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Experimental and theoretical study of the O3/O4 regioselectivity of glycosylation reactions of glucopyranosyl acceptors

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Abstract

The knowledge of the regioselectivity between different hydroxyl groups of glycosyl acceptors is valuable in planning simple strategies for the synthesis of oligosaccharides, minimizing the use of protecting groups. With the aim of obtaining deeper knowledge on this subject, we analyzed the relative reactivity of the OH-3 and OH-4 groups of 2,6-di-*O*-protected methyl α- and β-glucopyranosides in glycosylation reactions. The glycosyl acceptors were prepared by simple procedures, and galacto-pyranosyl and furanosyl trichloroacetimidates were evaluated as glycosyl donors. Experimental results were contrasted with those obtained by a molecular modeling approach. A fair agreement of the molecular modeling and experimental results was obtained. It has been shown, that by choosing the right anomer and protecting group, either the 1→3 or 1→4 linkage can selectively be installed using the appropriate glucosyl acceptor.

Introduction

Nowadays, the importance of specific oligosaccharides in biological processes is widely known, thus requiring simple and reproducible procedures for their synthesis.[1,2] Glycosylation reactions are a key factor in this process, though they often appears to complicate the synthesis due to the large number of free hydroxyl groups present in carbohydrates, many times with similar reactivities. This fact frequently makes it necessary to introduce protecting groups which have to be removed at the end, giving rise to a tedious, time-consuming and yield-depleting synthetic sequence. However, it is sometimes possible to plan selective glycosylations avoiding or minimizing the use of protecting groups if the reactivity of one of the hydroxyl groups is much larger than that of other one and limiting amounts of glycosyl donors are used. This approach requires comprehensive knowledge of the regioselectivity of glycosidation reactions, which are not always well understood, as they depend sometimes on subtle factors. Some attempts to rationalize the regioselectivity on theoretical basis have been fairly success,[3] although others have failed.

In a recent work, we analyzed the relative reactivity of the OH-3 and OH-4 of 2,6-*O*-diprotected methyl α- and β-galactopyranosyl derivatives in glycosylation reactions with different galactosyl donors. As expected, the formation of the 1→3 disaccharides was favored, given the equatorial orientation of the OH-3 group vs. the axial one of the OH-4 group. Nevertheless, the regioselectivities were very different, ranging from very high to quite low, depending on the substituents and the anomeric configuration of the acceptors, and the reactivities of the donors. The highest regioselectivities were achieved for the 2,6-di-*O*-benzylated acceptors and the per-*O*-benzoylated-galactopyranosyl trichloroacetimidate.[4] Nevertheless, for some donors derived from 2-phthalilamide-2-deoxy-glucopyranose the best regioselectivity was observed for a 2,6-di-*O*-benzoylated galactopuranosyl acceptor,[5] showing once again that the regioselectivity of glycosylation reactions is susceptible to a manifold of factors. Rationalization of the OH-3/OH-4 reactivities by different molecular modeling approaches agreed with the general trend but failed to explain subtler factors governing the difference in regioselectivity between some of the acceptors.[4]

The two most common monosaccharide residues in Nature are D-glucose and D-galactose, and consequently, these units have been used by carbohydrate chemists most frequently. Motifs D-Gal*p*-1→3-D-Glc*p* and D-Gal*p*-1→4-D-Glc*p* (lactose) are used as models for the action of β-galactosidases,[6] whereas other disaccharides and higher oligosaccharides containing these moieties can have important biological properties. Thus, knowledge of the selectivity between O-3/O-4 of glucosyl acceptors is significant in planning the synthesis of these oligosaccharides when intending to minimize the use of protecting groups. Although many studies related with the regioselective glycosylation of "less protected acceptors" have been reported,[5,7–9] the case of glucose, with all equatorial substituents, has been less attended, usually not involving the O-3/O-4 positions.[10–14]

The aim of the present work is to extend our previous study to the case of glucosyl acceptors, analyzing the glycosylation of 2,6-di-*O*-protected methyl glycosides **1α**,**β** and **2α**,**β**, which differ from the previous study[4] in the fact that both free OHs are equatorial (Figure 1). Trichloroacemidates **3** and **4** were used as donors, and the experimental results were compared with those obtained by molecular modeling. Differences with D-Gal acceptors are also analyzed.

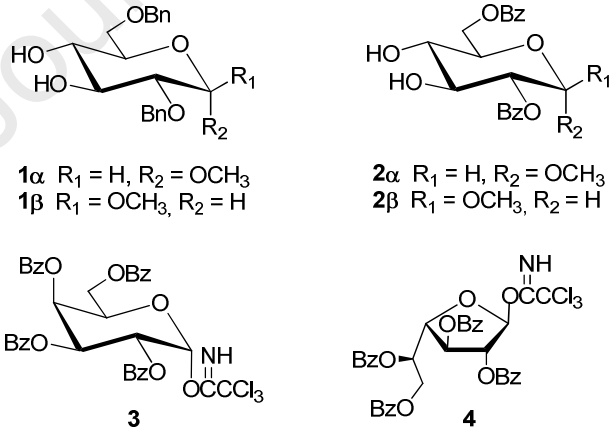
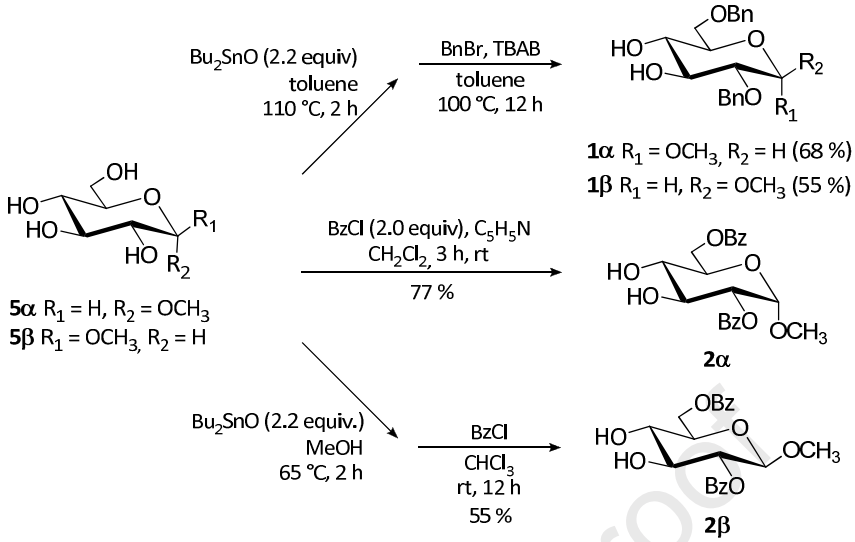


Figure 1. Studied glucosyl acceptors and galactosyl donors

1. Results and Discussion

1.1. Experimental study

For the objectives of the present study we required D-Glc*p* derivatives with OH-2 and OH-6 blocked. Many efforts have been made to develop simple procedures for the preparation of partially substituted derivatives of glucose,[13,15–20] which is more difficult to achieve, compared with mannose or galactose, due to the fact that all the secondary hydroxyl groups are equatorial and display comparable reactivity under various common conditions. We synthesized derivatives **1α,β** and **2α,β**, in order to evidence the possible differences between the electron withdrawing/not withdrawing character of the protecting groups in the glycosylation regioselectivity. α-Glucopyranosyl acceptors were synthesized from commercially available methyl α-D-Glc*p* (**5α**). For the β-anomers, instead, methyl β-D-Glc*p* (**5β**)[21] was prepared by $BF_3{\cdot}OEt_2$ promoted glycosidation[22] of per-*O*-benzoyl-Glc*p* under short reaction time to prevent α-anomerization, followed by Zemplèn de-*O*-benzoylation. Benzylated glycosides **1α**[17] and **1β**[18] were obtained by stannylene acetals-mediated substitutions, by treatment of **5α** or **5β** with Bu_2SnO under toluene reflux, followed by benzylation with benzyl bromide in the presence of TBAB (Scheme 1). The general procedure described by Zhou *et al.*[18] was followed. Benzoylated acceptor **2α**[23,24] was prepared by benzoylation with limited amounts of benzoyl chloride. It is worth mentioning, that this procedure was not effective for the β-anomer. Acceptor **2β**[16] was prepared also by stannylene acetals-mediated substitutions, but treatment with Bu_2SnO of **5β** was performed under MeOH reflux, followed by benzoylation in chloroform, as described by Dong *et al.*[25] (Scheme 1). The regioselective substitutions proceeded by formation of the 4,6- and 2,3-stannylene acetals which are then substituted at the most reactive O-2 and O-6 possitions.[18]



Scheme 1. Synthesis of D-Glc*p* glycosyl acceptors **1α,β** and **2α,β**

With glucosyl acceptors **1α,β** and **2α,β** in hand, we assayed glycosylation reactions with galactosyl donors **3** and **4**, with participating groups at position 2, and thus giving rise only to β glycosides. Trichloroacetimidates **5**[26] and **6**[27] were prepared by treatment with trichloroacetonitrile and DBU of the corresponding benzoylated hemiacetals. To avoid migration during the glycosylations, acetyl groups were not used either in the donors or the acceptors.[28,29] The coupling reactions were performed in CH_2Cl_2 solution, at low temperature, and the activation of the donor was acchieved with catalytic TMSOTf. The molar ratio of acceptor to donor was 1.4:1 to preclude double glycosylation of the acceptors.[4]

When TLC showed the disappearance of the donor, normally after 2 h of reaction, relative yields of the disaccharides were determined by integration of the ^{1}H NMR signals of the anomeric or other well-resolved protons of the crude mixtures. Then, the mixtures were purified by column chromatography in order to characterize the condensation products and to confirm the yields of the isolated regioisomers. The structures of the disaccharides were univocally assigned on the basis of NMR spectra. The position of the interglycosidic linkages was verified from the deshielding of the ^{13}C NMR signals involved in such linkages.[30,31] For example, for disaccharide **6β**, linked (1→3), the main product of the coupling between **3** with **1β**, signals corresponding to C-3 and C-4 were observed at

