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Synthesis of the hexasaccharide from *Trypanosoma cruzi* mucins with the Gal*p*(1 → 2)Gal*f* unit constructed with a superarmed thiogalactopyranosyl donor

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ABSTRACT

Hexasaccharide β-D-Gal*p*-(1→2)-[β-D-Gal*p*-(1 → 3)]-β-D-Gal*p*-(1 → 6)-[β-D-Gal*p*-(1 → 2)-β-D-Gal*f*-(1 → 4)]-D-GlcNAc (**1**) was found O-linked in mucins of *Trypanosoma cruzi* epimastigotes and metacyclic trypomatigotes. Studies on the biological pathways and functionalities of the mucin oligosaccharides are prompted in order to understand the interactions of these molecules with the insect host. Trisaccharide constituent β-D-Gal*p*-(1 → 2)-β-D-Gal*f*-(1 → 4)-D-GlcNAc was constructed from the reducing to the non-reducing end. We discuss the difficulties to introduce a Gal*p* unit at the *O*-2 position of a partially protected galactofuranosyl unit which were overcome using an anchimerically superarmed donor. By this route and employing a [3 + 3] nitrilium convergent approach hexasaccharide **1** was synthesized in moderate yield.

1. Introduction

Around eight million people living mainly in rural areas of Latin America, and 300 thousand people in the United States are infected with *Trypanosoma cruzi*, the protozoan parasite responsible for Chagas disease [1]. Triatomine bugs act as transmission vectors in endemic areas. In recent years, migration to non-endemic regions and/or countries increased the risk of transmission by blood transfusion and organ transplants [2,3]. *T. cruzi* epimastigotes replicate in the midgut of a triatomine insect, and down the intestine, they transform into non-proliferative metacyclic trypomastigotes that pass from the hindgut to the feces. The infective metacyclic trypomastigotes enter the vertebrate host through a skin wound or through mucosal membranes, invade cells and differentiate into amastigotes which, after several cycles of binary division, differentiate back into trypomastigotes. The non-replicative trypomastigotes are released into circulation upon host-cell rupture [4].

T. cruzi presents a surface covered by a dense glycocalyx, whose composition depends on the differentiation stage of the living cycle [5,6]. The most abundant surface glycoproteins are GPI anchored, highly glycosylated, mucin-like proteins (Gp35/50 kDa). Mucins, composed by 60% in mass of O-linked oligosaccharides, become negatively charged by accepting sialic acid residues (SA) from host glycoconjugates [7]. A *trans*-sialidase from the parasite (TcTS), catalyzes the transfer of sialic acid, a crucial process in pathogenesis [8–10]. The enzyme has been cloned [11] and used for *in vitro* sialylation of oligosaccharides [12].

The structure of the mucin oligosaccharides depends not only on the differentiation stage of the parasite, but also on the strains which were grouped into six evolutionary lineages classified as TcI to TcVI on the basis of genetic and phenotypic characteristics [13]. A particular aspect of the *O*-type oligosaccharide chains from Gp35/50 kDa mucins is that they are α-linked to threonine residues via *N*-acetylglucosamine (GlcNAc) instead of *N*-acetylgalactosamine (GalNAc) as found in mammalian mucins. Depending on the parasite strain, up to 20% of these α-D-GlcNAc residues may remain unsubstituted or they may be elongated and branched, with various units of β-galactose in different types of linkages and forms. The O-linked oligosaccharides may be derived from two cores: β-D-Gal*p*(1 → 4)-GlcNAc or β-D-Gal*f*(1 → 4)-GlcNAc [5,14]. Whereas the oligosaccharides carring the first core contain galactose in the pyranose form [15–18], those with the latter core contain galactose in both forms [19–23]. The biological reason of the diversity of this family of oligosaccharides is still not known.

In addition to β-galactopyranose (βGal*p*) residues, TcI parasite strains display antigenic β-galactofuranose (βGal*f*) units in the oligosaccharides. Several of these oligosaccharides have been characterized [19–23] and most of them were chemically synthesized by our group

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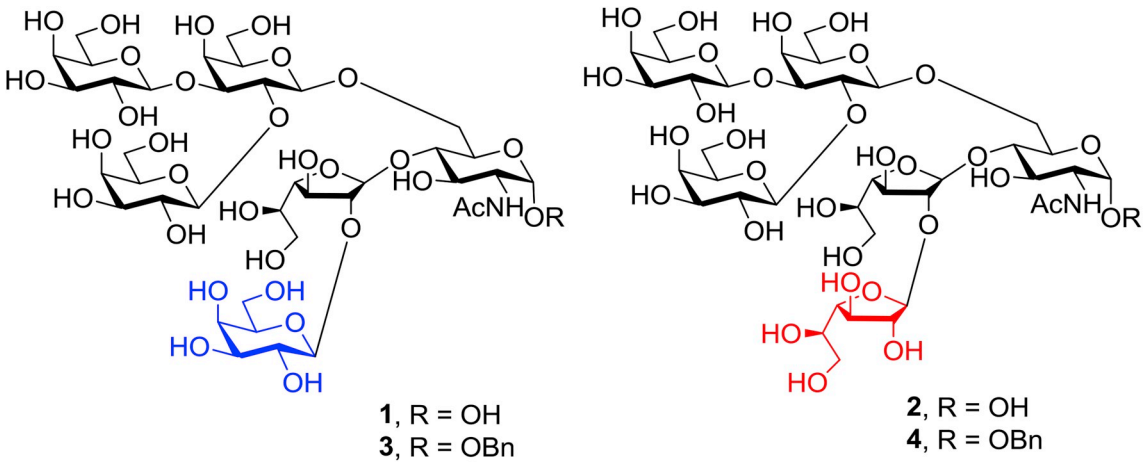


Fig. 1. Gal*f*-containing hexasaccharides found in mucins of *T. cruzi* epimastigotes.

[24–30]. It was recently shown that the trisaccharide (Gal*f*β1-4[Gal*p*β1-6]GlcNAcα) and higher oligosaccharides that contain this motif, in the mucins of O-linked glycans, inhibit the binding of *T. cruzi* epimastigotes to the insect hindgut tissues [31].

Very recently, the ^{13}C labeled tetrasaccharide alditol β-D-Gal*p*-(1→3)-β-D-Gal*p*-(1 → 6)[D-Gal*f*-(1 → 4)]-D-GlcNAc-ol, was synthesized and offered to the scientific community as a standard to facilitate analysis of native mucin *O*-glycans by mass spectroscopy [32]. The synthesis of a tetrasaccharide from the Y strain mucins which contains only Gal*p* has been described [33].

Hexasaccharides **1** and **2** (Fig. 1) are the largest oligosaccharides present in mucins of *T. cruzi* epimastigotes. These oligosaccharides were found not only in TcI strains but also in Tulahuen strains classified as TcVI [23]. Accordingly, several Tulahuen-derived clones displayed TcI-like features suggesting that the original 'strain' may have contained a mixture of parasite genotypes [34].

Isomers **1** and **2** differ in the form of the galactose residue β(1→2) linked to the internal galactofuranose, which is pyranose in **1** and furanose in **2**. We have already synthesized **1** and **2**, and their alditols [29,30], confirming the chemical structure found in the mucins. The synthesis of **2,** the Gal*f*(1 → 2)Gal*f* analogue, was accomplished with complete diastereoselectivity in each glycosylation step [29]. The benzyl glycoside **4** was used as substrate of sialylation with recombinant TcTS and a preparative synthesis allowed the characterization of the sialylated product establishing unequivocally the site of sialylation at the less hindered (1→3)linked galactopyranose [30].

In the case of the Gal*p*(1 → 2)Gal*f* analogue, HPAEC analysis of the *trans*-sialylation reaction on benzyl glycoside **3** showed two monosialylated products whose relative abundance changed with time. The low yield in the synthesis of **3** inhibited our ability to perform a preparative sialylation reaction for characterization of the products [30].

The previous synthesis of **1** [30] involved the synthesis of linear trisaccharide β-D-Gal*p*-(1 → 2)-β-D-Gal*f*-(1 → 4)-D-GlcNAc [28] using D-galactono-1,4-lactone (**5**) as the internal galactofuranosyl precursor. The glycosylation steps showed low stereoselectivity.

In order to afford enough material for biological studies, we looked for other strategies for the synthesis of hexasaccharide **1**. In the present work we describe a new synthesis of hexasaccharide **1** which was planned with the aim of improving the stereoselectivity of each glycosylation step.

2. Results and discussion

Hexasaccharide **1** could be obtained by a [3 + 3] nitrilium based glycosylation of branched trisaccharide imidate **6** [27] and a trisaccharide acceptor **7** (Scheme 1). Trisaccharide donor **6** has a 2-*O*-galactopyranosyl substituent, thus, lacking anchimeric assistance and was previous successfully used for the synthesis of **1** [30] and other mucin oligosaccharides [27,29].

