A comparative study of the influence of some protecting groups on the reactivity of D-glucosamine acceptors with a galactofuranosyl donor

María L. Bohn, a María I. Colombo, a Carlos A. Stortz b and Edmundo A. Ruveda a,*

a Instituto de Química Orgánica y de Síntesis (CONICET-UNR), Facultad de Ciencias Bioquímicas y Farmacéuticas, Suipacha 531, 2000 Rosario, Argentina
b Departamento de Química Orgánica-CHHIDECAR, Facultad de Ciencias Exactas y Naturales, UBA, Ciudad Universitaria, Pab. 2. 1428 Buenos Aires, Argentina

Received 13 February 2006; received in revised form 13 March 2006; accepted 22 March 2006
Available online 21 April 2006

This paper is dedicated to Professor Rosa M. de Lederkremer for her outstanding contributions to the chemistry of carbohydrates

Abstract—Competitive glycosylation experiments with a galactofuranosyl trichloroacetimidate donor were performed with glucosamine acceptors having a free 4-OH group and carrying different protecting groups at N-2, O-3, and O-6. The most reactive acceptor is the N-dimethylmaleimido 3,6-di-O-benzylated derivative (6c), which reacts even faster than the oxazolidinone 1a. Molecular orbital calculations have helped to rationalize these experimental facts in terms of a hard–hard reaction occurring between the donor and the acceptor.

Keywords: Glucosamine acceptors; N-Dimethylmaleoyl group; Galactofuranosyl donor; Competitive glycosylations; DFT

1. Introduction

Many oligosaccharides and glycoconjugates carry 4-linked N-acetylglucosamine moieties. 1 It is well known that the low reactivity at this 4-position carrying protecting groups at O-3 and O-6 sometimes hampers the synthesis of these biologically important compounds in acceptable yields. 2 This low reactivity has been attributed to steric factors and to the formation of a deactivating hydrogen bond in which the amido group is involved. 2,3 Recent observations that the amide group may itself be glycosylated by active species leading to O-glycosyl imidates provide a third explanation to the low reactivity of N-acetylglucosamine acceptors. 4,5 As a consequence, protection and synthetic strategies for the 2-amino moiety of D-glucosaminyl acceptors play a critical role in glycosylation reactions and post-glycosylation chemical manipulation to obtain target glycosides. 6

To the best of our knowledge there are only two reports directed to assess the effect of N-protecting groups on the relative reactivity of glucosamine acceptors. In one of them, Crich and Dudkin 3 determined, through a competitive experiment, the acceptor reactivity of the 4-OH group of N-acetyl, N-naphthalimido (Phth) and 2-azido-2-deoxyglucose derivatives when coupled with a mannosyl sulfoxide activated with triflic anhydride. They demonstrated that, under these conditions, the azido acceptor is 10 times more reactive than the N-acetylglucosamine acceptor, whereas the Phth acceptor has an intermediate reactivity. In addition, these authors have also shown that N,N-diacetylglucosamine and N-acetyl-N-benzylglucosamine derivatives, with a reactivity comparable to that of the Phth acceptor, were not worthwhile because of the instability of the imide function in the N,N-diacetyl derivative under those conditions.
conditions. Furthermore, the complicated NMR spectra obtained for the N-acetyl-N-benzyl derivative indicates instability also for this compound.

More recently, in a comparative study of the reactivity of the N-acetyl, N-Phth, and N-2,2,2-trichloroethoxy-carbonyl (Troc)-protected glucosamine derivatives, Matta and co-workers\(^7\) showed that the N-Troc group afforded greater 4-OH reactivity than the N-Phth and N-acetylglucosamine acceptors when coupled with a galactopyranosyl imidate donor in a trimethylsilyl triflate (TMSOTf) promoted glycosylation. The 4-OH of galactopyranosyl imidate donor in a trimethylsilyl triacetamido-3,6-di-O-benzyl-2-deoxy-α-D-glucopyranoside (1a)\(^10\) suggested that these derivatives were the ideal acceptors for the synthesis of oligosaccharides carrying 4-linked N-acetylglucosamine moieties.

The recent discovery of furanoside components in a wide variety of natural products of biological significance\(^11–13\) prompted us to study the reactivity of a wide variety of natural products of biological significance. Lederkremer and co-workers\(^14\) that galactofuranosides such molecules to be isolated. It has been shown by de Lederkremer and co-workers\(^14\) that galactofuranosides are good donors even when coupled with N-acetylgulcosamine derivatives. They found that the coupling of penta-O-benzoyl-D-galactofuranose (2a) with benzyl 2-acetamido-3,6-di-O-benzyl-2-deoxy-α-D-glucopyranoside (3a), employing tin(IV) chloride as catalyst, gave the disaccharide 4a in 85% yield. They also found that the coupling of tetra-O-benzoyl trichloroacetimidate 2b with the glucosamine derivative 3b in the presence of TMSOTf gave 4b in 77% yield (Scheme 1). These disaccharides were shown to be the β-anomers on the basis of the small galactofuranose \(^1J\_\text{CH}^\text{C1}\) anomic coupling constant and by the C-1\(^13\)C NMR chemical shift.\(^15\)

Herein we report some observations, through competitive experiments with a galactofuranosyl trichloroacet-imidate donor that clearly establish the influence of the N-dimethylmaleoyl group and electron-withdrawing and electron-donating groups at O-3 and O-6 on the reactivity of the 4-OH group of D-glucosamine acceptors.