86.0 and 68.7 ppm, respectively. Instead, for the minor product **7β** (1→4) such signals were observed at 75.3 (C-3) and 81.4 ppm (C-4). The deshielding effect of the glycosidation can be clearly observed in Table 1, where C-3 and C-4 chemical shifts (126 MHz, Cl_3CD) of acceptors **1α,β** and **2α,β** and dissacharides **6-13** are listed, as well as the Δδ values between the dissacharides and the corresponding acceptor and the difference between the Δδ of C-3 and C-4 (ΔΔδ). ΔΔδ values are > 0 for 1→3 dissaccharides and < 0 for 1→4. Information provided by HMBC experiments and observation in the ^{1}H NMR spectrum of the signal corresponding to the free OH and the corresponding $^3J_{H,OH}$ coupling constant, also supported the regiochemistry of the products. The stereochemistries of the newly formed glycosidic linkages were established from the $^3J_{1',2'}$ coupling constants, which were around 8 Hz for disaccharides obtained from pyranosic donor **3** and 1-2 Hz for those obtained from furanosic donor **4**.[32]

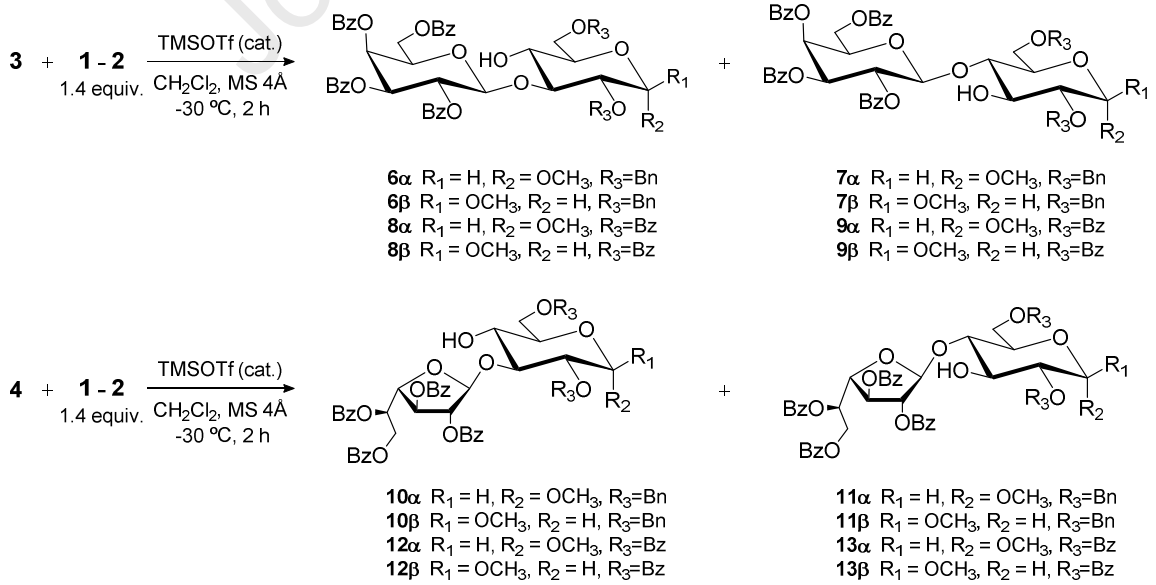
Table 1. ^{13}C NMR chemical shifts for C-3 and C-4 of the glycosyl acceptors **1α,β** and **2α,β** and the product dissacharides **6-13**; $\Delta\delta_n$: difference between $\delta_{C\text{-}n}$ of each disaccharide and $\delta_{C\text{-}n}$ of the corresponding acceptor; ΔΔδ: $\Delta\delta_3 - \Delta\delta_4$.

Acceptors								
	3α	**3β**	**4α**	**4β**				
C-3 (δ)	72.9	75.9	71.4	75.2				
C-4 (δ)	70.9	71.5	70.6	70.6				
1→3 Disaccharides								
	6α	**6β**	**8α**	**8β**	**10α**	**10β**	**12α**	**12β**
C-3 (δ)	84.2	86.0	82.0	85.9	81.3	82.9	79.7	83.0
$\Delta\delta_3$	11.3	10.1	10.6	10.7	8.4	7.0	8.3	7.8
C-4 (δ)	68.5	68.7	69.3	68.8	69.5	70.2	69.5	69.5
$\Delta\delta_4$	-2.4	-2.8	-1.3	-1.8	-1.4	-1.3	-1.1	-1.1
ΔΔδ	13.7	12.9	11.9	12.5	9.8	8.3	9.4	8.9
1→4 Disaccharides								
	7α	**7β**	**9α**	**9β**	**11α**	**11β**	**13α**	**13β**
C-3 (δ)	72.0	75.0	69.9	73.5	71.9	74.9	70.3	73.9
$\Delta\delta_3$	-0.9	-0.9	-1.5	-1.7	-1.0	-1.0	-1.1	-1.3
C-4 (δ)	81.4	81.5	82.7	82.3	77.2	77.1	79.8	79.5
$\Delta\delta_4$	10.5	10.0	12.1	11.7	6.3	5.6	9.2	8.9
ΔΔδ	-11.4	-10.9	-13.6	-13.4	-7.3	-6.6	-10.3	-10.2

In the glycosylation reactions, all the acceptors showed the same trend, except methyl 2,6-di-*O*-benzoyl-β-D-Glc*p* (**2β**, Table 2). Acceptors **1α**, **1β** and **2α** were preferentially glycosylated at O-3 (Table 2, entries 1-3 and 5-7), although it has been stated that both OHs have similar steric hindrance.[33] It was observed a higher selectivity for the benzylated derivative **1α** than for the benzoylated derivative **2α**. For **2β**, instead, the 1→4 disaccharide **9β** was the main product with **3** as glycosyl donor (entry 4), and no regioselectivity was observed hen donor **4** was utilized (entry 8). For donor **3**, less reactive than **4**,[34,35] higher regioselectivities were achieved (entries 1-4 vs- 5-8).

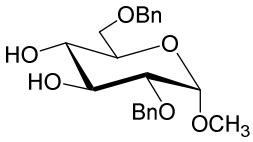
These results show that a trend increasing the reactivity of O-4 is observed when passing from benzylated derivatives to benzoylated derivatives, and when passing from α-anomers to β-anomers. This trend leads to O-4 preferential regioselectivity only for compound **2β**. Exactly the same trend was observed when assaying the O-3/O-4 regioselectivity of α-and β-methylglycosides of 2-deoxy-2-dimethylmaleoyl glucosamine derivatives protected with benzoyl and benzyl groups at O-6.[34] However, in that case, the trend led to preferential O-4 reactivity for both β-anomers. The reversal of regioselectivity observed in these cases shows that the remote anomeric configuration plays a significant role in the O-3/O-4 regioselectivity.

It is worth noticing that the yield was excellent in all glycosylations, showing once again the robustness of the trichloroacetimidate method.

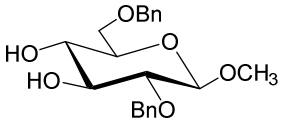


Scheme 2. Glycosylation of D-Glc*p* acceptors **1α,β** and **2α,β** with donors **3** and **4**.

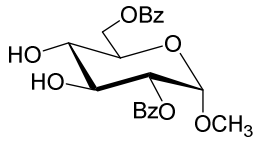
Table 2. Ratios and yields of 1→3 and 1→4 disaccharides obtained by reaction of donors **3-5** with acceptors **3α,β** and **4α,β**



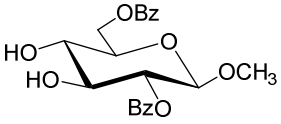
1α



1β



2α



2β

Entry	Donor	Acceptor	Products		Ratio[a]	Yields[b]	
			1→3	1→4	1→3 : 1→4	%[a]	%[c]
1	**3**	**1α**	**6α**	**7α**	6.5:1	96	91
2	**3**	**1β**	**6β**	**7β**	2:1	96	92
3	**3**	**2α**	**8α**	**9α**	2.1:1	71	75
4	**3**	**2β**	**8β**	**9β**	1:5.6	100	90
5	**4**	**1α**	**10α**	**11α**	3.45:1	92	89
6	**4**	**1β**	**10β**	**11β**	1.75:1	90	87
7	**4**	**2α**	**12α**	**13α**	2:1	100	92
8	**4**	**2β**	**12β**	**13β**	1:1	100	91

[a]Determined from the ^{1}H NMR spectrum of the crude reaction mixture.
[b]Combined yield of 1→3 and 1→4 regioisomers.
[c]Yields refers to isolated pure products after column chromatography on the basis of the donor amount used in the reaction.

2.2. Molecular modeling study

We have carried out molecular modeling calculations in order to determine the atomic partial charges and condensed-to-atom Fukui functions[36] in an attempt to rationalize the observed reactivity of the OH-3/OH-4 groups of acceptors **1α,β** and **2α,β,** as we did with the equivalent galactose acceptors.[4] As OH-3 in galactose was equatorial and OH-4 was axial, and both are equatorial in glucose, it could be expected a less sharp trend shown by modeling. The charge density was calculated for both methods using the regular Mulliken charges.