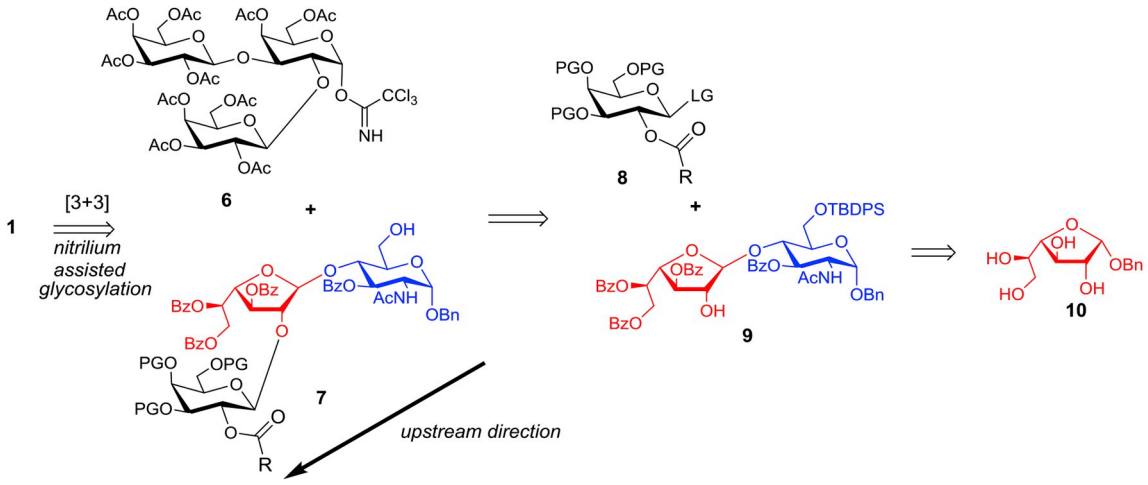
Compound **7** (Scheme 1) would be synthesized by glycosylation of known acceptor **9** [29] with galactopyranosyl donor **8** carrying an acyl group at O-2. Acceptor **9** was previously used for synthesis of the analogous trisaccharide with a terminal β-D-Gal*f* unit. Now **9** was synthesized using benzyl α-D-galactofuranoside **10**, obtained in one step from free galactose. This strategy would ensure the β-diastereoselective construction of the β-D-Gal*p*(1 → 2)-D-Gal*f* glycosidic bond considering the 2-*O*-acyl substituent in compound **8**.

2.1. Synthesis of trisaccharide 7 following the upstream elongation

For glycosylation of **9** we first employed the trichloroacetimidate method [35]. However, with the acyl trichloroacetimdate donors **11** [36] and **12** [37] (Table 1), no glycosylation product was obtained (entries 1 and 2, Table 1) and the acceptor **9** was recovered together with the by-product of hydrolysis of the donor (**13** and **14**). These unfavorable results suggested that a case of armed - disarmed donor or acceptor selective reciprocity RDAS should be considered [38]. For this reason, a glycosylation reaction was tested with a more reactive armed donor, the tetra benzyl trichloroacetimidate **15** [39], using acetonitrile as solvent to favor the formation of the β product by the nitrile effect [40–45] (entry 3, Table 1). However, the expected trisaccharide product was not detected and acceptor **9** was recovered unaltered together with trichloroacetamide **16** [46]. The formation of transposition products in the trichloroacetimidate method is associated with glycosylation reactions in the presence of poorly nucleophilic or sterically hindered acceptors [28,35,47,48].

We then tried the thioglycoside method of glycosylation without success. Reaction of **9** with donor **17** [49] using NIS/TfOH [50] as the promoting system gave 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1 → 2)-1,3,4,6-tetra-*O*-acetyl-β-D-galactopyranose (**18**) in 46% yield and **9** was recovered unaltered (entry 4). Disaccharide **18** was previously obtained by condensation of 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide with 1,3,4,6-tetra-*O*-acetyl-α-D-galactopyranose as acceptor [51].

When the benzoyl analogue of **17**, the thiogalactopyranoside **19** was employed, a similar by-product **20** was obtained (entry 5). Disaccharides **18** and **20** could be formed by an internal migration of the acyl group at *O*-2 to the anomeric carbon, through a nucleophilic attack of triflic acid to the carbon of the acyloxonium. A proton rearrangement in the trioxolane ion would leave the *O*-2 position free to react as an acceptor and yield the self-condensed product disaccharide **18** or **20**, respectively (Scheme 2).



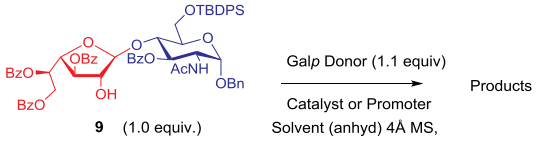
Scheme 1. Retrosynthesis of **1** using linear trisaccharide **7** constructed in the upstream direction.

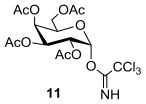
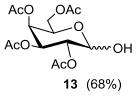
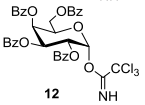
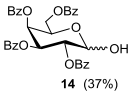
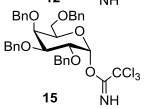
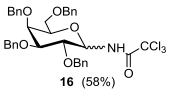
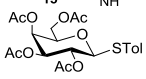
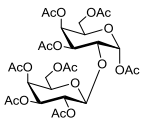
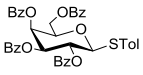
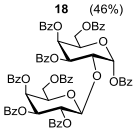
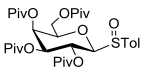
The spectroscopic signals for the anomeric H and C of compound **18**, matched with those previously described [51]. In the case of **20**, anomeric ^{1}H and ^{13}C NMR displacements and $J_{1,2}$ couplings were unambiguously assigned by comparison to those of **18**. Furthermore, HRMS analysis showed that the molecular formula matched the proposed structure.

Due to the unusual result obtained using thioglycoside **19**, we carried out the glycosylation of **21** [29], a galactofuranosyl derivative having the 2-OH free as a model reaction (Scheme 3). In this case, only glycosylation products were obtained and neither acyl transfer nor self-condensation by-product were detected, indicating that the sugar linked to the Gal*f* in **9** was responsible for the failure to glycosylate **17** or **19**. Despite the anchimeric assistance provided by the benzoyl group, a mixture of disaccharides **22** (β1→2) and **23** (α1→2) were produced in 9.5:1 β/α ratio (Scheme 3).

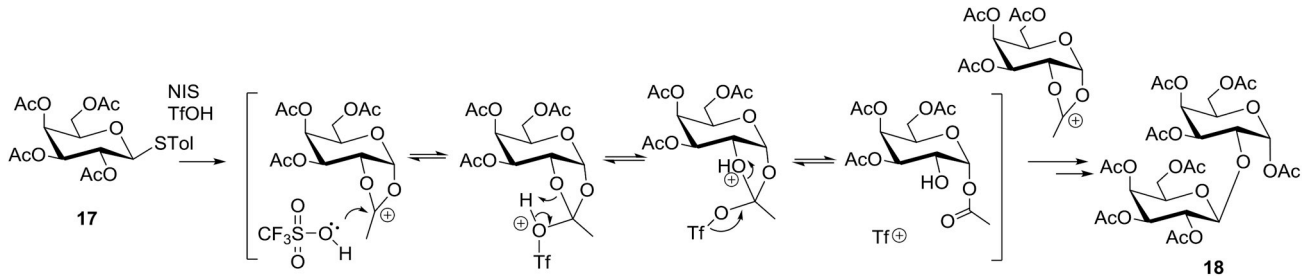
We also used the pivaloylated thiosulfoxide donor **24**, taking into account that the pivaloyl group has a lesser migratory aptitude, compared to benzoyl and acetyl groups [52] and the higher reactivity of the

Table 1
Attempts of glycosylation of acceptor **9** with Gal*p* donors **11**, **12**, **15**, **17**, **19** and **24**.



Entry	Donor	Catalyst or promoter	Solvent	T/°C (time)	By-Product (yield)	9 (% recovered)
1	 **11**	TMSOTf	CH_2Cl_2	−15 → 0 °C (72 h)	 **13** (68%)	72%
2	 **12**	TMSOTf	CH_2Cl_2	−20 → 0 °C (48 h)	 **14** (37%)	81%
3	 **15**	TMSOTf	CH_3CN	−15 → 0 °C (72 h)	 **16** (58%)	77%
4	 **17**	NIS/TfOH	CH_2Cl_2	−20 °C	 **18** (46%)	72%
5	 **19**	NIS/TfOH	CH_2Cl_2	−18 °C	 **20** (53%)	75%
6	 **24** (***R/S***)	DTBMP/Tf_2O	CH_2Cl_2	−78 °C → rt (20 h)	*	49%

[a] in all cases, concentration of acceptor **9** was 1–10 × 10^{-2} M *: undetermined.



Scheme 2. Suggested mechanism for the formation of disaccharide **18** (and its benzoylated analogue **20**).

thiosulfoxide. Although after 20 h of reaction [54,55] thin layer chromathography showed total consumption of **24**, several products of higher polarity, difficult to separate, were formed (entry 6, Table 1) and no glycosylation product was detected.