2. Results and discussion

The purpose of this work is to study the relative reactivity of acceptors 5, 6a, 6b, and 6c in competition with the highly reactive N-acetylgalactosaminyl protected glucosamine 1a by coupling with trichloroacetimidate 2b. We considered it interesting to include in this study the N-dimethylmaleimido (DMM) glucosaminyl acceptors 6a, 6b, and 6c, not only because this protecting group removes the deactivating hydrogen bond as well as the tendency toward amide glycosylation, but also because, as was shown by Schmidt and co-workers,\(^16\) the N-DMM protected sugars are easily prepared, are stable enough to acid and nonnucleophilic bases, and are readily transformed into their corresponding N-acetyl derivatives.

The acceptors 1a, 5, 6a, 6b, and 6c were prepared by standard techniques. The preparation of 1a was carried out following the sequence described by Crich and Vinod\(^10\) from glucosamine hydrochloride, except that for the transformation of the 4,6-O-benzylidene-protected glucosamine 7 into the corresponding oxazolidinone 8 (Scheme 2), we found it more convenient to use triphosgene [bis(trichloromethyl)carbonate] in anhydrous EtOAc and Et\(_3\)N,\(^17\) instead of p-nitrophenyl chloroformate and Amberlyst IR-120 resin. Under these
conditions the yield of 8 is excellent (87% after a column chromatography) and the workup is very simple.

For the preparation of 5 we have followed the sequence previously described.18 Acceptors 6a, 6b, and 6c were readily prepared from the known triol 9b16,19 (Scheme 3). Regioselective benzylation of 9b afforded 6a in 69% yield, while 4,6-O-benzylidene protection of 9b, followed by benzylation or benzoylation and reductive opening of the benzylidene group, gave 6b or 6c in 89% and 51% overall yield, respectively.

Each acceptor was then coupled with the tetra-O-benzoyl galactofuranosyl trichloroacetimidate donor (b) benzaldehyde dimethyl acetal, CSA, DMF; (c) benzoyl chloride,

Scheme 2.

With authentic samples of disaccharides 11, 12, 13a, 13b, and 13c in hand, we carried out a series of competitive glycosylation experiments in which 2b was allowed to react under the same reaction conditions with an equimolecular mixture of 1a and acceptors 5, 6a, 6b, and 6c, respectively. The crude reaction mixtures were then analyzed by HPLC to determine the ratios of the disaccharides obtained. As expected, the oxazolidinone-protected glucosamine acceptor 1a was shown to be eleven times more reactive than the acetamido derivative 5 (12:11, 11:1), whereas 1a was seven times more reactive than the N-DMM-protected acceptor 6a (12:13a, 7:1).

Interestingly enough, the competitive experiment between 1a and 6b showed that both acceptors displayed rather similar reactivities (12:13b, 1.5:1), whereas 6c was shown to be quite more reactive than 1a (12:13c, 1:4). The ratios were also determined by 1H NMR spectroscopy of the mixtures as explained in Section 3. Although the slightly higher reactivity of 1a in comparison with 6b can be attributed to the restricted nature of the protection on O-3 that minimizes the steric hindrance on 4-OH,10 and the known activating and deactivating effect of electron-donating and electron-withdrawing groups can be invoked to explain that 6c was the most reactive acceptor and that 6b was more reactive than 6a (3,6-di-O-benzyl in 6c and 3-O-benzoyl-6-O-benzyl in 6b vs 3,6-di-O-benzyl in 6a),21 it seemed to us interesting to rationalize the differential reactivity of these acceptors.

Analogs of 1a, 6a, 6b, and 6c where the benzoyl groups were replaced by a formyl moiety, and the benzyl groups were replaced by a methyl group (1c, 6d, 6e, and 6f, respectively) were submitted to minimization using DFT at the B3LYP/6-31+G** level. This level of basis set was considered necessary to yield good results on carbohydrates.22 As Table 1 shows, the conformation showing a hydrogen bond between H(O)-4 and O-6 is preferential in the cases where a methyl group resides on O-6. Modeling of the compound 6d, which carries a formyl group on C-6, shows the alternative conformation as the main one (Table 1), indicating than it can predict a weaker hydrogen bonding on a oxygen involved in an ester function. The same result is observed by looking at the distance between H(O)-4 and O-3 in the alternative conformation for the four compounds (Table 1): the shortest is that occurring for the 3-O-methylated compound, as expected considering its enhanced nucleophilic character.23 Although it is not expected that a methyl or a formyl group can reproduce exactly the features of a benzyl or a benzoyl, respectively, it is expected that they should show the projected trend, considering that modeling of such large groups is difficult at this level of theory.