For the sake of simplicity, instead of acceptors **1α,β** and **2α,β**, analogs carrying acetyl groups instead of benzoyl, and methyl groups instead of benzyl moieties were used[37] (Figure 2). The lower energy conformers were found by molecular mechanics calculations (MM3), and then optimized with B3LYP/6-311+G**, and then, single-point calculations with M06-2X/6-311+G** (Figure A.1 and Table A.1, Electronic Supplementary Information File 1). A whole set of low-energy conformers was calculated for each of the four compounds, in order to carry out a Boltzmann-averaging to obtain the

local charges and Fukui functions (Table A.2 and Table A.3, Electronic Supplementary Information File 1). It has been shown that in several systems the electron density distribution can define the reactivity, i.e. the position with a larger net charge (*q*) will be that preferably attacked by a hard electrophiles.[38] However, in order to describe the interaction between nucleophile and electrophile corresponding to a soft–soft interaction in the context of the hard and soft acid and base principle (HSAB), the most common numerical descriptor is the local Fukui function (*f*-), related to the electron density in the HOMO frontier molecular for an electrophilic attack.[36,39–41] It should be expected to obtain higher absolute values for *q* and for *f* for the more reactive site. Fukui functions have already been successfully used in order to assess the reactivity of secondary hydroxyl groups of carbohydrate.[42–44]

Figure 2 and Table 3 show that the charge and Fukui determinations predict that OH-3 should be the more reactive site for the benzylated derivatives **1α** and **1β**, whereas OH-4 should be the more reactive site for the benzoylated derivatives **2α** and **2β**. This indicates, that the experimental reactivity for the benzylated derivatives is predicted correctly by modeling. On the other hand, for benzoylated derivatives, modeling predicts correctly the higher HO-4 reactivity of **2β**, but fails to perform correctly for **2α**. The highest regioselectivity observed for **1α** appears not to be predicted either by using modeling, suggesting that subtle factors not considered in the charge determinations, or by energy errors which might lead to an inadequate Boltzmann-averaging of the low-energy conformers can have some influence on the final outcome. The *q* and *f* values calculated for both oxygens are very similar, much more than in the case of D-Gal*p* acceptors,[4] making the prediction of the regioselectivity less reliable, given the low significance of the differences. The recognized limitation of the DFT to reproduce the conformational dynamics of flexible systems,[45–49] and the influence of the donor structure, not considered in the present study, but certainly important,[50] could be some reasons for this divergence with the experimental results for **1α**. In any case, a fair agreement of the experimental results and the modeling has been determined.

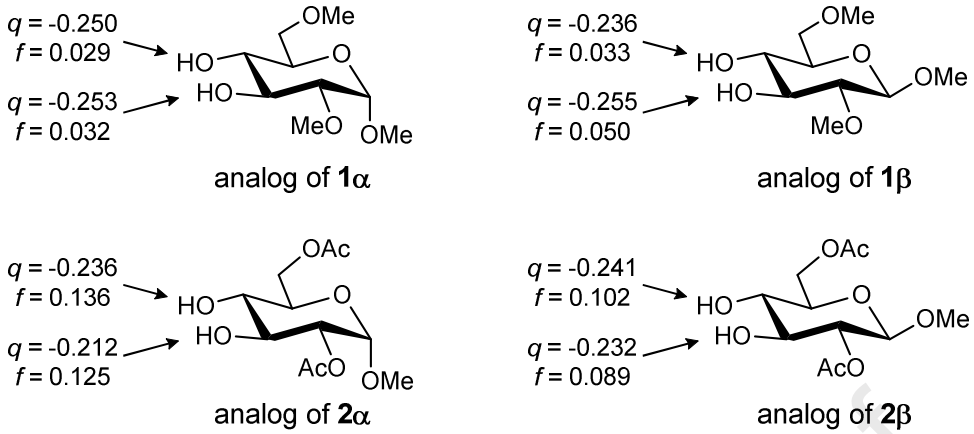


Figure 2. Model D-Glc*p* 3,4-diol acceptors and data obtained with B3LYP.

Table 3. Ratio of charges and Fukui functions on O-3/O-4 observed for analogs of acceptors **1-2** (calculations with M06-2X/6-311+G**//B3LYP/6-311+G**)

	$q_{O\text{-}3}/q_{O\text{-}4}$	$f_{O\text{-}3}/f_{O\text{-}4}$ [a]
Analog of **1α**	1.006	1.12
Analog of **1β**	1.079	1.54
Analog of **2α**	0.898	0.93
Analog of **2β**	0.962	0.87

2. Conclusions

Glucosyl acceptors **1α,β** and **2α,β** were obtained by simple procedures. For alkylated acceptors **1α,β**, a higher reactivity for OH-3 was experimentally observed in accordance with the theoretical results. For acylated acceptors **2α** and **2β** modeling predicts a slightly higher reactivity of OH-4 which only agrees with the experimental results for **2β**. A trend towards a relatively enhanced reactivity of O-4 with regards to O-3 in the changes α → β and Bz → Bn was observed, in agreement with previous reports. The regioselectivities experimentally achieved in some of the cases, could significantly simplify

the synthesis of D-Gal*p*-1→3-D-Glc*p* and D-Gal*p*-1→4-D-Glc*p* motifs in the synthesis relevant molecules.

3. Experimental section

4.1 General Methods

The solvents used were distilled, dried and stored according to standard procedures. Analytical thin layer chromatography (TLC) was performed on Silica Gel 60 F254 (Merck) aluminium supported plates (layer thickness 0.2 mm). Visualization of the spots was effected by exposure to UV light and charring with a solution of 5% (v/v) sulfuric acid in EtOH, containing 0.5% *p*-anisaldehyde. Column chromatography was carried out with Silica Gel 60 (230–400 mesh, Merck). Optical rotations were measured with a Perkin-Elmer 343 digital polarimeter. Nuclear magnetic resonance (NMR) spectra were recorded with a Bruker AMX 500 instrument. Chemical shifts (δ) are reported in ppm, with residual chloroform (δ 7.26 for ^{1}H and δ 77.16 for ^{13}C) as internal references. Assignments of ^{1}H and ^{13}C NMR spectra were assisted by 2D ^{1}H COSY and HSQC experiments. High resolution mass spectra (HRMS) were obtained by Electrospray Ionization (ESI) and Q-TOF detection.

*4.2. General procedure for glycosylations using trichloroacetimidates **5** or **6** as glycosyl donors*

Powdered 4 Å molecular sieves (0.5 g) were added to a solution of trichloroacetimidate **5**[26] **or 6**[27] (73 mg, 0.1 mmol) and glycosyl acceptor **1α,β** or **2 α,β** (0.14 mmol, 1.4 equiv) in anhyd. CH_2Cl_2 (8 mL), and the suspension was stirred under Ar for 30 min. Then, the mixture was cooled to −30 °C and TMSOTf (8 μL, 0.044 mmol) was added. When TLC analysis showed optimal conversion (normally 2 h of stirring at −30°C) the reaction mixture was quenched with triethylamine, filtered and coevaporated under reduced pressure with toluene. The residue was purified by column chromatography, as indicated in each case.

*4.2.2. Methyl 2,3,4,6-tetra-O-benzoyl-β-D-galactopyranosyl-(1→3)-2,6-di-O-benzyl-α-D-glucopyranoside (**6α**) and methyl 2,3,4,6-tetra-O-benzoyl-β-D-galactopyranosyl-(1→4)-2,6-di-O-benzyl-a-D-glucopyranoside (**7α**)*

Obtained according to the general procedure by condensation of **3** with **1α**. ^{1}H NMR spectrum of the crude mixture showed the presence of two regioisomers, **6α** and **7α,** in a 6.5:1 ratio according to the integration of the signals corresponding to H-3' of both disaccharides (5.63 and 5.50 ppm). TLC analysis of the crude mixture showed, among the excess of **1α**, a single product of *Rf* 0.37 (9:1 toluene-EtOAc), which after column chromatography purification (90:10→88:12 toluene/EtOAc) was resolved in two components with similar *Rf*. The first fractions afforded a mixture of disaccharides enriched in **7α** (0.03 g, 28%). ^{1}H NMR(500 MHz, $CDCl_3$) signals for **7α**: δ 8.15-7.18 (m, 30H, aromatic), 5.95 (dd, 1H, $J_{4',5'}$ = 0.8 Hz, $J_{3',4'}$ = 3.5 Hz, H-4'), 5.80 (dd, 1H, $J_{1',2'}$ = 8.1 Hz, $J_{2',3'}$ = 10.5 Hz, H-2'), 5.50 (dd, 1H, $J_{3',4'}$ = 3.5 Hz, $J_{2',3'}$ = 10.5 Hz, H-3'), 4.86 (d, H, J_{gem} = 12.4 Hz, CH_2Ph), 4.74 (d, 1H, $J_{1',2'}$ = 8.1 Hz, H-1'), 4.63 (d, H, J_{gem} = 12.4 Hz, CH_2Ph), 4.60 (dd, 1H, $J_{5',6a'}$ = 4.9 Hz, J_{gem} = 11.6 Hz, H-6'a), 4.54 (d, 1H, $J_{1,2}$ = 3.6 Hz, H-1), 4.53 (dd, 1H, $J_{5',6b'}$ = 7.9 Hz, J_{gem} = 11.6 Hz, H-6'b), 4.35 (d, 1H, J_{gem} = 12.2 Hz, CH_2Ph), 4.32 (ddd, 1H, $J_{4',5'}$ = 0.8 Hz, $J_{5',6a'}$ = 4.9 Hz, $J_{5',6b'}$ = 7.9 Hz, H-5'), 4.13 (d, 1H, J_{gem} = 12.2 Hz, CH_2Ph), 4.12 (ddd, 1H, $J_{3,OH}$ = 0.7 Hz, $J_{3,4}$ = 8.1 Hz, $J_{2,3}$ = 9.5 Hz, H-3), 4.03 (d, 1H, $J_{3,OH}$ = 0.7 Hz, OH-3), 3.69 (dd, 1H, $J_{3,4}$ = 8.1 Hz, $J_{4,5}$ = 9.9 Hz, H-4), 3.65 (ddd, 1H, $J_{5,6b}$ = 1.6 Hz, $J_{5,6a}$ = 3.2 Hz, $J_{4,5}$ = 9.9 Hz, H-5), 3.47 (dd, 1H, $J_{5,6a}$ = 3.2 Hz, J_{gem} = 10.8 Hz, H-6a), 3.38 (dd, 1H, $J_{1,2}$ = 3.6 Hz, $J_{2,3}$ = 9.5 Hz, H-2), 3.35 (dd, 1H, $J_{5,6b}$ = 1.6 Hz, J_{gem} = 10.8 Hz, H-6b), 3.28 (s, 3H, CH_3O) ppm. ^{13}C NMR (126 MHz, $CDCl_3$): δ 101.6 (C-1'), 98.6 (C-1), 81.4 (C-4), 78.3 (C-2), 73.6 (CH_2Ph), 73.1 (CH_2Ph), 72.0 (C-3), 71.9 (C-5'), 71.4 (C-3'), 69.6 (C-2'), 68.4 (C-5), 67.9 (C-4'), 67.7 (C-6), 62.4 (C-6'), 55.3 (CH_3O) ppm.