To overcome the poor reactivity of acceptor **9,** the anchimerically superarmed [56–58] donor *p*-tolyl 3,4,6-tri-*O*-benzyl-2-*O*-benzoyl-1-thio-β-D-galactopyranoside (**25**) [59] was synthesized as previously described, with slight modifications. Compound **25** was previously used as donor with several pyranosyl acceptors [59–62].

Coupling of donor **25** with acceptor **9** (Table 2), in the presence of NIS/TfOH system [50] in CH_2Cl_2 at 0 °C gave a new spot of higher polarity by TLC monitoring. As condensation products could not be isolated by column chromatography, the fraction obtained was analyzed by high resolution mass spectrometry (HRMS) and NMR, proving the presence of a mixture of trisaccharides with terminal β-D-Gal*p*-(1 → 2) and α-D-Gal*p*-(1 → 2) units (**26αβ**). The mixture could be separated after removal of the silyl ether group to afford the β-diastereomer -**27**- ($J_{1,2}$ = 8.0 Hz) and α-diastereomer **28**- ($J_{1,2}$ = 4.0 Hz) (total yield 62%, β/α 1.0:1.2) (Table 2, entry 1).

In order to improve the stereoselectivity in favor of the desired β-diastereomer, different temperatures, solvent systems and activators (conditions) were assayed (Table 2, entry 2–5). At −20 °C during 24 h (entry 2) the same yield of 62% was reached, but the diastereoselectivity was in a small degree reversed (β/α 1.1:1.0). At −78 °C (entry 3), we expected to obtain an improvement of the selectivity, but to our surprise, we did not obtain any glycosylation product. In an attempt to improve the diastereoselectivity by the nitrile effect, a mixture of CH_2Cl_2 with CH_3CN was used as solvent in 1:6 v/v ratio at −18 °C (entry 4). Although the diastereoselectivity was substantially improved (β/α 2.5:1.0), the total yield of the glycosylation reaction dropped to 25%. Also, the use of *p*-nitrophenyl sulfenyl chloride (*p*-NO_2PhSCl)/silver triflate as activator system [63] (entry 5) afforded a mixture of diastereomers with low yield (20%), favoring the unwanted diastereomer (β/α 1.0:1.4).

In summary, the best result was obtained using NIS/TfOH as the promoting system in CH_2Cl_2 as solvent at −20 °C. Under these conditions, glycosylation reached 62% of total yield with 1.1:1.0 β/α ratio, after two steps (Table 2, entry 4).

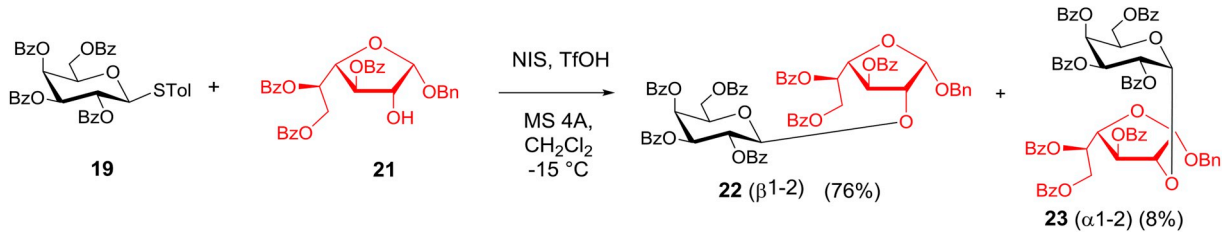
Although coupling of donor **25** with acceptor **9** gave a diastereomeric mixture, this result was encouraging, since, after many efforts, it was the first time that a galactopyranosyl unit could be introduced. This would indicate that previously assayed donors (Table 1) lacked the appropriate reactivity, and the "O-2/O-5 cooperative effect" [56–58] in **25** would be a requirement for glycosylation.

The unexpected formation of the α-D-galactopyranoside diastereomer from **25** carrying an *O*-2 participating group was reported [61] as well as from other 2-*O*-acyl substituted galactopyranosyl donors [28,64,65].

Beyond having obtained a mixture of diastereomers, the conclusion that emerges is that the increase in reactivity provided by the mixed profile of protecting groups in **25** was the main responsible for the introduction of the Gal*p* unit to the unreactive acceptor **9** in the O-2 glycosylation reaction. The present synthesis of trisaccharide Gal*p*(β1→2)Gal*f*(β1→4)GlcNAc involving a non-reducing end elongation is a better option than the previously described following the opposite direction [28].

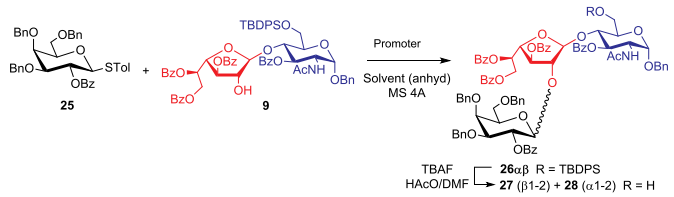
2.2. Synthesis of hexasaccharide 1

Despite the absence of anchimeric participation for glycosyation in trisaccharide donor **6**, our previous experience using this donor [27,29,30] moved us to follow a [3 + 3] convergent glycosylation with **27** using acetonitrile as participating solvent. The coupling reaction afforded the desired β-isomer **29** (Scheme 4) and no α-isomer was detected. Compound **29** co-eluted with the hydrolyzed product of trisaccharide imidate **6**. To isolate **29**, acetylation was carried out, affording hexasaccharide **29** with 43% yield after two steps. The by-product 1,4,6-tri-*O*-acetyl-2,3-di-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-D-galactopyranose (**31**) [66] was obtained in 16% yield and unreacted acceptor 27 was also recovered (34%) (Scheme 4). In our previous synthesis of hexasaccharide **1** [30], the [3 + 3] glycosylation step gave 33% yield but acetylation of the column chromatography fraction was not required. Signals in the ^{1}H and ^{13}C NMR spectra of hexasaccharide **29** were unequivocally assigned by COSY, HSQC and NOESY experiments. The βconfiguration of the new Gal*p*(1 → 6)GlcNAc glycosidic linkage was evident by the fact that the C-1^D signal of the internal β-D-Gal*p* moiety appeared at 102.0 ppm in the HSQC, and correlated with H-1^D (δ 4.68) with a coupling constant of $^1J_{H\text{-}H}$ = 7.5 Hz. The H-1^D signal was partially overlapped with a benzyl methylene hydrogen. This assignment was in agreement with the NOESY H–H correlation signal between H-1^D of the internal β-D-Gal*p* moiety and H-6a^A and H-6b^A of the GlcNAc moiety (see Supporting Information). Hexasaccharide **29** was further deprotected by treatment with MeONa in MeOH to give **32** (78%) and in this case, H-1^D appeared



Scheme 3. Glycosylation of **21** with donor **19**.

Table 2
Glycosylation of superarmed donor **25** with disaccharide acceptor **9**.



Entry	Promoter	Solvent	Temp.	Time	Method	Total Yield 27 + 28 (β/α ratio)
1	NIS/TfOH	CH_2Cl_2	0 °C	5 h	B	62% (1.0:1.2)
2	NIS/TfOH	CH_2Cl_2	−20 °C	24 h	A	62% (1.1:1.0)
3	NIS/TfOH	CH_2Cl_2	−78 °C	45 h	C	0%
4	NIS/TfOH	CH_2Cl_2/CH_3CN 1:6 v/v	−18 °C	94 h	D	25% (2.5:1)
5	*p*-NO_2PhSCl/AgTfO	CH_2Cl_2	−78 °C	80 h	E	20% (1.0:1.4)

at δ 4.48 ($J_{1,2}$ 7.9 Hz). Hydrogenolysis of **32** gave **1** with excellent yields. The spectra of hexasaccharide **1** matched those previously described [30].

3. Conclusion

Hexasaccharide **1** was synthesized by a new strategy using benzyl α-D-galactofuranoside, as a precursor of internal Gal*f*. An improvement of the overall yield with respect to the previous synthetic route [30] was obtained. Construction of the linear trisaccharide from the reducing end to the non-reducing end ensured the complete diastereoselectivity of the galactofuranosyl linkage. However, disaccharide acceptor **9** turned out to have limited reactivity. The use of anchimerically superarmed donor *p*-tolyl 3,4,6-tri-*O*-benzyl-2-*O*-benzoyl-1-thio-β-D-galactopyranoside (**19**) enabled to overcome this issue, but diastereoselectivity could not be controlled with a *O*-2 participating group.