Chemical reactivity can be discussed within the framework of density functional theory (DFT).24–26 The introduction of numerical descriptors24–28 has provided quantitative concepts for the reactivity of different compounds, as well as of sites within the same compound with nucleophilic, electrophilic, and radical reagents. One of the more common descriptors of reactivity is the Fukui function, related to the electron density in
the frontier molecular orbitals HOMO (for electrophilic attack) and LUMO (for nucleophilic attack).\textsuperscript{24,26–28} However, this approach seems to be valid when, in the application of the hard and soft acids and base principle (HSAB), the interaction between nucleophile and electrophile corresponds to a soft–soft interaction.\textsuperscript{26,29} On the other hand, the hard–hard interactions are not frontier controlled, and a different descriptor of reactivity is needed.\textsuperscript{26} For such systems, it was postulated that either a minimal value of the Fukui function\textsuperscript{26} or a maximum value of the net charge is required at the reaction site.\textsuperscript{29}

For each conformer, charge and Fukui functions were determined. It is not expected that vacuum calculations will reproduce the behavior of these molecules in a dichloromethane solution (containing small amounts of acetonitrile). However, probably the low polarity of the solvent will not introduce large differences, and thus the present modeling can help just as an approximation to the real system. Table 2 shows the results for each of the compounds carrying conformation b as the main one (1c, 6e, and 6f), considering Boltzmann-averaged populations. The less reactive oxazolidinone derivative (1a/1c) shows a smaller charge and a higher Fukui function on O-4 (Table 2) than the more reactive 6c/6f. Either data agree with a reaction that is not frontier controlled (hard–hard).\textsuperscript{26,29} Charge considerations are also supportive of the similar reactivity of 1c and 6f analogs, but the Fukui function is always higher for the oxazolidinone derivatives. However, when the hardness/softness of the compound is taken into account,\textsuperscript{29} the local softness on O-4 is smaller for the more reactive compound (analog of 6f) and similar for the other two compounds, as expected for a hard–hard interaction. Within the N-DMM-substituted derivatives, calculations of the Fukui function made by evaluation of the HOMO orbital\textsuperscript{26} also show decreasing values with increasing reactivity, as postulated by Li and Evans for hard–hard interactions.\textsuperscript{26}

In conclusion, based on our experimental observations together with the rationalization of the differential reactivity of several glucosaminyl acceptors, we believe that the easily prepared acceptor 6c, bearing Schmidt’s N-DMM protecting group, represents an interesting alternative for the synthesis of 4-linked glucosamine oligosaccharides.

### 3. Experimental

#### 3.1. General methods

Melting points were determined on an Electrothermal 9100 apparatus and are uncorrected. The $^1$H and $^{13}$C NMR spectra were recorded on a Bruker AC 200 spectrometer for CDCl$_3$ solutions with Me$_4$Si as internal standard. For the 2D and COSY experiments, Bruker standard software was employed. Mass spectra were measured using MALDI-TOF HRMS and HRFABMS at the UCR Mass Spectrometry Facility (Department of Chemistry, University of California Riverside, USA) and Kent Mass Spectrometry (Kent, UK). Optical rotations were measured with a Jasco DIP-1000 polarimeter. High-performance liquid chromatography (HPLC) was performed using a HP 1100 with Lichrospher RP-8 (5 μm), a Quat pump, and gradient controller equipped with a variable wavelength UV detector. 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.

### Table 1. Energy and selected geometry data on the two main conformers (a and b) obtained for compounds 1c, 6d, 6e, and 6f calculated at the B3LYP/6-31+G** level

<table>
<thead>
<tr>
<th>Compound</th>
<th>Relative $E$ (kcal)</th>
<th>$\theta_{O-5\cdot\cdot\cdotC-5\cdot\cdot\cdotO-6\cdot\cdot\cdotC-6}$ (°)</th>
<th>$\theta_{H-4\cdot\cdot\cdotC-4\cdot\cdot\cdotO-4\cdot\cdot\cdotH-4}$ (°)</th>
<th>$d_{HO-4\cdot\cdot\cdotO-3}$ (Å)</th>
<th>$d_{HO-4\cdot\cdot\cdotO-6}$ (Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1c, a</td>
<td>2.76</td>
<td>73</td>
<td>-170</td>
<td>3.23</td>
<td>2.00</td>
</tr>
<tr>
<td>1c, b</td>
<td>0.00</td>
<td>178</td>
<td>69</td>
<td>-</td>
<td>2.78</td>
</tr>
<tr>
<td>6d, a</td>
<td>0.00</td>
<td>72</td>
<td>-94</td>
<td>2.77</td>
<td>2.18</td>
</tr>
<tr>
<td>6d, b</td>
<td>2.23</td>
<td>180</td>
<td>61</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>6e, a</td>
<td>0.83</td>
<td>70</td>
<td>-96</td>
<td>2.77</td>
<td>-</td>
</tr>
<tr>
<td>6e, b</td>
<td>0.00</td>
<td>-179</td>
<td>69</td>
<td></td>
<td>2.03</td>
</tr>
<tr>
<td>6f, a</td>
<td>1.31</td>
<td>71</td>
<td>-65</td>
<td>2.38</td>
<td>-</td>
</tr>
<tr>
<td>6f, b</td>
<td>0.00</td>
<td>180</td>
<td>70</td>
<td></td>
<td>2.01</td>
</tr>
</tbody>
</table>