Further elution of the column afforded syrupy compound ***6α*** (0.064 g, 63%), $[\alpha]_D$ +65 (*c* 1, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.12-7.21 (m, 30H, aromatic), 5.99 (dd, 1H, $J_{4',5'}$ = 0.8 Hz, $J_{3',4'}$ = 3.5 Hz, H-4'), 5.93 (dd, 1H, $J_{1',2'}$ = 8.1 Hz, $J_{2',3'}$ = 10.4 Hz, H-2'), 5.63 (dd, 1H, $J_{3',4'}$ = 3.5 Hz, $J_{2',3'}$ = 10.4 Hz, H-3'), 5.11 (d, 1H, $J_{1',2'}$ = 8.1 Hz, H-1'), 4.58 (dd, 1H, $J_{5',6a'}$ = 5.1 Hz, J_{gem} = 11.7 Hz, H-6'a), 4.56 and 4.53 (2d, 2H, J_{gem} = 12.3 Hz, 2x CH_2Ph), 4.52 (dd, 1H, $J_{5',6b'}$ = 7.7 Hz, J_{gem} = 11.7 Hz, H-6'b), 4.40 (ddd, 1H, $J_{4',5'}$ = 0.8 Hz, $J_{5',6a'}$ = 5.1 Hz, $J_{5',6b'}$ = 7.7 Hz, H-5'), 4.31 (d, 1H, J_{gem} = 12.8 Hz, CH_2Ph), 4.25 (d, 1H, $J_{1,2}$ = 3.6

Hz, H-1), 4.01 (d, 1H, J_{gem} = 12.8 Hz, CH_2Ph), 3.95 (dd, 1H, $J_{3,4}$ = 8.1 Hz, $J_{2,3}$ = 9.5 Hz, H-3), 3.89 (br. s, 1H, OH-4), 3.74-3.59 (m, 4H, H-4, H-5, H-6a and H-6b), 3.37 (dd, 1H, $J_{1,2}$ = 3.6 Hz, $J_{2,3}$ = 9.5 Hz, H-2), 3.23 (s, 3H, CH_3O) ppm. ^{13}C NMR (126 MHz, $CDCl_3$): δ 165.9, 165.4, 165.3 (*C*OPh), 138.2, 138.0, 133.6, 133.36, 133.31, 133.2, 129.9, 129.77, 129.71, 129.2, 129.0, 128.7, 128.6, 128.5, 128.38, 128.33, 128.27, 128.25, 128.23, 127.8, 127.6, 127.46, 127.45 (aromatic), 102.1 (C-1'), 98.0 (C-1), 84.2 (C-3), 77.9 (C-2), 73.6 (*C*H_2Ph), 73.4 (*C*H_2Ph), 71.8 (C-5'), 71.6 (C-3'), 70.3 (C-5), 69.6 (C-2'), 69.0 (C-6), 68.5 (C-4), 68.0 (C-4'), 62.2 (C-6'), 54.9 (*C*H_3O) ppm. ESIMS: *m/z* calcd for $C_{55}H_{52}NaO_{15}$ $[M+Na]^+$ 975.3198. Found: 975.319547.

*4.2.3. Methyl 2,3,4,6-tetra-O-benzoyl-β-D-galactopyranosyl-(1→3)-2,6-di-O-benzyl-β-D-glucopyranoside (**6β**) and methyl 2,3,4,6-tetra-O-benzoyl-β-D-galactopyranosyl-(1→4)-2,6-di-O-benzyl-β-D-glucopyranoside (**7β**)*

Obtained according to the general procedure by condensation of **3** with **1β**. After 2 h TLC analysis showed total consumption of **3** and a single spot of *Rf* 0.47 (85:15 toluene-EtOAc) composed, according to the ^{1}H NMR spectrum of the crude mixture, by two regioisomers in 2:1 ratio. The products could not be separated by column chromatography (0.09 g; 91%) but all the NMR signals could be assigned.

The major product was identified as **6β**. ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.98 (dd, 1H, $J_{4',5'}$ = 0.7 Hz, $J_{3',4'}$ = 3.5 Hz, H-4'), 5.88 (dd, 1H, $J_{1',2'}$ = 8.1 Hz, $J_{2',3'}$ = 10.5 Hz, H-2'), 5.62 (dd, 1H, $J_{3',4'}$ = 3.5 Hz, $J_{2',3'}$ = 10.5 Hz, H-3'), 5.14 (d, 1H, $J_{1',2'}$ = 8.1 Hz, H-1'), 4.63, 4.59 (2d, 2H, J_{gem} = 12.2 Hz, CH_2Ph), 4.59-4.50 (m, 3H, H-6'a, H-6'b y CH_2Ph), 4.38 (ddd, 1H, $J_{4',5'}$ = 0.7 Hz, $J_{5',6'a}$ = 5.9 Hz, $J_{5',6'b}$ = 6.8 Hz, H-5'), 4.23 (d, 1H, $J_{1,2}$ = 7.9 Hz, H-1), 4.19 (d, 1H, J_{gem} = 11.5 Hz, CH_2Ph), 3.88 (dd, 1H, $J_{5,6a}$ = 1.8 Hz, J_{gem} = 10.9 Hz, H-6a), 3.71-3.65 (m, 2H, H-3 y H-6b), 5.98 (t, 1H, $J_{3,4}$ = 9.3 Hz, $J_{4,5}$ = 9.3 Hz, H-4), 3.46 (s, 3H, CH_3O), 3.43-3.36 (m, 1H, H-5), 3.29 (dd, 1H, $J_{1,2}$ = 7.6 Hz, $J_{2,3}$ = 8.9 Hz, H-2) ppm. ^{13}C NMR (126 MHz, $CDCl_3$): δ 104.5 (C-1), 101.9 (C-1'), 86.0 (C-3), 80.6 (C-2), 75.2 (C-5), 74.0 (*C*H_2Ph), 73.5 (*C*H_2Ph), 71.7 (C-5'), 71.5 (C-3'), 69.7 (C-2'), 69.68 (C-6), 68.7 (C-4), 68.0 (C-4'), 62.0 (C-6'), 57.0 (*C*H_3O) ppm.

The minor product was identified as **7β**. ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.95 dd, 1H, $J_{4',5'}$ = 0.7 Hz, $J_{3',4'}$ = 3.4 Hz, H-4'), 5.81 (dd, 1H, $J_{1',2'}$ = 8.1 Hz, $J_{2',3'}$ = 10.4 Hz, H-2'), 5.53

(dd, 1H, $J_{3',4'}$ = 3.4 Hz, $J_{2',3'}$ = 10.4 Hz, H-3'), 4.85 (d, 1H, $J_{1',2'}$ = 8.1 Hz, H-1'), 4.80, 4.77 (2d, 2H, J_{gem} = 11.2 Hz, CH_2Ph), 4.64-4.59 (m, 1H, H-6'a), 4.51 (dd, 1H, $J_{5',6'b}$ = 7.9 Hz, J_{gem} = 11.7 Hz, H-6'b), 4.35-4.31 (m, 1H, H-5'), 4.33 (d, 1H, J_{gem} = 12.3 Hz, CH_2Ph), 4.24 (d, 1H, $J_{1,2}$ = 8.0 Hz, H-1), 4.16 (d, 1H, J_{gem} = 12.3 Hz, CH_2Ph), 3.84 (dd, 1H, $J_{2,3}$ = 8.7 Hz, $J_{3,4}$ = 8.7 Hz, H-3), 4.74-4.69 (m, 1H, H-4), 3.49 (s, 3H, CH_3O), 4.74-4.69 (m, 1H, H-4), 3.46 (s, 1H, H-6a y H-6b), 4.43-4.36 (m, 1H, H-5), 3.29 (dd, 1H, $J_{1,2}$ = 8.0 Hz, $J_{2,3}$ = 8.7 Hz, H-2) ppm. ^{13}C NMR (126 MHz, $CDCl_3$): δ 103.9 (C-1), 101.7 (C-1'), 81.5 (C-4), 81.4 (C-2), 75.0 (C-3), 74.8 (CH_2Ph), 73.4 (C-5), 73.0 (CH_2Ph), 72.0 (C-5'), 71.4 (C-3'), 69.64 (C-2'), 67.99 (C-4'), 67.94 (C-6), 62.4 (C-6'), 57.0 (CH_3O) ppm.

*4.2.4. Methyl 2,3,4,6-tetra-O-benzoyl-β-D-galactopyranosyl-(1→3)-2,6-di-O-benzoyl-a-D-glucopyranoside (**8α**) and methyl 2,3,4,6-tetra-O-benzoyl-β-D-galactopyranosyl-(1→4)-2,6-di-O-benzoyl-a-D-glucopyranoside (**9α**)*

Obtained according to the general procedure by condensation of **3** with **2α**. The crude mixture showed by TLC, among the excess of **2α** (*Rf* 0.05, 85:15 toluene/EtOAc), the presence of a major product of *Rf* 0.40 and a minor product of *Rf* 0.37. According to the integration of the ^{1}H NMR signals corresponding to the OCH_3 groups (3.31 and 3.34 ppm) of both products they were in a 2.1:1.0 ratio.

Column chromatography purification (92:8 toluene-EtOAc) afforded fractions enriched in each regioisomer. The first fractions afforded syrupy compound ***8α*** (0.05 g, 49%), ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.16-7.13 (m, 30H, aromatic), 5.98 (dd, 1H, $J_{4',5'}$ = 0.9 Hz, $J_{3',4'}$ = 3.5 Hz, H-4'), 5.82 (dd, 1H, $J_{1',2'}$ = 8.0 Hz, $J_{2',3'}$ = 10.5 Hz, H-2'), 5.58 (dd, 1H, $J_{3',4'}$ = 3.4 Hz, $J_{2',3'}$ = 10.5 Hz, H-3'), 5.04 (d, 1H, $J_{1',2'}$ = 8.0 Hz, H-1'), 5.02 (dd, 1H, $J_{1,2}$ = 3.8 Hz, $J_{2,3}$ = 9.6 Hz, H-2), 4.98 (d, 1H, $J_{1,2}$ = 3.8 Hz, H-1), 4.69 (dd, 1H, $J_{5,6a}$ = 1.0 Hz, J_{gem} = 12.0 Hz, H-6a), 4.64-4.54 (m, 3H, H-6b, H-6'a and H-6'b), 4.47 (ddd, 1H, $J_{4',5'}$ = 0.9 Hz, $J_{5',6a'}$ = 5.5 Hz, $J_{5',6b'}$ = 7.4 Hz, H-5'), 4.20 (dd, 1H, $J_{3,4}$ = 8.6 Hz, $J_{2,3}$ = 9.6 Hz, H-3), 3.90-3.88 (m, 2H, H-4 and H-5), 3.31 (s, 3H, CH_3O) ppm. ^{13}C NMR (126 MHz, $CDCl_3$): δ 166.3, 166.1, 165.48, 165.45, 165.14, 165.10 (*C*OPh), 133.7, 133.37, 133.34, 133.2, 133.0, 132.7, 130.0, 129.97, 129.94, 129.7, 129.6, 129.2, 129.00, 128.69, 128.67, 128.4, 128.38, 127.35, 128.31, 128.2, 128.1, 127.9 (aromatic), 102.0 (C-1'), 96.6 (C-1),

82.0 (C-3), 72.3 (C-2), 71.9 (C-5'), 71.4 (C-3'), 69.38 (C-2'), 69.31 (C-4), 68.6 (C-5), 67.8 (C-4'), 63.4 (C-6), 61.9 (C-6'), 55.1 (*C*H_3O) ppm.