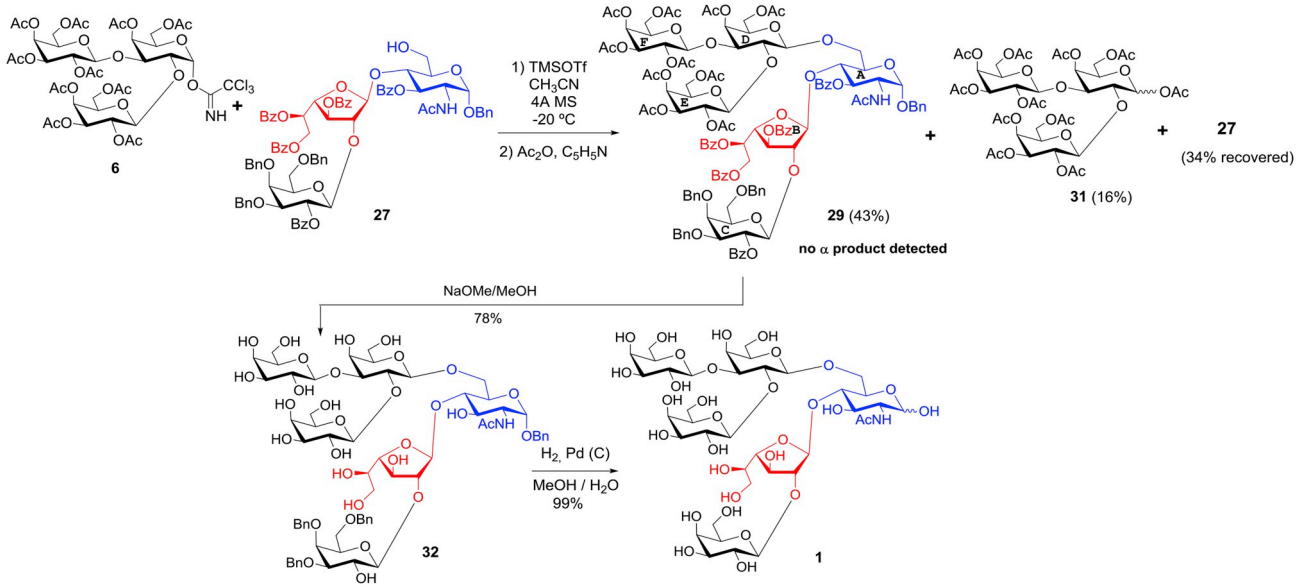
4. Experimental

4.1. General methods

TLC was performed on 0.2 mm Silica Gel 60 F254 aluminium supported plates. Detection was effected by exposure to UV light or by spraying with 10% (v/v) H_2SO_4 in EtOH and charring. Column chromatography was performed on silica gel 60 (230–400 mesh). NMR spectra were recorded with a Bruker AVANCE II 500 spectrometer at 500 MHz (^{1}H) and 125.8 MHz (^{13}C) or with a Bruker AC 200 at 200 MHz (^{1}H) and 50.3 MHz (^{13}C). Chemical shifts are given relative to the signal of internal acetone standard at 2.22 and 30.89 ppm for ^{1}H NMR and ^{13}C NMR spectra, respectively when recorded in D_2O. ^{1}H and ^{13}C assignments were supported by 2D COSY, and HSQC experiments. High resolution mass spectra (HRMS) were recorded on a BRUKER micrOTOF-Q II electrospray ionization mass spectrometer. Optical rotations were measured with a Perkin-Elmer 343 polarimeter with a path length of 1 dm at 25 °C.

4.2. General procedure of glycosylation reaction using the trichloroacetimidate method

A dry solution of acceptor benzyl 3,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1 → 4)-2-acetamido-3-*O*-benzoyl-6-*O*-*ter*-butyldiphenylsilyl-2-deoxy-α-D-glucopyranoside (**9,** 1 equiv.) and the trichloroacetimidates **11**, **12** or **15** (1.1 equiv., Table 1) in freshly distilled anhyd solvent (1 mL/10 mg of **9**), activated powdered 4 Å molecular sieves (30 mg/mL of solvent) was added and the mixture was vigorously stirred at rt under argon. After 10 min, the mixture was cooled to the corresponding temperature, TMSOTf (0.25 equiv.) was added. The reaction was quenched by addition of Et_3N after TLC monitoring showed



Scheme 4. Convergent synthesis of hexasaccharide **1**.

starting material disappeard. Then mixture was filtered over celite and washed, the filtrates concentrated under vacuum and the residue was purified by silica gel chromatography.

4.3. 2,3,4,6-Tetra-O-acetyl-β-D-galactopyranosyl-(1 → 2)-1,3,4,6-tetra-O-acetyl-α-D-galactopyranose (18)

To a solution of *p*-tolyl tetra-*O*-acetyl-1-thio-β-D-galactopyranoside (**17**, 33.5 mg, 0.074 mmol, 1.4 equiv.) [49], *N*-iodosuccinimide (16.0 mg, 0.071 mmol, 1.3 equiv.) and benzyl 3,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1 → 4)-2-acetamido-3-*O*-benzoyl-6-*O*-*tert*-butyl-diphenylsilyl-2-deoxy-α-D-glucopyranoside [29] (**9**, 60 mg, 0.053 mmol, 1.0 equiv.) in anhyd CH_2Cl_2 (2.5 mL), activated powdered 4 Å molecular sieves was added under argon atmosphere. After 5 min of vigorous stirring at − 20 °C, TfOH (3.5 μL, 0.040 mmol, 0.7 equiv.) was added. The stirring continued for 22 h when a TLC analysis showed unchanged acceptor **9** (R_f 0.31, 3: 2 hexane-EtOAc), a new polar compound (R_f 0.08, 1:1 hexane-EtOAc) and consumption of donor **17**. The reaction was quenched by addition of Et_3N (6 μL, 0.043 mmol), filtered through Celite washing with CH_2Cl_2. The filtrate was washed with $Na_2S_2O_3$ (2 × 30 mL), $NaHCO_3$ (2 × 30 mL), H_2O (1 × 30 mL), dried (Na_2SO_4), filtered and concentrated to give a residue which was purified by silica column chromatography (2:1 hexane-EtOAc).

The first fraction from the column gave unreacted acceptor **9** (49 mg, 72%). The next fraction gave an amorphous solid which was identified as 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1 → 2)-1,3,4,6-tetra-*O*-acetyl-α-D-galactopyranose (**18**, 11.4 mg, 46% from donor **17**) with diagnostic signals similar to ^{1}H NMR spectrum already described [51]. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 6.32 (d, 1H, J = 3.9 Hz, H-1), 5.42 (dd, 1H, J = 3.4, 1.4 Hz, H-4 or H-4′), 5.36–5.31 (m, 1H, H-4 or H-4′), 5.31 (dd, J = 10.7, 3.3 Hz, H-3), 5.10 (dd, J = 10.6, 7.6 Hz, H-2′), 4.95 (dd, J = 10.5, 3.4 Hz, H-3′), 4.57 (d, J = 7.7 Hz, H-1′), 4.27–3.87 (m, 7H, H-2, H-5, H-6a, H-6b, H-5′, H-6a', H-6b), 2.17, 2.16, 2.15, 2.06, 2.04, 2.02, 1.99, 1.97 (8s, 24H, C*H*$_3$CO). ^{13}C NMR ($CDCl_3$, 50.3 MHz): δ 167.5 (CH$_3$*C*O), 101.5 (C-1′), 90.7 (C-1), 72.4, 70.7 (x 2), 69.1, 68.8, 68.1, 67.6, 66.7, 61.01 (C-6 or C-6′), 60.96 (C-6 or C-6′), 20.8, 20.6, 20.5 (*C*H$_3$CO).

4.4. 2,3,4,6-Tetra-O-benzoyl-β-D-galactopyranosyl-(1 → 3)-1,3,4,6-tetra-O-benzoyl-α-D-galactopyranose (20)