\* Using the Fukui function determined from charges obtained according to the CHelp scheme.\textsuperscript{31}
\* Using the Fukui function determined from Mulliken charges.\textsuperscript{27}
\* Using the Fukui function determined from fitting to the electrostatic potential.\textsuperscript{30}
\* Using the Fukui function determined from charges obtained according to the CHelpG scheme.\textsuperscript{32}

\* Using the Fukui function determined from Mulliken charges.\textsuperscript{27}
\* Using the Fukui function determined from Mulliken charges.\textsuperscript{27}

\* Using the Fukui function determined from Mulliken charges.\textsuperscript{27}
\* Using the Fukui function determined from Mulliken charges.\textsuperscript{27}

\* Using the Fukui function determined from Mulliken charges.\textsuperscript{27}
\* Using the Fukui function determined from Mulliken charges.\textsuperscript{27}

\* Using the Fukui function determined from Mulliken charges.\textsuperscript{27}
\* Using the Fukui function determined from Mulliken charges.\textsuperscript{27}
(except where noted). Analytical TLC was carried out using Kieselgel GF254 (E. Merck) with a thickness of 0.20 mm. The homogeneity of all disaccharides 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.

3.2. Methyl 2,3,5,6-tetra-O-benzoyl-β-D-galactofuranosyl-(1→4)-2-acetamido-3,6-di-O-benzoyl-2-deoxy-α-D-glucopyranoside (11)

A suspension of the known acceptor 518 (248 mg, 0.56 mmol), donor 2b11,12,20 (436.5 mg, 0.59 mmol), activated 4 Å molecular sieves (127.5 mg) in anhyd CH2Cl2 (19 mL), and CH3CN (640 μL) was stirred at room temperature. After 40 min, the mixture was cooled to −30 °C, TMSOTf (106 μL, 0.58 mmol) was slowly added, and the stirring was continued for 4 h. The mixture was then neutralized by addition of solid NaHCO3 (200 mg) and evaporated. The residue was chromatographed to yield 11 (167 mg, 29%), as a foamy solid: [α]D20 +44.8 (c 1.01, CHCl3); Rf 0.41 (1:4 hexane–EtOAc); 1H NMR: δ 8.15–7.00 (m, 30H, ArH), 7.77 (d, 1H, J6H,2 9.5 Hz, NH), 5.68–5.55 (m, 2H, H-5), 5.65 (dd, 1H, J3,4 9.0, J3,3 10.7 Hz, H-3), 5.43 (s, 1H, H-2'), 5.33 (s, 1H, H-1'), 4.89 (dd, 1H, J6a,5 1.5, J6a,b 12.0 Hz, H-6a), 4.78 (d, 1H, J1,2 3.7 Hz, H-1), 4.64 (dd, 1H, J6b,5 3.6 Hz, H-6b), 4.58–4.44 (m, 1H, H-2), 4.37–4.16 (m, 5H, H-6'a, H-6'b, H-4', H-4, H-5), 3.45 (s, 3H, OCH3), 1.81 (s, 3H, COCH3); 13C NMR: δ 169.65 (COCH3), 165.90–164.90 (CO × 6), 132.75–132.58, 129.50–127.31 (C-Ar), 106.51 (C-1'), 98.12 (C-2'), 81.92 (C-4'), 77.03 (C-3'), 74.54 (C-5), 71.82 (C-3), 69.64 (C-5'), 68.64 (C-4), 62.91 (C-6'), 62.44 (C-6), 54.79 (OCH3), 51.64 (C-2), 22.22 (COCH3); MALDI-TOF HRMS: calcd for C53H51NO17 [M+Na]+, m/z 1044.3055; found, m/z 1044.3033.

3.3. Preparation of 1a

Compound 1a was prepared essentially as previously described and identified by comparison of NMR data with that reported,10 except for the transformation of 7 into 8.

3.3.1. Preparation of 8. To a stirred solution of compound 7 (1.83 g, 6.51 mmol) in anhyd EtOAc (108 mL) was added anhyd Et3N (1.8 mL, 12.8 mmol) and then triphosgene (644 mg, 14.6 mmol). The reaction mixture was stirred at room temperature for 1 h (TLC) after which time it was filtered, concentrated, and purified by flash chromatography with 55:45 hexane–EtOAc to give 8 as a white solid (1.74 g, 87%); mp 209.3–210.6 °C, lit.10 198 °C. This compound was identified by comparison of NMR data with that previously reported.

3.4. Methyl 2,3,5,6-tetra-O-benzoyl-β-D-galactofuranosyl-(1→4)-2-acetamido-6-O-benzyl-2-N,3-O-carbonyl-2-deoxy-α-D-glucopyranoside (12)