Further elution from the column afforded a fraction enriched in **9α** (0.02 mg, 26%). ^{1}H NMR (500 MHz, $CDCl_3$) signals for **9α**: δ 8.14-7.14 (m, 30H, aromatic), 5.97 (d, 1H, $J_{3',4'}$ = 3.4 Hz, H-4'), 5.91 (dd, 1H, $J_{1',2'}$ = 8.0 Hz, $J_{2',3'}$ = 10.5 Hz, H-2'), 5.58 (dd, 1H, $J_{3',4'}$ = 3.4 Hz, $J_{2',3'}$ = 10.5 Hz, H-3'), 5.08 (dd, 1H, $J_{1,2}$ = 3.7 Hz, $J_{2,3}$ = 10.0 Hz, H-2), 5.03 (d, 1H, $J_{1',2'}$ = 8.0 Hz, H-1'), 4.92 (d, 1H, $J_{1,2}$ = 3.7 Hz, H-1), 4.67 (d, 1H, *J* = 7.8 Hz, H-6'a), 4.46-4.41 (m, 3H, H-5', H-6a and H-6'b), 3.37 (ddd, 1H, $J_{3,OH}$ = 1.6 Hz, $J_{3,4}$ = 8.4 Hz, $J_{2,3}$ = 10.0 Hz, H-3), 4.19 (dd, 1H, $J_{5,6b}$ = 4.1 Hz, J_{gem} = 12.0 Hz, H-6b), 4.02 (ddd, 1H, $J_{5,6a}$ = 1.6 Hz, $J_{5,6b}$ = 4.1 Hz, $J_{4,5}$ = 9.9 Hz, H-5), 3.83 (dd, 1H, $J_{3,4}$ = 8.4 Hz, $J_{4,5}$ = 9.9 Hz, H-4), 3.34 (s, 3H, C*H*$_3$O) ppm. ^{13}C NMR (126 MHz, $CDCl_3$): δ 166.1, 166.0, 165.5, 165.4, 165.3, 165.2 (*C*OPh), 133.7, 133.5, 133.3, 133.2, 133.1, 130.0, 129.9, 129.8, 129.7, 129.68, 129.66, 129.62, 129.5, 129.2, 128.8, 128.7, 128.45, 128.40, 128.37, 127.33, 128.31, 128.2, 128.0 (aromatic), 102.2 (C-1'), 97.1 (C-1), 82.7 (C-4), 72.5 (C-2), 72.4 (C-5'), 71.5 (C-3'), 69.9 (C-3), 69.4 (C-2'), 67.9 (C-4'), 67.3 (C-5), 62.7 (C-6'), 62.3 (C-6), 55.4 (*C*H_3O) ppm.

4.2.5. Methyl 2,3,4,6-tetra-O-benzoyl-β-D-galactopyranosyl-(1→3)-2,6-di-O-benzoyl-β-D-galactopyranoside ***(8β)*** *and methyl 2,3,4,6-tetra-O-benzoyl-β-D-galactopyranosyl-(1→4)-2,6-di-O-benzoyl-β-D-glucopyranoside* ***(9β)***

Obtained according to the general procedure by condensation of **3** with **2β**. TLC analysis of the crude reaction showed total consumption of **3** and a single spot of *Rf* 0.57 (85:15 toluene-EtOAc) composed, according to the ^{1}H NMR spectrum of the crude mixture, by two regioisomers in 1:5.6 ratio. The products could not be separated by column chromatography (0.08 g; 90%) but all the NMR signals were assigned.

The minor product was identified as **8β**. ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.94 (d, 1H, $J_{3',4'}$ = 3.5 Hz, H-4'), 5.82 (dd, 1H, $J_{1',2'}$ = 8.0 Hz, $J_{2',3'}$ = 10.5 Hz, H-2'), 5.51 (dd, 1H, $J_{3',4'}$ = 3.5 Hz, $J_{2',3'}$ = 10.5 Hz, H-3'), 5.28-5.22 (m, 1H, H-2), 4.93 (d, 1H, $J_{1',2'}$ = 8.0 Hz, H-1'), 4.77 (dd, 1H, $J_{5,6a}$ = 4.8 Hz, J_{gem} = 11.5 Hz, H-6a), 4.66 (dd, 1H, $J_{5',6'a}$ = 2.0 Hz, J_{gem} = 11.8 Hz, H-6'a), 4.57-4.48 (m, 2H, H-6b y H-6'b), 4.47-4.39 (m, 2H, H-1 y H-5'), 4.25 (d, 1H, $J_{4,OH}$ = 0.8 Hz, OH-4), 3.96 (ddd, 1H, $J_{4,OH}$ = 0.8 Hz, $J_{3,4}$ = 8.3 Hz, $J_{4,5}$ = 9.6 Hz, H-4), 3.92-4.87 (m, 1H, H-3), 3.63 (ddd, 1H, $J_{5,6b}$ = 2.0 Hz, $J_{5,6a}$ = 4.8 Hz, $J_{4,5}$ = 9.6 Hz, H-5),

3.36 (s, 3H, CH_3O) ppm. ^{13}C NMR (126 MHz, $CDCl_3$): δ102.1 (C-1'); 102.0 (C-1); 85.9 (C-3); 73.8 (C-5); 72.0 (C-2); 71.9 (C-5'); 71.3 (C-3'); 69.3 (C-2'); 68.8 (C-4); 67.8 (C-4'); 63.4 (C-6); 62.2 (C-6'); 56.5 (CH_3O) ppm.

The major product was identified as **9β**. ^1H NMR (500 MHz, $CDCl_3$): δ 8.13-7.01 (m, 30H, aromatic), 5.96 (d, 1H, $J_{3',4'}$ = 3.4 Hz, H-4'), 5.90 (dd, 1H, $J_{1',2'}$ = 8.0 Hz, $J_{2',3'}$ = 10.4 Hz, H-2'), 5.58 (dd, 1H, $J_{3',4'}$ = 3.4 Hz, $J_{2',3'}$ = 10.4 Hz, H-3'), 5.23 (dd, 1H, $J_{1,2}$ = 8.0 Hz, $J_{2,3}$ = 9.5 Hz, H-2), 5.03 (d, 1H, $J_{1',2'}$ = 8.0 Hz, H-1'), 4.68 (dd, 1H, $J_{5',6a'}$ = 1.1 Hz, J_{gem} = 9.6 Hz, H-6'a), 4.54 (d, 1H, $J_{3,OH}$ = 1.8 Hz, OH-3), 4.51 (dd, 1H, $J_{5,6a}$ = 1.6 Hz, J_{gem} = 12.0 Hz, H-6a), 4.50 (d, 1H, $J_{1,2}$ = 8.0 Hz, H-1), 4.43-4.36 (m, 2H, H-5' and H-6'b), 4.16 (dd, 1H, $J_{5,6b}$ = 4.2 Hz, J_{gem} = 12.0 Hz, H-6b), 4.09 (ddd, 1H, $J_{3,OH}$ = 1.8 Hz, $J_{3,4}$ = 8.5 Hz, $J_{2,3}$ = 9.5 Hz, H-3), 3.91 (dd, 1H, $J_{3,4}$ = 8.5 Hz, $J_{4,5}$ = 9.8 Hz, H-4), 3.75 (ddd, 1H, $J_{5,6a}$ = 1.6 Hz, $J_{5,6b}$ = 4.2 Hz, $J_{4,5}$ = 9.8 Hz, H-5), 3.40 (s, 3H, CH_3O) ppm. ^{13}C NMR (126 MHz, $CDCl_3$): δ 166.1, 165.4, 165.37, 165.30, 165.29, 165.25 (*C*OPh), 133.7, 133.5, 133.3, 133.2, 133.0, 132.9, 129.99, 129.97, 129.8, 129.76, 129.70, 129.6, 129.59, 129.55, 129.4, 129.0, 128.7, 128.67, 128.64, 128.42, 128.40, 128.38, 128.34, 128.28, 128.23, 128.20, 128.19, 128.15, 128.0 (aromatic), 102.3 (C-1'), 101.6 (C-1), 82.3 (C-4), 73.5 (C-3), 72.7 (C-2), 72.5 (C-5'), 72.0 (C-5), 71.4 (C-3'), 69.4 (C-2'), 67.9 (C-4'), 62.7 (C-6'), 62.2 (C-6), 56.7 (*C*H_3O) ppm.