To a solution of *p*-tolyl 2,3,4,6-tetra-*O*-benzoyl-1-thio-β-D-galactopyranoside (**19**, 55 mg, 0.079 mmol, 1.5 equiv.), *N*-iodosuccinimide (17 mg, 0.077 mmol, 1.5 equiv.) and **9** (58 mg, 0.052 mmol, 1.0 equiv.) in anhyd CH_2Cl_2 (3 mL), activated 4 Å powdered molecular sieves was added under argon atmosphere. The mixture was cooled to − 18 °C and after 20 min of vigorous stirring, TfOH (3 μL, 0.034 mmol, 0.7 equiv.) was added and stirring continued for 2 h. Then the reaction was allowed to warm to 0 °C and the stirring continued for 16 h. TLC analysis showed the presence of unreacted acceptor **9** (R_f 0.25 3:2 hexane – EtOAc) and a new product (R_f 0,38 3:2 hexane – EtOAc). The reaction was quenched by addition of Et_3N (7 μL, 0.050 mmol) at 0 °C, filtered over Celite washing with CH_2Cl_2. The filtrate was washed with saturated aq. $Na_2S_2O_3$ (2 × 30 mL), saturated aq. $NaHCO_3$ (2 × 30 mL) and H_2O (1 × 30 mL), dried (Na_2SO_4) and concentrated under vacuum. The residue was purified by silica gel column chromatography (30 : 1 toluene – EtOAc) to give, in the first fraction, a colorless syrup identified as 2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl-(1 → 2)-1,3,4,6-tetra-*O*-acetyl-α-D-galactopyranose (**20**, 25 mg, 53% from **19**). $[\alpha]_D$ +85.3 (*c* 1, $CHCl_3$), ^{1}H NMR ($CDCl_3$, 500 MHz): δ 8.24–7.14 (38H, H-arom.), 7.02 (d, 1H, J = 3.8 Hz, H-1), 6.94–6.91 (m, 2H, arom.), 6.01 (dd, 1H, J = 3.4, 1.0 Hz, H-4), 5.90 (dd, 1H, J = 3.4, 0.8 Hz, H-4′), 5.85 (dd, 1H, J = 10.4, 3.4 Hz, H-3), 5.63 (dd, 1H, J = 10.5, 7.8 Hz, H-2′), 5.44 (dd, 1H, J = 10.5, 3.4 Hz, H-3′), 5.05 (d, 1H, J = 7.8 Hz, H-1′), 4.70 (dd, 1H, J = 11.3, 6.4 Hz, H-6a'), 4.68–4.62 (m, 1H, H-5), 4.57 (dd, 1H, J = 11.3, 6.2 Hz H-6a), 4.55 (dd, 1H, J = 10.4, 3.8 Hz, H-2), 4.42 (dd, 1H, J = 11.3, 6.8 Hz, H-6b'), 4.37–4.33 (m, 1H, H-5′), 4.32 (dd, 1H, J = 11.3, 7.3 Hz, H-6b). ^{13}C NMR ($CDCl_3$, 125.8 MHz): δ 166.1, 165.9, 165.3 (x 2), 165.2, 165.07, 165.01, 164.7 (Ph*C*O), 136.6–128.0 (arom.), 102.1 (C-1′), 92.2 (C-1), 74.0 (C-2), 71.4 (C-3′), 71.2 (C-5′), 69.7 (C-3), 69.5 (C-2′), 68.6 (C-5), 68.3 (C-4), 67.6 (C-4′), 61.7 (C-6), 61.6 (C-6′). HRMS (ESI/APCI) *m/z* calcd for $C_{68}H_{54}O_{19}Na$ (M + Na^+): 1197.3152. Found 1197.3183.

Unreacted **9** (49 mg, 75%) was recovered.

4.5. Benzyl 2,3,4,6-tetra-O-benzoyl-β-D-galactopyranosyl-(1 → 2)-3,5,6-tri-O-benzoyl-α-D-galactofuranoside (22) and benzyl 2,3,4,6-tetra-O-benzoyl-α-D-galactopyranosyl-(1 → 2)-3,5,6-tri-O-benzoyl-α-D-galactofuranoside (23)

A solution of *p*-tolyl 2,3,4,6-tetra-*O*-benzoyl-1-thio-β-D-galactopyranoside (**19**, 655 mg, 0.93 mmol, 1.5 equiv.) and dry benzyl 3,5,6-tri-*O*-benzoyl-α-D-galactofuranoside [29] (**21**, 361 mg, 0.62 mmol, 1.0 equiv.) in freshly distilled anhyd CH_2Cl_2, *N*-iodosuccinimide (209 mg, 0.93 mmol, 1.5 equiv.) and activated 4 Å powdered molecular sieves (300 mg) was added under argon atmosphere. The mixture was cooled to – 15 °C, the stirring continued for 20 min and TfOH (38 μL, 0.43 mmol, 0.7 equiv.) was added. After 2.5 h, a TLC showed no acceptor **21** left (R_f 0.60, 3:2 hexane – EtOA) and the reaction was quenched by addition of Et_3N (65 μL, 0.47 mmol), filtered washing with CH_2Cl_2. The organic phase was washed with $Na_2S_2O_3$ (2 × 60 mL), $NaHCO_3$ (2 × 70 mL), H_2O (1 × 60 mL), dried (Na_2SO_4), filtered, and concentrated. The residue was purified by silica gel column chromatography (2.5:1 hexane-EtOAc) to give in the first fraction 56 mg of **23** (8%) as a glassy solid. $[\alpha]_D$ +128.4 (*c* 0.7, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz): δ 8.03–7.12 (40H, H-arom.), 6.09 (dd, 1H, J = 7.3, 6.5 Hz, H-3), 6.01 (dd, 1H, J = 10.6, 3.4 Hz, H-3′), 5.98 (dd, 1H, J = 3.4, 1.1 Hz, H-4′), 5.76 (m, 1H, H-5), 5.72 (dd, 1H, J = 10.6, 3.7 Hz, H-2′), 5.54 (d, 1H, J = 3.7 Hz, H-1′), 5.15 (d, 1H, J = 4.4 Hz, H-1), 4.78 (d, 1H, J = 11.9, 4.4 Hz, H-6a), 4.70, 4.48 (2d, 2H, J = 12.3 Hz, C*H*$_2$Ph), 4.67 (dd, 1H, J = 11.9, 6.2 Hz, H-6b), 4.67 (dd, 1H, J = 7.3, 4.4 Hz, H-2), 4.67–4.63 (m, 1H, H-5′), 4.46 (dd, 1H, J = 6.5, 5.0 Hz, H-4), 4.39 (dd, 1H, J = 11.2, 6.4 Hz, H-6a'), 4.26 (dd, 1H, J = 11.2, 6.9 Hz, H-6b');^{13}C NMR ($CDCl_3$, 125.8 MHz): δ 166.2, 165.9, 165.68, 165.67, 165.5, 165.4, 165.2 (Ph*C*O), 136.6, 133.5, 133.4, 133.11, 133.07, 133.0 (arom.), 130.0–127.7 (arom.), 98.1 (C-1), 95.9 (C-1′), 79.3 (C-2′), 79.0 (C-4), 75.3 (C-3), 71.5 (C-5), 69.5 (*C*H$_2$Ph), 68.82, 68.80 (C-2′, C-4′), 68.1 (C-3′), 67.4 (C-5′), 62.9 (C-6), 61.9 (C-6′). HRMS (ESI/APCI) *m/z* calcd for $C_{68}H_{56}NaO_{18}$ (M + Na^+): 1183.3359. Found 1183.3382.

The next fraction gave **22** (546 mg, 76%) as a glassy solid: R_f 0.4 (11: 1 toluene-EtOAc), $[\alpha]_D$ +96,7 (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz): δ 8.10–6.97 (40H, H-arom.), 6.02 (dd, 1H, J = 8.1, 6.8 Hz, H-3), 5.95 (dd, 1H, J = 3.4, 0.8 Hz, H-4′), 5.89 (dd, 1H, J = 10.4, 8.0 Hz, H-2′), 5.70 (m, 1H, H-5), 5.51 (dd, 1H, J = 10.4, 3.4 Hz, H-3′), 5.24 (d, 1H, J = 4.4 Hz, H-1), 5.03 (d, 1H, J = 8.0 Hz, H-1′), 4.95, 4.65 (2d, 2H, J = 12.3 Hz, C*H*$_2$Ph), 4.73 (dd, 1H, J = 11.9, 4.2 Hz, H-6a), 4.59 (dd, 1H, J = 11.9, 5.2 Hz, H-6b), 4.58 (dd, 1H, J = 8.1, 4.4 Hz, H-2), 4.46 (dd, 1H, J = 6.8, 5.3 Hz, H-4), 4.36 (dd, 1H, J = 11.4, 6.8 Hz, H-6a'), 4.34 (dd, 1H, J = 11.4, 4.6 Hz, H-6b), 4.24 (m, 1H, H-5′); ^{13}C NMR ($CDCl_3$, 125.8 MHz): δ 165.90, 165.89, 165.7, 165.54, 165.47, 165.0, 164.9 (Ph*C*O), 137.4, 133.7, 133.4, 133.3, 133.1, 132.92, 132.89, 132.8 (arom.), 130.0–127.6 (arom.), 101.9 (C-1′), 100.4 (C-1), 82.9 (C-2), 78.2 (C-4), 74.6 (C-3), 71.69, 71.66, 71.6 (C-3′, C-5, C-5′), 70.0 (*C*H$_2$Ph), 69.3 (C-2′), 68.0 (C-4′), 62.9 (C-6), 62.0 (C-6′). HRMS (ESI/APCI) *m/z* calcd for $C_{68}H_{56}NaO_{18}$ (M + Na^+): 1183.33589. Found: 1183.34158.