A suspension of acceptor 1a10 (183.7 mg, 0.52 mmol), donor 2b11,12,20 (411.7 mg, 0.56 mmol), activated 4 Å molecular sieves (120 mg) in anhyd CH2Cl2 (10 mL), and CH3CN (595 μL) was stirred at room temperature. After 40 min, the mixture was cooled to −30 °C, TMSOTf (100 μL, 0.55 mmol) was slowly added, and the stirring was continued for 4 h. The mixture was then neutralized by addition of solid NaHCO3 (200 mg) and filtered through a silica gel pad with copious washings with CH2Cl2. The filtrate was dried (Na2SO4) and evaporated. The residue was chromatographed to yield 12 (308.6 mg, 64%), as a foamy solid: [α]D20 +43.6 (c 1.01, CHCl3); Rf 0.61 (1:4 hexane–EtOAc); 1H NMR: δ 8.20–7.00 (m, 25H, ArH), 6.15–6.05 (m, 1H, H-5'), 5.60 (br s, 1H, H-1'), 5.59 (d, 1H, J5',7 3.2 Hz, H-3'), 5.31 (d, 1H, J7,8 1.1 Hz, H-2'), 5.24 (br s, 1H, H-1'), 4.91 (dd, 1H, J5',7 5.9 Hz, H-4'), 4.83 (s, 1H, H-6'b), 4.80 (br s, 1H, H-6'a), 4.64 (d, 1H, J12 12.0 Hz, H-3), 4.52 (d, 1H, CH2Ph), 4.32 (dd with appearance of t, 1H, J4,5 9.2 Hz, H-4), 3.94 (dd, 1H, J6a,5 2.6, J6a,b 10.8 Hz, H-6a), 3.86–3.65 (m, 3H, H-2, H-5, H-6b), 3.44 (s, 3H, OCH3), 2.50 (s, 3H, COCH3); 13C NMR: δ 171.08 (COCH3), 165.96 (CO), 165.64 (CO × 2), 165.58 (CO × 2), 152.88 (CO), 137.65, 133.4–132.78, 129.78–127.57 (C-Ar), 105.37 (C-1'), 96.78 (C-1), 82.28 (C-2'), 81.12 (C-4'), 77.30 (C-3'), 75.48 (C-3), 73.40 (CH2Ph), 72.52 (C-5'), 72.43 (C-4'), 70.14 (C-5'), 67.31 (C-6), 64.08 (C-6'), 59.97 (C-2'), 55.81 (OCH3), 23.64 (COCH3); MALDI-TOF HRMS: calcd for C53H47NO17 [M+Na]+, m/z 952.2793; found, m/z 952.2748.

3.5. Methyl 2,3,5,6-tetra-O-benzoyl-β-D-galactofuranosyl-(1→4)-3,6-di-O-benzoyl-2-deoxy-2-dimethylamino-β-D-glucopyranoside (13a)

A mixture of the triacetate 9a16,19 (1.54 g, 3.62 mmol), anhyd MeOH (36 mL) and NaOMe solution (0.169 M, 2.2 mL) was stirred at room temperature. After 2.30 h, the solution was neutralized with Amberlite IR-120 (H+) resin, filtered, and evaporated. The residue was chromatographed (9:1 EtOAc-MeOH) to yield 9b (1.1 g, 100%), as a foamy solid. To a stirred solution of 9b (393 mg, 1.30 mmol) in anhyd pyridine (7.2 mL) at −40 °C was added dropwise freshly distilled benzoyl chloride (390 μL, 3.36 mmol). The reaction mixture was stirred for 2 h between −35 and −30 °C, and then
it was allowed to slowly warm to room temperature. After 15 h, water was added, the mixture was stirred for 10 min and then extracted with CH2Cl2. The organic layer was washed with 1 N HCl, satd aq NaHCO3, and brine, dried (Na2SO4), and evaporated. The residue was chromatographed to yield 6a (454.6 mg, 69%), as a foamy solid: [α]D25 +63.76 (c 1.01, CHCl3); 1H NMR: δ 8.10–7.90 (m, 2H, ArH), 6.70–7.25 (m, 4H, ArH), 5.98 (dd, 1H, J3,4 9.2, J3,2 10.3 Hz, H-3), 5.53 (s, 1H, CHPh), 5.27 (d, 1H, J1,2 8.4 Hz, H-1), 4.50–4.37 (m, 1H, H-5), 4.22 (dd, 1H, H-2), 3.89 (dd, 1H, J6a,6b 2.5, J6a,b 9.4 Hz, H-6a), 3.87–3.52 (m, 2H, H-4, H-6b), 3.48 (s, 3H, OCH3), 1.88 (s, 6H, CH2CH2×2). 13C NMR: δ 171.26 (CO), 137.02 (CO), 136.85, 128.96–126.07 (C–Ar), 101.51 (CHPh), 99.65 (C-1), 81.88 (C-4), 68.27 (C-3), 68.36 (C-6), 65.81 (C-5), 56.64 (OCH3), 56.10 (C-2), 8.48 (CCH3×2).