*4.2.6. Methyl 2,3,5,6-tetra-O-benzoyl-β-D-galactofuranosyl-(1→3)-2,6-di-O-benzyl-a-D-glucopyranoside (**10α**) and methyl 2,3,5,6-tetra-O-benzoyl-β-D-galactofuranosyl-(1→4)-2,6-di-O-benzyl-a-D-glucopyranoside (**11α**)*

Obtained according to the general procedure by condensation of **4** with **1α.** TLC of the crude mixture showed the formation of two regioisomers of R_f 0.46 and 0.50 (85:15 toluene/EtOAc), which according to the integration of the ^1H NMR signals were in a 3.45:1 ratio. After purification by column chromatography (92:8 toluene/EtOAc) fractions of *Rf* 0.50 afforded compound **11α** (0.02 g; 20%) impurified with 2,3,4,6-*O*-Bz-α,β-Gal*p*. From the mixture, signals corresponding to **11α:** ^1H NMR (500 MHz, $CDCl_3$): δ 6.01 (dt, 1H, $J_{4',5'}$ = 4.0 Hz, $J_{5',6'a}$ = 4.0 Hz, $J_{5',6'b}$ = 6.7 Hz, H-5'), 5.66-5.63 (m, 1H, H-3'), 5.36 (d, 1H, $J_{2',3'}$ = 1.4 Hz, H-2'), 5.31 (s, 1H, H-1'), 5.00 (dd, 1H, $J_{4',5'}$ = 4.0 Hz, $J_{3',4'}$ = 5.4 Hz, H-4'), 5.66-5.63 (m, 3H, H-6'a, H-6'b y CH_2Ph), 4.64 (d, 1H, J_{gem} = 12.1 Hz, CH_2Ph), 4.62 (d, 1H,

$J_{1,2}$ = 3.6 Hz, H-1), 4.56, 4.51 (2d, 2H, J_{gem} = 12.0 Hz, CH_2Ph), 4.03 (ddd, 1H, $J_{3,OH}$ = 2.4 Hz, $J_{3,4}$ = 8.5 Hz, $J_{2,3}$ = 9.5 Hz, H-3), 3.87 (dd, 1H, $J_{5,6a}$ = 3.0 Hz, J_{gem} = 10.8 Hz, H-6a), 3.83 (dd, 1H, $J_{3,4}$ = 8.5 Hz, $J_{4,5}$ = 9.9 Hz, H-4), 3.81-3.77 (m, 1H, H-5), 3.70 (dd, 1H, $J_{5,6b}$ = 1.7 Hz, J_{gem} = 10.8 Hz, H-6b), 3.42 (dd, 1H, $J_{1,2}$ = 3.6 Hz, $J_{2,3}$ = 9.5 Hz, H-2), 3.34 (s, 3H, CH_3O), 3.09 (d, 1H, $J_{3,OH}$ = 2.4 Hz, OH-3) ppm. ^{13}C NMR (126 MHz, $CDCl_3$): δ 106.1 (C-1'), 101.0 (C-1), 82.3 (C-2'), 81.2 (C-4'), 79.1 (C-2), 77.2 (C-3' y C-4), 73.31 (CH_2Ph), 73.30 (CH_2Ph), 71.9 (C-3), 70.4 (C-5'), 69.2 (C-5), 68.0 (C-6), 63.4 (C-6'), 55.2 (CH_3O) ppm.

Fractions of *Rf* 0.46 afforded syrupy compound **10α** (0.07 g, 69%), $[\alpha]_D$ +1 (c 1, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.09-7.13 (m, 30H, aromatic), 5.98 (ddd, 1H, $J_{4',5'}$ = 4.2 Hz, $J_{5',6a'}$ = 4.4 Hz, $J_{5',6b'}$ = 6.4 Hz, H-5'), 5.67 (s, 1H, H-1'), 5.66-5.63 (m, 2H, H-2' and H-3'), 4.89 (dd, 1H, $J_{4',5'}$ = 4.2 Hz, $J_{3',4'}$ = 4.6 Hz, H-4'), 4.77 (dd, 1H, $J_{5',6a'}$ = 4.4 Hz, J_{gem} = 12.0 Hz, H-6'a), 4.76 (d, 1H, J_{gem} = 12.4 Hz, CH_2Ph), 4.71 (dd, 1H, $J_{5',6b'}$ = 6.4 Hz, J_{gem} = 12.0 Hz, H-6'b), 4.62 (d, 1H, J_{gem} = 12.4 Hz, CH_2Ph), 4.60 (d, 1H, $J_{1,2}$ = 3.6 Hz, H-1), 4.58 (d, 1H, J_{gem} = 12.3 Hz, CH_2Ph), 4.55 (d, 1H, J_{gem} = 12.3 Hz, CH_2Ph), 3.98 (dd, 1H, $J_{3,4}$ = 8.8 Hz, $J_{2,3}$ = 9.6 Hz, H-3), 3.73-3.64 (m, 3H, H-5, H-6a and H-6b), 3.60 (ddd, 1H, $J_{4,OH}$ = 3.1, $J_{3,4}$ = 8.8 Hz, $J_{4,5}$ = 9.5 Hz, H-4), 3.54 (dd, 1H, $J_{1,2}$ = 3.6 Hz, $J_{2,3}$ = 9.6 Hz, H-2), 3.36 (s, 3H, CH_3O), 3.21 (d, 1H, $J_{4,OH}$ = 3.1 Hz, OH-4) ppm. ^{13}C NMR (126 MHz, $CDCl_3$): δ 166.1, 165.6, 165.5, 165.1 (*C*OPh), 138.0, 137.9, 133.5, 133.3, 133.2, 133.0, 129.7, 129.5, 129.3, 128.99, 128.96, 128.8, 128.4, 128.36, 128.33, 128.32, 128.2, 128.1, 128.8, 127.8, 127.5 (aromatic), 107.3 (C-1'), 98.0 (C-1), 81.63 (C-4'), 81.60 (C-2'), 81.3 (C-3), 78.2 (C-2), 77.6 (C-3'), 73.5 (CH_2Ph), 73.4 (CH_2Ph), 70.39 (C-5'), 70.30 (C-5), 69.5 (C-4), 69.2 (C-6), 63.2 (C-6'), 55.1 (CH_3O) ppm. ESIMS: *m/z* calcd for $C_{55}H_{52}NaO_{15}$ $[M+Na]^+$ 975.3198. Found: 975.3206

*4.2.7. Methyl 2,3,5,6-tetra-O-benzoyl-β-D-galactofuranosyl-(1→3)-2,6-di-O-benzyl-β-D-glucopyranoside (**10β**) and methyl 2,3,5,6-tetra-O-benzoyl-β-D-galactofuranosyl-(1→4)-2,6-di-O-benzyl-β-D-glucopyranoside (**11β**)*

Obtained according to the general procedure by condensation of **4** with **1β.** TLC of the crude mixture showed the formation of two regioisomers of R_f 0.58 and 0.50 (85:15 toluene/EtOAc), which according to the integration of the ^{1}H NMR anomeric signals were in a 1.75:1 ratio. After purification by column chromatography (9:1 toluene/EtOAc)

fractions of *Rf* 0.58 (85:15 toluene/EtOAc) afforded **11β** (0.03 g, 32%), $[\alpha]_D$ -25 (*c* 1, $CHCl_3$). ^{1}H NMR(500 MHz, $CDCl_3$): δ 8.11-7.11 (m, 30H, aromatic), 6.00 (apparent dt, 1H, $J_{4',5'}$ = 3.8 Hz, $J_{5',6a'}$ = 4.1 Hz, $J_{5',6b'}$ = 7.0 Hz, H-5'), 5.62 (d, 1H, $J_{3',4'}$ = 5.4 Hz, H-3'), 5.39-5.38 (m, 2H, H-1' and H-2'), 4.97 (dd, 1H, $J_{4',5'}$ = 3.8 Hz, $J_{3',4'}$ = 5.4 Hz, H-4'), 4.90 (d, 1H, J_{gem} = 11.3 Hz, C*H*$_2$Ph), 4.78 (dd, 1H, $J_{5',6a'}$ = 4.1 Hz, J_{gem} = 12.1 Hz, H-6'a), 4.71 (dd, 1H, $J_{5',6b'}$ = 7.0 Hz, J_{gem} = 12.1 Hz, H-6'b), 4.69 (d, 1H, J_{gem} = 11.3 Hz, C*H*$_2$Ph), 4.59, 4.55 (2d, 2H, J_{gem} = 12.1 Hz, C*H*$_2$Ph), 4.31 (d, 1H, $J_{1,2}$ = 7.8 Hz, H-1), 3.86 (dd, 1H, $J_{5,6a}$ = 3.9 Hz, J_{gem} = 11.0 Hz, H-6^a), 3.83 (apparent t, 1H, $J_{3,4}$ = 9.0 Hz, $J_{4,5}$ = 9.8 Hz, H-4), 3.80 (dd, 1H, $J_{5,6b}$ = 1.8 Hz, J_{gem} = 11.0 Hz, H-6b), 3.70 (td, 1H, $J_{3,4}$ = 9.0 Hz, $J_{2,3}$ = 9.1 Hz, $J_{3,OH}$ = 1.7 Hz, H-3), 3.56 (s, 3H, C*H*$_3$O), 3.50 (ddd, 1H, $J_{5,6b}$ = 1.8 Hz, $J_{5,6a}$ = 3.9 Hz, $J_{4,5}$ = 9.8 Hz, H-5), 3.29 (dd, 1H, $J_{1,2}$ = 7.8 Hz, $J_{2,3}$ = 9.1 Hz, H-2), 3.09 (d, 1H, $J_{3,OH}$ = 1.7 Hz, OH-3) ppm.^{13}C NMR (126 MHz, $CDCl_3$): δ 166.1, 165.7, 165.6, 165.5 (*C*OPh), 138.4, 138.1, 133.5, 133.4, 133.3, 133.0, 130.0, 129.88, 129.84, 129.7, 129.5, 129.4, 128.9, 128.7, 128.6, 128.46, 128.41, 128.40, 128.3, 128.1, 128.0, 127.7, 127.5, 127.4 (aromatic), 106.3 (C-1'), 104.2 (C-1), 82.2 (C-2'), 81.5 (C-2), 81.2 (C-4'), 77.3 (C-3'), 77.1 (C-4), 74.9 (C-3), 74.5 (*C*H$_2$Ph), 74.4 (C-5), 73.2 (*C*H$_2$Ph), 68.3 (C-5'), 68.3 (C-6), 63.5 (C-6'), 57.0 (*C*H$_3$O) ppm. ESIMS: *m/z* calcd for $C_{55}H_{52}NaO_{15}$ $[M+H]^+$ 975.3198. Found: 975.3201.