4.6. (S) and (R)-p-tolyl 2,3,4,6-tetra-O-pivaloyl-1-thio-β-D-galactopyranoside S-oxide (24)

The procedure described for the phenyl analogue [53] was followed. To a stirred solution of *p*-tolyl 1-thio-β-D-galactopyranoside

(185 mg, 0.64 mmol) and DMAP (39 mg, 0.32 mmol) in anhyd pyridine (1.9 mL) at rt, freshly distilled pivaloyl chloride (0.80 mL, 0.64 mmol) was added and warmed to 110 °C. After 18 h of stirring at 110 °C, the reaction was cooled to rt, and quenched by slow addition of methanol (2.5 mL) and diluted with CH_2Cl_2 (15 mL). The mixture was washed with 1 N HCl (3 × 15 mL), saturated aq. $NaHCO_3$ (2 × 15 mL) and H_2O (1 × 15 mL), dried (Na_2SO_4) and concentrated. The residue was purified by column chromatography (25:1 hexane – EtOAc) to give *p*-tolyl 2,3,4,6-tetra-*O*-pivaloyl-1-thio-β-D-galactopyranoside (159 mg, 40%). R_f 0.45 (10:1 hexane – EtOAc); $[\alpha]_D$ – 9.0° (*c* 1.2, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$): δ 8.09 (d, 2H, *J* = 8.0 Hz, arom.), 7.89 (d, 2H, *J* = 8.0 Hz, arom.), 5.39 (d, 1H, *J* = 3.0 Hz, H-4), 5.17 (t, 1H, *J* = 9.8 Hz, H-2), 5.10 (dd, 1H, *J* = 10.0, 3.1 Hz, H-3), 4.65 (d, 1H, *J* = 9.7 Hz, H-1), 4.18 (m, 1H, H-6a), 4.02–3.98 (m, 2H, H-5, H-6b), 2.34 (s, 3H, C*H*$_3$Ph), 1.22, 1.18, 1.14, 1.09 (4s, 36H, (C*H*$_3$)$_3$CCO). ^{13}C NMR ($CDCl_3$, 125.8 MHz): δ 177.9, 177.2, 176.8, 176.4 ((CH$_3$)$_3$*C*CO), 138.7, 134.3, 129.6, 127.3 (arom.), 85.9 (C-1), 74.6 (C-5), 72.2 (C-3), 66.9 (C-4), 66.6 (C-2), 61.4 (C-6), 38.9, 38.7, 38.7, 38.7 ((CH$_3$)$_3$*C*CO), 27.2, 27.1, 27.04, 26.95 ((*C*H$_3$)$_3$CCO), 21.2 (*C*H$_3$Ph). HRMS (ESI/APCI) *m/z* calcd for $C_{33}H_{50}O_9SNa$ ($M + Na^+$): 645.30677. Found: 645.30775.

To a solution of *p*-tolyl 2,3,4,6-tetra-*O*-pivaloyl-1-thio-β-D-galactopyranoside (43 mg; 0.069 mmol) in CH_2Cl_2 (0.8 mL) cooled at −75 °C, was added *m*-CPBA (77%) (20.4 mg, 0.083 mmol) in CH_2Cl_2 (0.4 mL). After 3 h of vigorous stirring, TLC showed no starting material left and two more-polar products (R_f 0.32 and 0.17, 4: 1 hexane - AcOEt). The mixture was quenched by addition of saturated aq. $NaHCO_3$ (3 mL) stirring for 5 min at rt. The mixture was diluted with CH_2Cl_2 (20 mL) and organic phase was extracted with saturated aq. $NaHCO_3$ (2 × 10 mL), H_2O (1 × 10 mL), dried (Na_2SO_4) and concentrated. Purification of the residue by silica gel column chromatography (4:1 hexane – EtOAc) gave a first fraction of the column afforded less polar tentatively assigned (*R*)-diastereomer (R_f 0.32, 1:1 hexane – EtOAc) of **24** (12.6 mg, 28%) as a colorless syrup. ^1H NMR ($CDCl_3$, 200 MHz): δ 7.54 (d, 2H, *J* = 8.3 Hz, arom.), 7.33 (d, 2H, *J* = 8.4 Hz, H arom.), 5.53 (t, 1H, *J* = 9.8 Hz, H-2), 5.36 (d, 1H, *J* = 3.0 Hz, H-4), 5.16 (dd, 1H, *J* = 9.9, 3.0 Hz, H-3), 4.42 (d, 1H, *J* = 9.7 Hz, H-1), 4.14–3.88 (m, 3H, H-5, H-6a, H-6b), 2.42 (s, 3H, C*H*$_3$Ph), 1.23, 1.13, 1.11, 1.10 (4s, 36H, (C*H*$_3$)$_3$CCO). HRMS (ESI/APCI) *m/z* calcd for $C_{33}H_{50}NaO_{10}S$ ($M + Na^+$): 661.30169. Found: 661.30210.

The second fraction from the column gave 24.3 mg of a syrup tentatively identified as (*S*)-diasteromer of **24** (55%). ^1H NMR ($CDCl_3$, 200 MHz): δ 7.66 (d, 2H, *J* = 8.1 Hz, arom.), 7.33 (d, 2H, *J* = 8.1 Hz, arom.), 5.30 (d, 1H, *J* = 2.8 Hz, H-4), 5.14 (dd, 1H, *J* = 9.6, 2.8 Hz, H-3), 5.02 (t, 1H, *J* = 9.8 Hz, H-2), 4.64 (dd, 1H, *J* = 9.9 Hz, H-1), 4.15–4.00 (m, 2H), 3.82–3.70 (m, 1H), 2.42 (s, 3H, C*H*$_3$Ph), 1.24, 1.15, 1.07, 0.93 (4s, 36H, (C*H*$_3$)$_3$CCO). HRMS (ESI/APCI) *m/z* calcd for $C_{33}H_{50}NaO_{10}S$ ($M + Na^+$): 661.30169. Found 661.30354.

^{13}C NMR of (*R*) and (*S*) mixture: ($CDCl_3$, 50.3 MHz): δ 129.6, 129.3, 127.3, 126.0, 92.0 (C-1 maj.), 89.2 (C-1 min.), 75.5 (min.), 74.9 (maj.), 72.2 (min.), 71.8 (maj.), 66.5 (min.), 66.1 (maj.), 64.9 (maj.), 64.3 (min.), 61.1 (min.), 60.2 (maj.), 38.9, 38.8, 38,7, 27.1, 27.0, 26.9, 26.7, 21.4 (*C*H$_3$Ph). The absolute sulfoxide configuration of each diastereomer was tentatively assigned based on differences in the chemical shifts C-1 in ^{13}C NMR spectra comparing to the acetyl analogues [67].

*4.7. Reaction of benzyl 3,5,6-tri-O-benzoyl-β-D-galactofuranosyl-(1 → 4)-2-acetamido-3-O-benzoyl-6-O-ter-butyldiphenylsilyl-2-deoxy-α-D-glucopyranoside (9) with (S)/(R)-p-tolyl 2,3,4,6-tetra-O-pivaloyl-1-thio-β-D-galactopyranoside S-oxide (**24**)*

To a stirred mixture of (*R/S*)-**24** (33 mg, 0.051 mmol), 2,6-di-*tert*-butyl-4-methylpyridine (DTBMP) (21 mg, 0.10 mmol, 2 equiv.) and activated 4 Å powdered molecular sieves, in anhyd CH_2Cl_2 (3.5 mL) cooled to – 78 °C, was added Tf_2O (10 μL, 0.059 mmol, 1.2 equiv.) under argon atmosphere. After 30 min of vigorous stirring, a solution of acceptor **9** (59 mg, 0.052 mmol, 1 equiv.) in CH_2Cl_2 (1.8 mL) was added. The mixture was kept at −78 °C for 19 h until a TLC examination showed disappearance of thiosulphoxide derivatives and several dispersed spots which are presumed as decomposition products, together with a spot that comigrated with the acceptor **9**. The reaction was quenched by addition of saturated aq. $NaHCO_3$ (5 mL) and the organic phase was succesively washed with saturated aq. $NaHCO_3$ (2 × 15 mL) and H_2O (2 × 15 mL), dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The residue was purified by column chromatography (6:1 hexane – EtOAc) giving several fractions lacking glycosylation product. 26 mg of unreacted acceptor **9** was recovered (49%).