To a stirred suspension of 6a (116.9 mg, 0.30 mmol) in anhyd pyridine (600 μL) in an ice-water bath was added dropwise freshly distilled benzoyl chloride (140 μL, 1.21 mmol). The reaction mixture was allowed to slowly warm to room temperature. After 15 h, water was added, and the mixture was stirred for 10 min and then extracted with CH2Cl2. The organic layer was washed with brine, dried (Na2SO4), and evaporated. The residue was chromatographed to yield 10b (119.3 mg, 81%), as a foamy solid: 1H NMR: δ 8.00–7.90 (m, 2H, ArH), 7.60–7.25 (m, 8H, ArH), 5.98 (dd, 1H, J3,4 9.2, J3,2 10.3 Hz, H-3), 5.53 (s, 1H, CHPh), 5.27 (d, 1H, J1,2 8.4 Hz, H-1), 4.50–4.37 (m, 1H, H-5), 4.22 (dd, 1H, H-2), 3.89 (dd, 1H, J6a,6b 2.5, J6a,b 9.4 Hz, H-6a), 3.87–3.52 (m, 2H, H-4, H-6b), 3.48 (s, 3H, OCH3), 1.88 (s, 6H, CH2CH2×2). 13C NMR: δ 171.26 (CO×2), 164.47 (CO), 137.29 (C–Ar), 137.02 (C×2), 133.74–126.14 (C–Ar), 101.44 (CHPh), 99.38 (C-1), 81.23 (C-4), 68.88 (C-6), 66.97 (C-5), 62.42 (C-3), 55.47 (OCH3), 53.93 (C-2), 8.80 (CCH3×2).

To a solution of 10b (29.5 mg, 0.06 mmol) and BH3(N(CH3)3) (9.8 mg, 0.14 mmol) in CH3CN (600 μL) in an ice-water bath was added dropwise BF3·OEt2 (15 μL, 0.14 mmol). After 1 h at this temperature, the solution was stirred at room temperature for an additional 0.5 h (TLC). NaHCO3 (8.5 mg) was then added, and the solution was evaporated to dryness. The crude product in CH2Cl2 was washed with brine, dried (Na2SO4), and evaporated. The residue was chromatographed to yield 6b (26.4 mg, 89%), as a foamy solid: [α]D25 +48.95 (c 0.98, CHCl3); 1H NMR: δ 8.00–7.90 (m, 2H, ArH), 7.63–7.25 (m, 8H, ArH), 5.70 (dd, 1H, J3,4 8.6, J3,2 10.8 Hz, H-3), 5.17 (d, 1H, J1,2 8.6 Hz, H-1), 4.67 (d, 1H, J 12.2 Hz, CH2Ph), 4.59 (d, 1H, CH2Ph), 4.19 (dd, 1H, H-2), 3.97–3.82 (m, 3H, H-6a, H-6b, H-4), 3.80–3.67 (m, 1H, H-5), 3.47 (s, 3H, OCH3), 3.24 (d, 1H, JOD,4 2.8 Hz, OH), 1.87 (s, 6H, CCH3×2); 13C NMR: δ 171.32 (CO×2), 166.66 (CO), 137.65 (C×2), 137.12, 132.22–127.52 (C–Ar), 98.83 (C-1), 74.47 (C-5), 74.38 (C-3), 74.15 (C-4), 69.62 (C-6), 56.63 (OCH3), 54.02 (C-2), 8.48 (CCH3×2) (the assignments for signals marked with an asterisk (*) may be reversed).

To a stirred solution of 9b (107 mg, 0.36 mmol), in anhyd DMF (1.8 mL) was added benzaldehyde dimethyl acetal (109 μL, 0.19 mmol) and a catalytic amount of camphorsulfonic acid, and the mixture was stirred over-night at room temperature. The reaction mixture was neutralized with solid Na2CO3 and diluted with EtOAc.

The organic layer was washed with brine, dried (Na2SO4), and evaporated. The residue was chromatographed to yield 10a (116.9 mg, 85%), as a foamy solid: 1H NMR: δ 7.52–7.32 (m, 5H, ArH), 5.55 (s, 1H, CHPh), 5.03 (d, 1H, J1,2 8.4 Hz, H-1), 4.46 (dd, 1H, J3,4 8.8, J3,2 10.5 Hz, H-3), 4.37 (dd, 1H, J6a,5 4.3, J6a,b 10.9 Hz, H-6a), 3.98 (dd, 1H, H-2), 3.83 (dd with appearance of t, 1H, J5,9 9.9 Hz, H-4), 3.61–3.51 (m, 2H, H-5, H-6b), 3.44 (s, 3H, OCH3), 1.96 (s, 6H, CCH3×2); 13C NMR: δ 171.61 (CO×2), 137.02 (C×2), 136.85, 128.96–126.07 (C–Ar), 101.51 (CHPh), 99.65 (C-1), 81.88 (C-4), 68.27 (C-3), 68.36 (C-6), 65.81 (C-5), 56.64 (OCH3), 56.10 (C-2), 8.48 (CCH3×2).

3.6 Methyl 2,3,5,6-tetra-O-benzoyl-β-D-galactofuranosyl-(1→4)-3-O-benzoyl-6-O-benzyl-2-deoxy-2-dimethylmaleimido-β-D-glucopyanoside (13b)