Fractions of *Rf* 0.50 afforded compound **10β** (0.05 g, 55%), $[\alpha]_D$ -8 (*c* 1, $CHCl_3$). ^{1}H NMR(500 MHz, $CDCl_3$): δ 8.13-7.05 (m, 30H, aromatic), 5.98 (apparent dt ddd, 1H, $J_{4',5'}$ = 4.8 Hz, $J_{5',6a'}$ = 4.6 Hz, $J_{5',6b'}$ = 6.5 Hz, H-5'), 5.63 (s, 1H, H-1'), 5.60 (dd, 1H, $J_{2',3'}$ = 1.4 Hz, $J_{3',4'}$ = 5.1 Hz, H-3'), 5.56 (d, 1H, $J_{2',3'}$ = 1.4 Hz, H-2'), 4.87 (dd, 1H, $J_{4',5'}$ = 4.0 Hz, $J_{3',4'}$ = 5.1 Hz, H-4'), 4.83 (d, 1H, J_{gem} = 10.9 Hz, C*H*$_2$Ph), 4.74 (dd, 1H, $J_{5',6a'}$ = 4.6 Hz, J_{gem} = 11.9 Hz, H-6'a), 4.72 (d, 1H, J_{gem} = 10.9 Hz, C*H*$_2$Ph), 4.70 (dd, 1H, $J_{5',6b'}$ = 6.5 Hz, J_{gem} = 11.9 Hz, H-6'b), 4.60, 4.57 (2d, 2H, J_{gem} = 12.3 Hz, C*H*$_2$Ph), 4.30 (d, 1H, $J_{1,2}$ = 7.9 Hz, H-1), 3.77 (dd, 1H, $J_{5,6a}$ = 3.3 Hz, J_{gem} = 10.6 Hz, H-6^a), 3.67 (apparent t, 1H, $J_{3,4}$ = 9.0 Hz, $J_{2,3}$ = 9.1 Hz, H-3), 3.66 (dd, 1H, $J_{5,6b}$ = 5.3 Hz, J_{gem} = 10.6 Hz, H-6b), 3.57 (s, 3H, C*H*$_3$O), 3.54 (td, 1H, $J_{4,OH}$ = 2.8 Hz, $J_{3,4}$ = 9.0 Hz, $J_{4,5}$ = 9.5 Hz, H-4), 3.43 (ddd, 1H, $J_{5,6a}$ = 3.3 Hz, $J_{5,6b}$ = 5.3 Hz, $J_{4,5}$ = 9.5 Hz, H-5), 3.42 (dd, 1H, $J_{1,2}$ = 7.9 Hz, $J_{2,3}$ = 9.1 Hz, H-2), 3.28 (d, 1H, $J_{4,OH}$ = 2.8 Hz, OH-4) ppm. ^{13}C NMR (126 MHz, $CDCl_3$): δ 166.1, 165.6, 165.5, 165.1 (*C*OPh), 138.1, 138.0, 133.5, 133.3, 133.2, 133.0, 129.95, 129.92, 129.8, 129.7, 129.5, 129.3, 128.9, 128.8, 128.4, 128.39, 128.38, 128.35, 128.31, 128.26, 128.25,

128.1, 127.66, 127.64, 127.5 (aromatic), 106.9 (C-1'), 104.5 (C-1), 82.9 (C-3), 81.69 (C-2'), 81.66 (C-4'), 80.6 (C-2), 77,5 (C-3'), 74.58 (*C*H_2Ph), 74.54 (C-5), 73.5 (*C*H_2Ph), 70.3 (C-5'), 70.2 (C-4), 69.9 (C-6), 63.2 (C-6'), 57.1 (*C*H_3O) ppm. ESIMS: *m/z* calcd for $C_{55}H_{52}NaO_{15}$ $[M+H]^+$ 975.3198. Found: 975.3168.

*4.2.8. Methyl 2,3,5,6-tetra-O-benzoyl-β-D-galactofuranosyl-(1→3)-2,6-di-O-benzoyl-a-D-glucopyranoside (**12α**) and methyl 2,3,5,6-tetra-O-benzoyl-β-D-galactofuranosyl-(1→4)-2,6-di-O-benzoyl-a-D-glucopyranoside (**13α**)*

Obtained according to the general procedure by condensation of **4** with **2α**. After 2h of reaction TLC analysis showed consumption of compound **4** and the formation of products of *Rf* 0.28 and 0.32 (85:15 Toluene-EtOAc). According to the integration of the ^{1}H NMR signals corresponding to H-4, they were in 2.0:1.0 ratio.

After purification by column chromatography (9:1 Toluene-EtOAc) fractions of Rf 0.32 afforded **12α** (0.04 g; 38%), impurified with **13α**, ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.12-7.21 (m, 30H, aromatic), 5.98 (dt, 1H, $J_{4',5'}$ = 4.1 Hz, $J_{5',6a'}$ = 4.6 Hz, $J_{5',6b'}$ = 6.6 Hz, H-5'), 5.68 (dd, 1H, $J_{2',3'}$ = 1.8 Hz, $J_{3',4'}$ = 5.5 Hz, H-3'), 5.49 (dd, 1H, $J_{1',2'}$ = 0.7 Hz, $J_{2',3'}$ = 1.8 Hz, H-2'), 5.48 (s, 1H, H-1'), 5.07-5.02 (m, 2H, H-1 and H-2), 4.94 (dd, 1H, $J_{4',5'}$ = 4.1 Hz, $J_{3',4'}$ = 5.5 Hz, H-4'), 4.79 (dd, 1H, $J_{5',6a'}$ = 4.6 Hz, J_{gem} = 12.0 Hz, H-6'a), 4.78 (dd, 1H, $J_{5,6a}$ = 1.9 Hz, J_{gem} = 11.9 Hz, H-6a), 4.69 (dd, 1H, $J_{5,6b}$ = 4.4 Hz, J_{gem} = 11.9 Hz, H-6b), 4.68 (dd, 1H, $J_{5',6b'}$ = 6.6 Hz, J_{gem} = 12.0 Hz, H-6'b), 4.31 (td, 1H, $J_{3,OH}$ = 3.2 Hz, $J_{3,4}$ = 8.8 Hz, $J_{2,3}$ = 8.9 Hz, H-3), 4.14 (ddd, 1H, $J_{5,6a}$ = 1.9 Hz, $J_{5,6b}$ = 4.4 Hz, $J_{4,5}$ = 10.0 Hz, H-5), 3.88 (dd, 1H, $J_{3,4}$ = 8.8 Hz, $J_{4,5}$ = 10.0 Hz, H-4), 3.64 (d, 1H, $J_{3,OH}$ = 3.2 Hz, OH-3), 3.42 (s, 3H, C*H*$_3$O) ppm. ^{13}C NMR (126 MHz, $CDCl_3$): δ 166.19, 166.12, 165.65, 165.61, 163.5 (*C*OPh), 133.6, 133.4, 133.3, 133.2, 133.1, 133.0, 129.9, 129.88, 129.85, 129.7, 129.6, 129.3, 129.1, 128.9, 128.7, 128.6, 128.49, 128.40, 128.35, 128.34 (aromatic), 107.5 (C-1'), 97.1 (C-1), 82.7 (C-2'), 81.6 (C-4'), 79.8 (C-4), 77.1 (C-3'), 73.4 (C-2), 70.3 (C-3), 70.3 (C-5'), 67.9 (C-5), 63.0 (C-6), 62.9 (C-6'), 55.5 (*C*H_3O) ppm.

Fractions of *Rf* 0.28 afforded a sample enriches in **13α** (0.05 g; 54%). ^{1}H NMR (500 MHz, $CDCl_3$): 8.10-7.13 (m, 30H, aromatic), 5.96 (ddd, 1H, $J_{4',5'}$ = 4.3 Hz, $J_{5',6a'}$ = 4.7 Hz, $J_{5',6b'}$ = 6.3 Hz, H-5'), 5.62 (dd, 1H, $J_{2',3'}$ = 1.9 Hz, $J_{3',4'}$ = 5.5 Hz, H-3'), 5.55 (s, 1H, H-1'), 5.42 (dd, 1H, $J_{1',2'}$ = 0.8 Hz, $J_{2',3'}$ = 1.9 Hz, H-2'), 5.14 (dd, 1H, $J_{1,2}$ = 3.7 Hz, $J_{2,3}$ = 10.0

Hz, H-2), 5.07 (d, 1H, $J_{1,2}$ = 3.7 Hz, H-1), 4.9 (dd, 1H, $J_{4',5'}$ = 4.3 Hz, $J_{3',4'}$ = 5.5 Hz, H-4'), 4.79 (dd, 1H, $J_{5',6a'}$ = 4.7 Hz, J_{gem} = 11.9 Hz, H-6'a), 4.72 (dd, 1H, $J_{5',6b'}$ = 6.3 Hz, J_{gem} = 11.9 Hz, H-6'b), 4.65-4.57 (m, 2H, H-6a and H-6b), 4.25 (dd, 1H, $J_{3,4}$ = 9.0 Hz, $J_{2,3}$ = 10.0 Hz, H-3), 3.98 (ddd, 1H, $J_{5,6a}$ = 2.9 Hz, $J_{5,6b}$ = 4.6 Hz, $J_{4,5}$ = 9.9 Hz, H-5), 3.71 (dd, 1H, $J_{3,4}$ = 9.0 Hz, $J_{4,5}$ = 9.9 Hz, H-4), 3.64 (br. s, 1H, OH-4), 3.39 (s, 3H, CH_3O) ppm. ^{13}C NMR (126 MHz, $CDCl_3$): δ 166.6, 166.2, 165.8, 165.6, 165.5, 164.8 (*C*OPh), 133.5, 133.3, 133.2, 133.17, 133.13, 133.0, 129.96, 129.92, 129.88, 129.82, 129.7, 129.46, 129.40, 129.3, 129.0, 128.7, 128.5, 128.4, 128.3, 128.27, 128.23, 128.20, (aromatic), 107.4 (C-1'), 97.0 (C-1), 81.9 (C-2'), 81.6 (C-4'), 79.7 (C-3), 77.05 (C-3'), 72.7 (C-2), 70.3 (C-5'), 69.7 (C-3), 69.5 (C-4), 63.6 (C-6), 63.0 (C-6'), 55.2 (*C*H_3O) ppm.