*4.8. Benzyl 3,5,6-tri-O-benzyl-2-O-benzoyl-β-D-galactopyranosyl-(1 → 2)-3,5,6-tri-O-benzoyl-β-D-galactofuranosyl-(1 → 4)-2-acetamido-3-O-benzoyl-2-deoxy-α-D-glucopyranoside (**27**) and benzyl 3,5,6-tri-O-benzyl-2-O-benzoyl-α-D-galactopyranosyl-(1 → 2)-3,5,6-tri-O-benzoyl-β-D-galactofuranosyl-(1 → 4)-2-acetamido-3-O-benzoyl-2-deoxy-α-D-glucopyranoside (**28**)*

4.8.1. Method A

To a vigorously stirred mixture of **9** (0.90 g, 0.80 mmol) and activated powdered 4 Å molecular sieves (800 mg) in 20 mL of freshly distilled anhyd CH_2Cl_2 under argon atmosphere, was added a solution of *p*-tolyl 3,4,6-tri-*O*-benzyl-2-*O*-benzoyl-1-thio-β-D-galactopyranoside [59] (**25**, 744 mg, 1.24 mmol, 1.6 equiv.) and *N*-iodosuccinimide (305 mg, 1.36 mmol, 1.7 equiv.) in 30 mL of freshly distilled CH_2Cl_2 via *cannula*. The mixture was vigorously stirred for 10 min at rt and then cooled to −20 °C under argon. After 5 min, TfOH (50 μL, 0.57 mmol, 0.7 equiv.) was added. After 24 h of stirring at −20 °C, a TLC showed no donor left and a new spot (R_f 0.28, 7: 4 hexane-EtOAc, twice developed). The mixture was diluted in CH_2Cl_2 (100 mL), washed with saturated aq. $Na_2S_2O_3$ (3 × 70 mL), saturated aq. $NaHCO_3$ (2 × 70 mL) and H_2O (2 × 70 mL), dried ($N_{a2}SO_4$), filtered and concentrated. Purification by column chromatography (7: 4 hexane-EtOAc) gave a fraction that was analyzed by ^1H and ^{13}C NMR spectroscopy and showed the presence of two diastereomers benzyl 3,5,6-tri-*O*-benzyl-2-*O*-benzoyl-β-D-galactopyranosyl-(1 → 2)-3,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1 → 4)-2-acetamido-3-*O*-benzoyl-6-*O*-*tert*-butyldiphenylsilyl-2-deoxy-α-D-glucopyranoside (**26β**) and benzyl 3,5,6-tri-*O*-benzyl-2-*O*-benzoyl-α-D-galactopyranosyl-(1 → 2)-3,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1 → 4)-2-acetamido-3-*O*-benzoyl-6-*O*-*tert*-butyldiphenylsilyl-2-deoxy-α-D-glucopyranoside (**26α**). ^{13}C NMR ($CDCl_3$, 50.3 MHz) δ: anomeric signals 107.0 (C-1′, β anomer), 105.5 (C-1′, α anomer), 100.5 (C-1″, β anomer), 96.1 (C-1″, α anomer), 95.9 (C-1, α and β anomers). HMRS (ESI/APCI) analysis of this fraction gave two peaks *m/z* $C_{99}H_{98}NO_{21}Si$ (MH^+): 1664.6395 and $C_{99}H_{97}NNaO_{21}Si$ ($M + Na^+$): 1686.6215, found 1664.6429 and 1686.6271, respectively.

To a stirred solution of a mixture of the isolated diastereomers **26αβ** (1.44 g) in DMF (15 mL) cooled to 0 °C, a solution of 1 M TBAF in anhyd THF (1.7 mL, 1.7 mmol) was added, followed by glacial acetic acid (104 μL, 1.8 mmol). After 24 h of stirring, a TLC showed no material left (R_f 0.65, 2:3 hexane-EtOAc, twice developed) and two new spots of higher polarity: R_f 0.27 and 0.25 (2: 3 hexane-EtOAc, twice developed). The mixture was diluted in CH_2Cl_2 (150 mL), washed with H_2O (4 × 110 mL), dried (Na_2SO_4), filtered, and concentrated under reduced pressure. Column chromatography of the residue (5: 4 hexane-EtOAc, then 1:1 hexane-EtOAc) gave a first fraction of **28** (341 mg, 30% two steps) as a glassy solid: R_f 0.27 (1:1 hexane – EtOAc, twice developed), $[\alpha]_D$ +82.9 (*c* 0.6, $CHCl_3$), ^1H NMR ($CDCl_3$, 500 MHz): δ 8.02 (dd, 2H, *J* = 8.3, 1.2 Hz, arom.), 7.95–7.92 (m, 4H, arom.), 7.87 (dd, 2H, *J* = 8.4; 1.3 Hz, arom.), 7.77 (dd, 2H, *J* = 8.4; 1.2 Hz, arom.), 7.61–7.14 (m, 35H, arom.), 5.69 (d, 1H, *J* = 9.6 Hz, N*H*), 5.55 (dd, 1H, *J* = 4.3, 1.2 Hz, H-3′), 5.47 (t, 1H, *J* = 10.2 Hz, H-3), 5.46 (d, 1H, *J* = 4.0 Hz, H-1″), 5.34 (dd, 1H, *J* = 10.3, 4.0 Hz, H-2″), 5.30 (ddd, 1H, *J* = 7.3, 4.7, 3.3 Hz, H-5′), 5.11 (s, 1H, H-1′), 4.94 (d, 1H, *J* = 3.7 Hz, H-1), 4.88 (d, 1H, *J* = 11.3 Hz, C*H*$_2$Ph), 4.67 (d, 1H, *J* = 11.9 Hz,

CH_2Ph), 4.63 (d, 1H, J = 11.8 Hz, CH_2Ph), 4.60 (d, 1H, J = 11.8 Hz, CH_2Ph), 4.54 (d, 1H, J = 11.3 Hz, CH_2Ph), 4.50 (d, 1H, J = 11.9 Hz, CH_2Ph), 4.44 (d, 1H, J = 11.9 Hz, CH_2Ph), 4.41 (ddd, 1H, J = 10.7, 9.6, 3.7 Hz, H-2), 4.39 (d, 1H, J = 11.9 Hz, CH_2Ph), 4.30 (dd, 1H, J = 12.3, 7.3 Hz, H-6a'), 4.30 (d, 1H, J = 1.2 Hz, H-2′), 4.25 (dd, 1H, J = 12.3, 3.3 Hz, H-6b'), 4.20 (t, 1H, J = 6.8 Hz, H-5″), 4.07 (t, 1H, J = 9.6 Hz, H-4), 4.02 (t, 1H, J = 4.7 Hz, H-4′), 3.98 (dd, 1H, J = 10.3, 2.8 Hz, H-3″), 3.95 (d, 1H, J = 2.8 Hz, H-4″), 3.75–3.65 (m, 3H, H-5, H-6a, H-6b), 3.55–3.47 (m, 2H, H-6a″, H-6b″), 2.48 (dd, 1H, J = 5.9, 2.8 Hz, -O*H*), 1.74 (s, 3H, CH_3CONH); ^{13}C NMR (CDl_3, 125.8 MHz): δ 169.8 (CH$_3$*C*ONH), 167.4 (Ph*C*O), 166.8 (Ph*C*O), 165.9 (Ph*C*O), 165.4 (Ph*C*O), 164.9 (Ph*C*O), 106.5 (C-1′), 96.9 (C-1), 96.0 (C-1″), 84.4 (C-2′), 81.2 (C-4′), 77.4 (C-3′), 76.8 (C-3″), 74.9 (*C*H$_2$Ph), 74.3 (C-4″), 73.3 (*C*H$_2$Ph), 73.1 (C-4), 72.9 (*C*H$_2$Ph), 72.6 (C-2″), 72.0 (C-3), 71.3 (C-5), 70.1 (*C*H$_2$Ph), 69.8 (C-5′), 69.6 (C-5″), 68.1 (C-6″), 63.0 (C-6′), 60.4 (C-6), 52.2 (C-2), 23.1 (CH$_3$*C*ONH). HRMS (ESI/APCI) *m/z* calcd for $C_{83}H_{79}NNaO_{21}$ (M + Na^+): 1448.5037. Found: 1448.5063.

Second fraction from the column afforded **27** (365 mg, 32%, two steps) as a glassy solid. (R_f 0.25, 1:1 hexane – EtOAc, twice developed); $[\alpha]_D$ +13,3 (*c* 0.6, $CHCl_3$); ^{1}H NMR (CDl_3, 500 MHz): δ 7.95–7.73 (m, 10H, arom.), 7.56–7.12 (m, 35H, arom.), 5.77 (d, 1H, J = 9.4 Hz, N*H*), 5.58 (dd, 1H, J = 10.0, 8.0 Hz, H-2″), 5.55 (dd, 1H, J = 10.8, 9.3 Hz, H-3), 5.37 (s, 1H, H-1′), 5.29 (m, 1H, H-5′), 5.23 (dd, 1H, J = 5.1, 1.9 Hz, H-3′), 4.98 (d, 1H, J = 3.6 Hz, H-1), 4.92 (d, 1H, J = 11.6 Hz, CH_2Ph), 4.73 (d, 1H, J = 11.9 Hz, CH_2Ph), 4.67 (d, 1H, J = 8.0 Hz, H-1″), 4.58 (d, 1H, J = 12.3 Hz, CH_2Ph), 4.55 (d, 1H, J = 11.6 Hz, CH_2Ph), 4.50 (d, 1H, J = 11.9 Hz, CH_2Ph), 4.46 (d, 1H, J = 12.2 Hz, CH_2Ph), 4.42 (ddd, 1H, J = 10.8, 9.4, 3.6 Hz, H-2), 4.40 (d, 1H, J = 11.7 Hz, CH_2Ph), 4.38 (s, 1H, H-2′), 4.33 (d, 1H, J = 11.7 Hz, CH_2Ph), 4.19 (t, 1H, H-4′), 4.15 (t, 1H, H-4), 4.06 (dd, 1H, J = 12.2, 3.3 Hz, H-6a'), 3.98 (dd, 1H, J = 12.2, 7.7 Hz, H-6b'), 3.92 (d, 1H, J = 2.7 Hz, H-4″), 3.91–3.87 (m, 1H, H-6a), 3.84–3.77 (m, 2H, H-5, H-6b), 3.61 (dd, 1H, J = 10.0, 2.7 Hz, H-3″), 3.54–3.50 (m, 3H, H-5″, H-6a″, H-6b″), 2.68 (t, 1H, J = 5.6 Hz, O*H*), 1.75 (s, 3H, CH_3CO); ^{13}C NMR (CDl_3, 125.8 MHz): δ 169.8 (CH$_3$*C*ONH), 167.1 (Ph*C*O), 165.6 (Ph*C*O), 165.2 (Ph*C*O), 138.3–127.6 (C arom.); 107.9 (C-1′), 100.7 (C-1″), 96.7 (C-1), 85.7 (C-2′), 80.9 (C-4′), 79.6 (C-3″), 77.7 (C-3′), 74.5 (*C*H$_2$Ph), 74.4 (C-4), 73.8 (C-5″), 73.4 (*C*H$_2$Ph), 72.6 (C-4″), 72.5 (C-3), 71.9 (*C*H$_2$Ph), 71.5 (C-5), 71.3 (C-2″), 70.1 (C-5′), 69.8 (*C*H$_2$Ph), 68.3 (C-6″), 62.9 (C-6′), 60.8 (C-6), 52.4 (C-2), 23.1 (*C*H$_3$CONH). HRMS (ESI/APCI) *m/z* calcd for $C_{83}H_{79}NNaO_{21}$ (M + Na^+): 1448.5037. Found: 1448.5099.