To a stirred solution of 9b (107 mg, 0.36 mmol), in anhyd pyridine (1.8 mL) was added benzaldehyde dimethyl acetal (109 μL, 0.19 mmol) and a catalytic amount of camphorsulfonic acid, and the mixture was stirred over-night at room temperature. The reaction mixture was neutralized with solid Na2CO3 and diluted with EtOAc.
A suspension of acceptor 6b (60 mg, 0.12 mmol), donor 2b,21,22,20 (117 mg, 0.16 mmol), activated 4 Å molecular sieves (51 mg) in anhyd CH₂Cl₂ (4.6 mL), and CH₃CN (155 µL) was stirred at room temperature. After 40 min, the mixture was cooled to −30 °C, TMSOTf (26 µL, 0.15 mmol) was slowly added, and the stirring was continued for 4 h. The mixture was then neutralized by addition of solid NaHCO₃ (220 mg) and filtered through a silica gel pad with copious washings with EtOAc. The filtrate was dried (Na₂SO₄) and evaporated. The residue was chromatographed to yield 13b (65.8 mg, 70% based on the recovered starting material, 16.8 mg), as a foamy solid: [α]D²⁷ +44.2 (c 1.00, CHCl₃); Rf 0.38 (1:1 hexane–EtOAc); ¹H NMR: δ 8.12−7.19 (m, 30H, ArH), 5.96 (dd, 1H, J₃,₂ 9.2, J₂,₃ 10.7 Hz, H-3), 5.59−5.51 (m, 2H, H-5', H-3'), 5.45 (s, 1H, H-1'), 5.30 (br s, 1H, H-2'), 5.22 (d, 1H, J₁,₂ 8.6 Hz, H-1), 4.64 (d, 1H, J 12.2 Hz, CH₂Ph), 4.55 (d, 1H, CH₂Ph), 4.36−4.07 (m, 5H, H-2, H-4, H-4', H-6'a, H-6'b), 4.02 (dd, 1H, J₆,a,₅ 3.0, J₆,b 11.4 Hz, H-6a), 3.88 (dd, 1H, J₆,b 1.5 Hz, H-6b), 3.83−3.76 (m, 1H, H-5), 3.48 (s, 3H, OCH₃), 1.84 (br s, 6H, CCH₃ × 2); ¹³C NMR: δ 171.28 (CO × 2), 165.69−165.28 (CO × 5), 137.13 (C × 2), 133.32−127.38 (C–Ar), 106.39 (C-1'), 99.05 (C-1), 82.28 (C-2'), 82.03 (C-4'), 77.18 (C-3'), 74.83 (C-4', C-5), 73.08 (CH₂Ph), 71.84 (C-3), 69.91 (C-5), 67.94 (C-6'), 63.39 (C-6), 56.59 (OCH₃), 54.71 (C-2), 8.50 (CCH₃ × 2); MALDI-TOF HRMS: calcd for C₆₁H₅₅NO₁₇ [M+Na]+, m/z 1096.3362; found, m/z 1096.3351.

3.7. Methyl 2,3,5,6-tetra-O-benzoyl-β-D-galactofuranosyl-(1→4)-3,6-di-O-benzyl-2-deoxy-2-dimethylamino-β-D-glucopyranoside (13c)

To a solution of 10a (198 mg, 0.51 mmol) in anhyd CH₂Cl₂ (1.0 mL) were added benzyl 2,2,2-trichloroacetimidate (190 µL, 1.02 mmol) and activated 4 Å molecular sieves (254 mg), and the mixture was stirred at room temperature for 45 min. Then the mixture was cooled in an ice-water bath, and a catalytic amount of TfOH (0.11 mmol) and activated 4 Å molecular sieves (56 mg) in anhyd CHCl₃ (4.3 mL), and CH₃CN (139 µL) was stirred at room temperature. After 40 min, the mixture was cooled to −30 °C, TMSOTf (23 µL, 0.13 mmol) was slowly added, and the stirring was continued for 4 h. The mixture was then neutralized by addition of solid NaHCO₃ (196 mg) and filtered through a silica gel pad with copious washings with EtOAc. The filtrate was dried (Na₂SO₄) and evaporated. The residue was chromatographed to yield 6c (90 mg, 51%), as a foamy solid: [α]D³⁵ +41.51 (c 0.40, CHCl₃); ¹H NMR: δ 7.40−7.08 (m, 10H, ArH), 4.90 (d, 1H, J₁,₂ 8.4 Hz, H-1), 4.77 (d, 1H, J 12.2 Hz, 3-OCH₂Ph), 4.64 (d, 1H, J 12.2 Hz, 6-OCH₂Ph), 4.56 (d, 1H, 6-OCH₂Ph), 4.50 (d, 1H, 3-OCH₂Ph), 4.08 (dd, 1H, J₃,₄ 8.1, J₃,₂ 10.8 Hz, H-3), 3.91 (dd, 1H, H-2), 3.81−3.70 (m, 3H, H-4, H-6a, H-6b), 3.63−3.53 (m, 1H, H-5), 3.37 (s, 3H, OCH₃), 2.95 (br s, 1H, OH), 1.82 (s, 6H, CCH₃ × 2); ¹³C NMR: δ 171.49 (CO × 2), 138.39, 137.50 (C–Ar), 136.77 (C × 2), 128.29−127.23 (C–Ar), 99.06 (C-1), 79.11 (C-4), 74.12 (CH₂Ph), 73.79 (C-3'), 73.54 (C-5'), 70.31 (C-6), 56.34 (OCH₃), 54.82 (C-2), 8.45 (CCH₃ × 2) (the assignments for signals marked with an asterisk (*) may be reversed).

