 (C5′), 69.76 (C5), 69.28 (C2′), 66.79 (C4′), 66.66 (C4), 60.86 (C6′), 55.57 (OCH_3), 51.60 (C2), 20.69–20.54 ($COCH_3$ × 4), 8.75 (CCH_3 × 2). ESI-HRMS: calcd for $[C_{34}H_{43}NO_{16} + H]^+$: 722.26546. Found, m/z: 722.26684.

Acetate 8b. $[\alpha]_D^{32}$ + 46.4 (*c* 0.44, $CHCl_3$); R_f 0.13 (1 : 1 hexane–EtOAc). ^{1}H NMR: δ 5.05 (1H, dd, $J_{4,3}$ = 3.1, $J_{4,5}$ = 10.5 Hz, H4). ESI-HRMS: calcd for $[C_{36}H_{45}NO_{17} + Na]^+$: 786.25797. Found, m/z: 786.25748.

Methyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→3)-6-*O*-benzyl-2-deoxy-2-dimethylmaleimido-β-D-allopyranoside (10a). 93.5%; as a foamy solid: $[\alpha]_D^{32}$ –66.1 (*c* 0.34, $CHCl_3$); R_f.0.15 (1 : 1 hexane–EtOAc). ^{1}H NMR (300 MHz; $CDCl_3$; Me_4Si): δ 7.40–7.27 (5H, m, ArH), 5.66 (1H, d, J = 8.7 Hz, H1), 5.30 (1H, d, $J_{4',3'}$ = 3.2 Hz, H4′), 5.09 (1H, dd, $J_{2',1'}$ = 7.8, $J_{2',3'}$ = 10.4 Hz, H2′), 4.94 (1H, dd, H3′), 4.60 (1H, d, J = 11.6 Hz, CH_2Ph), 4.52 (1H, d, CH_2Ph), 4.51 (1H, d, H1′), 4.08 (1H, dd with appearance of br t, H3), 3.90 (1H, dd, $J_{6'a,5}$ = 7.4, $J_{6'a,6'b}$ = 10.5 Hz, H6′a), 3.86–3.68 (6H, m, H2, H4, H5, H6a, H6b, and H5′), 3.54 (1H, dd, $J_{6'b,5'}$ = 6.4 Hz, H6′b), 3.47 (3H, s, OCH_3), 3.01 (1H, d, J = 2.8 Hz, H(O)4), 2.13 (3H, s, $COCH_3$), 2.02 (3H, s, $COCH_3$), 1.95 (6H, s, CCH_3 × 2), 1.93 (6H, s, $COCH_3$). ^{13}C NMR (75 MHz; $CDCl_3$; Me_4Si): δ 172.29–169.45 (CO), 137.94 (C), 137.11 (C-Ar), 135.97 (C), 128.58–127.99 (C-Ar), 102.01 (C1′), 97.96 (C1), 77.47 (C3), 74.00 (CH_2Ph), 71.57 (C6), 71.46 (C5), 70.89 (C4), 70.64 (C3′), 69.93 (C5′), 69.24 (C2′), 66.75 (C4′), 60.85 (C6′), 56.91 (OCH_3), 55.47 (C2), 20.77–20.54 ($COCH_3$ × 4), 8.67 (CCH_3), 8.48 (CCH_3). ESI-HRMS: calcd for $[C_{34}H_{43}NO_{16} + Na]^+$: 744.24741. Found, m/z: 744.24604.

Acetate 10b. $[\alpha]_D^{32}$ –7.29 (*c* 0.38, $CHCl_3$); R_f 0.20 (1 : 1 hexane–EtOAc). ^{1}H NMR: δ 4.98 (1H, dd, $J_{4,3}$ = 2.7, $J_{4,5}$ = 10.1 Hz, H-4). ESI-HRMS: calcd for $[C_{36}H_{45}NO_{17} + Na]^+$: 786.25797. Found, m/z: 786.25489.

Computational determinations

Quantum mechanical calculations were performed using Jaguar 6.0,[19] using default minimization methods and termination conditions. All the DFT calculations were made at the B3LYP/6-31+G(d,p) level, starting with different geometries around the exocyclic moieties, before and after the introduction of the methyl group. For the latter group, it has been shown that the group leads to a lower energy isomer when it is *gauche* (rather than *anti*) to the hydrogen attached to the carbon (dihedral angle Hn–Cn–On–Me ≈ ±60°). For the determination of the DE of methylation of compounds **1e** and **2e**, the lower-energy conformers for the non-methylated and methylated were used for the calculation, as well as a methyl carbocation optimized separately.

Conclusions

We have herein presented experimental evidences of the influence of a strong hydrogen bond in the regioselectivity of the α-anomer of the *N*-DMM-D-allosamine diol acceptor (**6**) when coupled with the disarmed donor **5**. Owing to the difficulties for predicting the regioselectivity of the glycosylation reaction of the β-anomer **7** (showing only weak hydrogen bonds) with the same donor, we have calculated by DFT the relative stabilities of the charged intermediates using a rather simple model, which assumes that these stabilities might account for the observed regioselectivity. On the basis of these calculations we have rationalized the experimental results. Furthermore, by applying the same approach we were able to rationalize the more complex regioselectivity outcome observed previously with the *N*-DMM-D-glucosamine acceptors **11a,b** and **12a,b**. The theoretical data have agreed with experimental results even better than we expected. Our intention to

identify trends was fulfilled with this approach, which in turn can be used hopefully by synthetic chemists to predict regioselectivity outcomes, and thus to improve the efficiency of glycosylation reactions.

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