*4.2.9. Methyl 2,3,5,6-tetra-O-benzoyl-β-D-galactofuranosyl-(1→3)-2,6-di-O-benzoyl-β-D-glucopyranoside (**12β**) and methyl 2,3,5,6-tetra-O-benzoyl-β-D-galactofuranosyl-(1→4)-2,6-di-O-benzoyl-β-D-glucopyranoside (**13β**)*

Obtained according to the general procedure by condensation of **4** with **2β**. ^{1}H NMR spectrum of the crude mixture evidenced the formation of two disaccharides in a 1:1 ratio, according to the integration of the H-3' signals. After purification by column chromatography (9:1 toluene-hexane) fractions of *Rf* 0.47 (85:15 toluene-hexane) afforded compound **13β** (0.04 g, 42%), $[\alpha]_D$ -46 (*c* 1, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$): ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.11-7.12 (m, 30H, aromatic), 5.95 (ddd, 1H, $J_{4',5'}$ = 4.1 Hz, $J_{5',6a'}$ = 4.6 Hz, $J_{5',6b'}$ = 6.5 Hz, H-5'), 5.66 (dd, 1H, $J_{2',3'}$ = 1.8 Hz, $J_{3',4'}$ = 5.5 Hz, H-3'), 5.47 (dd, 1H, $J_{1',2'}$ = 0.6 Hz, $J_{2',3'}$ = 1.8 Hz, H-2'), 5.45 (s, 1H, H-1'), 5.18 (dd, 1H, $J_{1,2}$ = 8.0 Hz, $J_{2,3}$ = 9.1 Hz, H-2), 4.92 (dd, 1H, $J_{4',5'}$ = 4.1 Hz, $J_{3',4'}$ = 5.5 Hz, H-4'), 4.83 (dd, 1H, $J_{5,6a}$ = 1.9 Hz, J_{gem} = 12.1 Hz, H-6a), 4.76 (dd, 1H, $J_{5',6a'}$ = 4.6 Hz, J_{gem} = 12.0 Hz, H-6'a), 4.66 (dd, 1H, $J_{5',6b'}$ = 6.5 Hz, J_{gem} = 12.0 Hz, H-6'b), 4.65 (dd, 1H, $J_{5,6b}$ = 4.4 Hz, J_{gem} = 12.1 Hz, H-6b), 4.57 (d, 1H, $J_{1,2}$ = 8.0 Hz, H-1), 3.99 (ddd, 1H, $J_{3,OH}$ = 3.6 Hz, $J_{3,4}$ = 8.7 Hz, $J_{2,3}$ = 9.1 Hz, H-3), 3.94 (apparent t, 1H, $J_{3,4}$ = 8.7 Hz, $J_{4,5}$ = 9.3 Hz, H-4), 3.85 (ddd, 1H, $J_{5,6a}$ = 1.9 Hz, $J_{5,6b}$ = 4.4 Hz, $J_{4,5}$ = 9.3 Hz, H-5), 3.75 (d, 1H, $J_{3,OH}$ = 3.6 Hz, OH-3), 3.48 (s, 3H, CH_3O) ppm. ^{13}C NMR (126 MHz, $CDCl_3$): δ 166.11, 166.10, 165.7, 165.6, 165.58, 165.54 (*C*OPh), 133.5, 133.39, 133.30, 133.15, 133.11, 132.9, 129.95, 129.93, 129.91, 129.86, 129.83, 129.7, 129.6, 129.3, 129.1, 129.0, 128.7, 128.46, 128.44,

128.38, 128.36, 128.33, 128.31, 128.30, 128.2 (aromatic), 107.5 (C-1'), 101.7 (C-1), 82.6 (C-2'), 81.6 (C-4'), 79.5 (C-4), 77.1 (C-3'), 73.9 (C-3), 73.8 (C-2), 72.6 (C-5), 70.3 (C-5'), 2 x 68.3 (C-6 and C-6'), 56.8 (*C*H_3O) ppm. ESIMS: *m*/*z* calcd for $C_{55}H_{48}NaO_{17}$ $[M+H]^+$ 1003.2784. Found: 1003.2771.

Fractions of R_f 0.43 (85:15 toluene-hexane) **afforded compound 12β** 0.05 g, 47%), $[\alpha]_D$ +8 (*c* 1, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.11-7.13 (m, 30H, aromatic), 5.93 (ddd, 1H, $J_{4',5'}$ = 4.3 Hz, $J_{5',6a'}$ = 4.8 Hz, $J_{5',6b'}$ = 6.2 Hz, H-5'), 5.59 (dd, 1H, $J_{2',3'}$ = 1.9 Hz, $J_{3',4'}$ = 5.4 Hz, H-3'), 5.43 (s, 1H, H-1'), 5.35 (dd, 1H, $J_{1',2'}$ = 0.8 Hz, $J_{2',3'}$ = 1.9 Hz, H-2'), 5.32 (dd, 1H, $J_{1,2}$ = 8.0 Hz, $J_{2,3}$ = 9.5 Hz, H-2), 4.86 (dd, 1H, $J_{4',5'}$ = 4.3 Hz, $J_{3',4'}$ = 5.4 Hz, H-4'), 4.78 (dd, 1H, $J_{5',6a'}$ = 4.8 Hz, J_{gem} = 11.9 Hz, H-6'a), 4.67 (dd, 1H, $J_{5',6b'}$ = 6.2 Hz, J_{gem} = 11.9 Hz, H-6'b), 4.64 (dd, 1H, $J_{5,6a}$ = 2.2 Hz, J_{gem} = 12.0 Hz, H-6a), 4.57 (dd, 1H, $J_{5,6b}$ = 5.1 Hz, J_{gem} = 12.0 Hz, H-6b), 4.50 (d, 1H, $J_{1,2}$ = 8.0 Hz, H-1), 3.91 (dd, 1H, $J_{3,4}$ = 8.7 Hz, $J_{2,3}$ = 9.6 Hz, H-3), 3.73 (ddd, 1H, $J_{4,OH}$ = 3.3 Hz, $J_{3,4}$ = 8.7 Hz, $J_{4,5}$ = 9.6 Hz, H-4), 3.67 (ddd, 1H, $J_{5,6a}$ = 2.2 Hz, $J_{5,6b}$ = 5.1 Hz, $J_{4,5}$ = 9.6 Hz, H-5), 3.66 (d, 1H, $J_{3,OH}$ = 3.3 Hz, OH-4), 3.48 (s, 3H, C*H*$_3$O) ppm. ^{13}C NMR (126 MHz, $CDCl_3$): δ 166.6, 166.1, 165.59, 165.54, 165.1, 164.7 (*C*OPh), 133.5, 133.3, 133.2, 133.1, 132.8, 132.9, 129.99, 129.92, 129.8, 129.78, 129.74, 129.6, 129.4, 129.2, 129.0, 128.9, 128.7, 128.59, 128.51, 128.45, 128.41, 128.3, 128.2, 128.1 (aromatic), 107.4 (C-1'), 101.9 (C-1), 83.0 (C-3), 82.0 (C-2'), 81.7 (C-4'), 76.8 (C-3'), 74.0 (C-5), 72.4 (C-2), 70.3 (C-5'), 69.5 (C-4), 63.6 (C-6), 62.9 (C-6'), 56.8 (*C*H_3O) ppm. ESIMS: *m*/*z* calcd for $C_{55}H_{48}NaO_{17}$ $[M+H]^+$ 1003.2784. Found: 1003.2808.

4.3. Computational methods

Molecular mechanics calculations were carried out using the program MM3(92) (QCPE, Indiana, USA)[51,52] Quantum mechanical calculations were carried out using Gaussian 09W (rev. C.01),[53] with standard termination options.

From a given rotamer, an automated routine was used to generate the starting conformations produced by rotation of ± 120º for each of the exocyclic dihedrals. Those conformers within the first 10 kcal/mol were submitted to DFT optimizations at the B3LYP/6-311+G** level and then to single point calculations with M06-2X at the same level. Charges were then obtained with M06-2X at the same level of theory, for both the

ground molecule and the radical cation, using the Mulliken charge distribution. Condensed-to-atom Fukui functions and charges were then obtained as Boltzmann average ratios

Stationary points were characterized by frequency calculations in order to verify that the minima had no imaginary frequencies. Population analysis was carried out using the Boltzmann equation, with a temperature of 298 K and using electronic energies for DFT.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Electronic Supplementary Information (ESI)

Supplementary data to this article (Additional figure and tables and NMR spectra for compounds **6-13**) can be found at https: doi:…….

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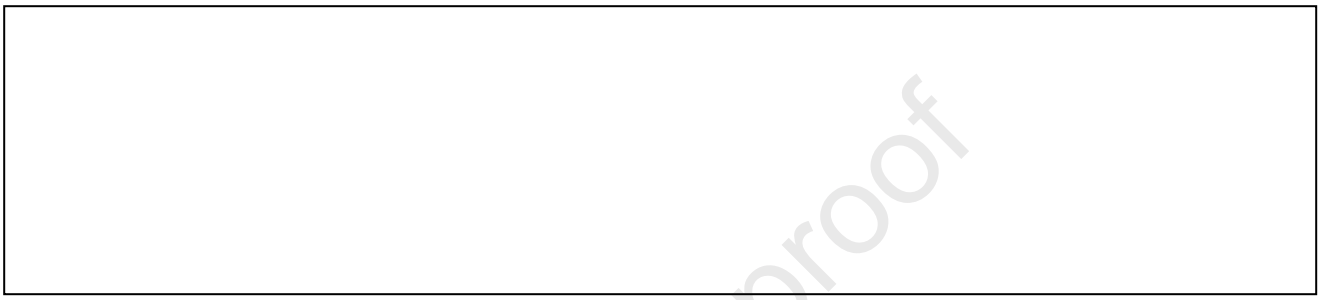
- ✓ Glucopyranosyl acceptors 2,6-di-*O*-protected were efficiently synthesized.
- ✓ Relative reactivity of O-3/O-4 of glucopyranosides in glycosylation reactions was analyzed.
- ✓ The α-anomers are preferentially glycosylated at O-3. The benzoylated β-anomer is preferentially glycosylated at O-4.
- ✓ Experimental regioselectivities and molecular modeling were compared.
- ✓ 1→3 or 1→4 Linkages can selectively be installed using the appropriate glucosyl acceptor.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:



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