A suspension of acceptor 6c (53.5 mg, 0.10 mmol), donor 2b,21,22,20 (101.6 mg, 0.14 mmol), activated 4 Å molecular sieves (56 mg) in anhyd CH₂Cl₂ (4.3 mL), and CH₃CN (139 µL) was stirred at room temperature. After 40 min, the mixture was cooled to −30 °C, TMSOTf (23 µL, 0.13 mmol) was slowly added, and the stirring was continued for 4 h. The mixture was then neutralized by addition of solid NaHCO₃ (196 mg) and filtered through a silica gel pad with copious washings with EtOAc. The filtrate was dried (Na₂SO₄) and evaporated. The residue was chromatographed to yield 13c (82 mg, 70%), as a foamy solid: [α]D³⁵ +30.3 (c 1.04, CHCl₃); Rf 0.11 (CH₂Cl₂); ¹H NMR: δ 8.05−7.10 (m, 30H, ArH), 5.91−5.83 (m, 1H, H-5'), 5.62 (d, 1H, J₉,y,₂ 4.9 Hz, H-3'), 5.59 (s, 1H, H-1'), 5.47 (s, 1H, H-2'), 4.89 (d, 1H, J₁,₂ 8.2 Hz, H-1), 4.84 (d, 1H, J 12.0 Hz, 3-OCH₂Ph), 4.72 (t, 1H, J₉,a,₄ 4.3, H-4'), 4.60 (dd, 1H, J₉,a,₄ 7.3, J₉,a,₄ 12.2 Hz, H-6'a), 4.60 (d, 1H, J 12.4 Hz, 6-OCH₂Ph), 4.51 (d, 1H, 6-OCH₂Ph), 4.49−4.37 (m, 1H, H-6'b), 4.41 (d, 1H, 3-OCH₂Ph), 4.25 (dd, 1H, J₄,₅ 8.8, J₃,₂ 10.3 Hz, H-3), 4.11 (dd with appearance of t, 1H, J₄,₅ 8.8 Hz, H-4'), 4.02 (dd, 1H, H-2), 3.96 (dd, 1H, J₉,a,₄ 5.4, J₉,a,₄ 11.2 Hz, H-6a), 3.82 (dd, 1H, J₉,b,₅ 0.9 Hz, H-6b), 3.68−3.57 (m, 1H, H-5), 3.39 (s, 3H, OCH₃), 1.78 (s, 6H, CCH₃ × 2); ¹³C NMR: δ 171.53 (CO × 2), 165.82−165.60 (CO × 4), 138.22−137.94 (C–Ar), 136.81 (C × 2), 133.39−127.31 (C–Ar), 106.14 (C-1'), 99.15 (C-1), 82.38 (C-2'), 82.05 (C-4'), 78.78 (C-3'), 77.79 (C-3'), 76.14 (C), 74.94 (C-5), 74.85 (3-OCH₂Ph), 73.05 (6-OCH₂Ph), 70.57 (C-5'), 68.11
mended by 1H NMR spectroscopy of the mixtures of basis sets and default minimization methods and terminal Gaussian 98W (version 5.2, revision A-7) with standard Quantum mechanical calculations were performed using HPLC. The ratios of disaccharides 12:11:12:13a, 12:13b, and 12:13c were 11:1, 7:1, 1.5:1, and 1:4, respectively. The ratios of 12:13b and 12:13c were also determined by 1H NMR spectroscopy of the mixtures of disaccharides after purification by column chromatography. By integration of the signals at 4.90 (dd, 1H, J6,5 3.2, J5,4 5.9 Hz, H-4') in 12 and 5.45 (s, 1H, H-1') in 13b and at 5.24 (br s, 1H, H-1') in 12 and 5.47 (s, 1H, H-2') in 13c, the ratios were 12:13b, 1:1 and 12:13c, 1:8, respectively.

3.8. Competition experiments

The mixtures of acceptors 1a and 5, 1a and 6a, 1a and 6b, and 1a and 6c (1.0 equiv of each) were glycosylated as described for the preparation of disaccharides 11, 12, 13a, 13b, and 13c, using 1.2 equiv of donor 2b. The resulting mixtures of products were analyzed using HPLC. The determination of net charge attributed to each atom was made by several methods, including the regular Mulliken analysis, fits to the electrostatic potential according to the Merz–Singh–Kollman scheme, 30 the CHelp scheme, 31 and the CHelpG scheme. 32 The values of charge and Fukui function obtained for the O-4 of both conformers of each compound were Boltzmann averaged. Selected results are shown in Table 2. Global softness is the inverse of chemical hardness, which was calculated as (EHOMO + ELUMO)/2, according to Pérez et al. 36 The local softness reported in the table is calculated by multiplying the global softness by the local Fukui function obtained using each charge distribution method.

Acknowledgements

We thank Dr. Manuel González-Sierra for spectral determinations and Dr. Cristóbal López for helpful suggestions. We also thank Drs. Crich and Nikolaev for sending us the experimental details for the preparation of different compounds. Financial support from UNR, UBA, and CONICET and a fellowship (M.L.B.) are also acknowledged. M.I.C., C.A.S., and E.A.R. are Research Members of the National Research Council of Argentina (CONICET).

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.carres.2006.03.030.

References