4.8.2. Method B

Same procedure described for Method A was followed at 0 °C for 5 h. Compound **9** (33 mg, 0.03 mmol) and **25** (25 mg, 0.04 mmol), gave 16 mg of **28** (33%) and 14 mg of **27** (29%).

4.8.3. Method C

Same procedure described for Method A was followed except that the reaction was carried out at −18 °C for 94 h using a mixture of 1:6 CH_2Cl_2–CH_3CN as solvent. Compound **9** (66 mg, 0.06 mmol) and **25** (47 mg, 0.07 mmol), gave 7 mg of **28** (7%) and 17 mg of **27** (18%).

4.8.4. Method D

Same procedure described for Method A was followed at −78 °C for 45 h. From 51 mg of **9** (0.05 mmol) and 36 mg of **25** (0.05 mmol), no products of glycosylation were obtained.

4.8.5. Method E

A suspension of donor **9** (40 mg, 0.04 mmol), **25** (51 mg, 0.05 mmol), silver triflate (30 mg, 0.11 mmol) and 4 Å molecular sieves in anhydrous CH_2Cl_2 (4 mL) was stirred, with the exclusion of light, at room temperature under argon. After 10 min, the mixture was cooled to −78 °C and a solution of *p*-nitrobenzenesulfenyl chloride (9.2 mg, 0.05 mmol) in anhydrous CH_2Cl_2 (0.5 mL) was added. The reaction was stirred for 80 h and was quenched by addition of saturated aq. NaHCO3 solution (0.5 mL). The mixture was diluted with CH_2Cl_2 (5 mL) and filtered. Then the mixture was treated as described in Method A to give 7 mg of **28** (12%) and 5 mg of **27** (8%).

4.9. Benzyl 2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl-(1 → 2)-[2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl-(1 → 3)]-4,6-di-O-acetyl-β-D-galactopyranosyl-(1 → 6)- [3,5,6-tri-O-benzyl-2-O-benzoyl-β-D-galactopyranosyl-(1 → 2)-3,5,6-tri-O-benzoyl-β-D-galactofuranosyl-(1 → 4)]-2-acetamido-3-O-benzoyl-2-deoxy-α-D-glucopyranoside (29)

To a flask containing dried *O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1 → 2)-[2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1 → 3)]-4,6-di-*O*-acetyl-β-D-galactopyranosyl trichloroacetimidate [27] (**6**, 358 mg, 0.33 mmol, 1.2 equiv.) and activated powdered 4 Å molecular sieves, a solution of acceptor **27** (389 mg, 0.27 mmol, 1.0 equiv.) in freshly distilled anhydr CH_3CN (16 mL) was added via *cannula*. The suspension was cooled to −20 °C and after vigorous stirring for 10 min, TMSOTf (11 μL, 0.061 mmol, 0.2 equiv.) was added. After 40 h of stirring, TLC analysis showwed no trisaccharide donor **6** left (R_f 0.48, 2: 3 toluene-EtOAc, TEA 0.2%) and a new product of higher polarity (R_f 0.33, 2: 3 toluene-EtOAc, TEA 0.2%). The reaction was quenched by addition Et_3N (10 μL, 0.072 mmol), filtered whashing with CH_3CN, and concentrated under reduced pressure to give a residue which was purified by column chromatography (1: 1 toluene-EtOAc, followed by 5:6 toluene-EtOAc). Unreacted acceptor **27** (133 mg, 34%, R_f 0.55, 2: 3 toluene-EtOAc) was recovered from the first fraction from the column. The next fraction afforded a mixture (405 mg) of the desired hexasaccharide **29** (R_f 0.23, 2:3 toluene-EtOAc) which co-eluted with hydrolyzed trisaccharide donor 2,3-di-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-4,6-di-*O*-acetyl-α,β-D-galactopyranose (**30**) as suggested by NMR spectroscopy [66]. This fraction was acetylated with acetic anhydride (4 mL) in anhydrous pyridine (4 mL) at room temperature for 40 min, followed by addition of MeOH at 0 °C. Evaporation of the mixture gave a residue which was purified by column chromatography (2:3 hexane-EtOAc, followed by 2:5 hexane-EtOAc). The first fraction afforded 1,4,6-tri-*O*-acetyl-2,3-di-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-D-galactopyranose (**31**) [66] (52 mg, 16%, R_f 0.35, 1:2 hexane–EtOAc). The next fraction afforded **29** (270 mg, 43% two steps, R_f 0.25 (1:2 hexane – EtOAc)) as a glassy solid: $[\alpha]_D$ +11.3 (*c* 1, $CHCl_3$), ^{1}H NMR (CDl_3, 500 MHz): δ 8.01–7.64 (m, 10H, arom.), 7.55–7.04 (m, 35H, arom.), 5.83 (d, 1H, J = 9.3 Hz, N*H*), 5.56 (dd, 1H, J = 10.7, 8.60 Hz, H-3^A), 5.53 (dd, 1H, J = 9.7, 8.3 Hz, H–2^C), 5.41 (m, 2H, H–3^B, H-4^E), 5.38 (d, 1H, J = 2.9 Hz, H–4^F), 5.33 (s, 1H, H–1^B), 5.31 (d, 1H, J = 3.6 Hz, H-4^D), 5.26 (dd, 1H, J = 10.3, 8.2 Hz, H–2^F), 5.18 (dd, 1H, J = 10.5, 3.3 Hz, H-3^E), 5.12 (dd, 1H, J = 10.4, 7.5 Hz, H-2^E), 5.03 (dd, 1H, J = 10.5, 3.5 Hz, H–3^F), 4.99 (m, 1H, H–5^B), 4.99 (d, 1H, J = 4.0 Hz, H-1^A), 4.99 (d, 1H, J = 7.5 Hz, H–1^F), 4.96 (d, 1H, J = 7.6 Hz, H-1^E), 4.90 (d, 1H, J = 11.6 Hz, CH_2Ph), 4.88 (d, 1H, J = 7.7 Hz, H–1^C), 4.79 (d, 1H, J = 11.9 Hz, CH_2Ph), 4.69 (d, 1H, J = 7.5 Hz, H-1^D), 4.68 (d, 1H, J = 12.1 Hz, CH_2Ph), 4.53 (d, 1H, J = 12.2 Hz, CH_2Ph), 4.50 (d, 1H, J = 11.7 Hz, CH_2Ph), 4.45 (m, 1H, H–2^B), 4.37 (d, 1H, J = 11.6 Hz, CH_2Ph), 4.36 (d, 2H, J = 12.6 Hz, CH_2Ph), 4.35 (bs, 2H, H-2^A, H-6a^E), 4.28–4.24 (m, 1H, H–4^B), 4.23–4.08 (m, 9H, H-6a^A, H-3^D, H-4^A, H-5^E, H–5^F, H-6a^B, H-6b^E, H-6a^F, H-6b^F), 4.01–3.86 (m, 8H, H-2^D, H–4^C, H-5^A, H-5^D, H-6b^A, H-6b^B, H-6a^D, H-6b^D), 3.61–3.56 (m, 3H, H–3^C, H–5^C, H-6a^C), 3.53 (m, 1H, H–6^C), 2.17, 2.05, 2.03, 2.00, 2.00, 1.99, 1.96, 1.94, 1.94, 1.90 (10s, 30H, CH_3CO), 1.74 (s, 3H, CH_3CONH). ^{13}C NMR (CDl_3, 125.8 MHz): δ 170.4 (x 3), 170.3, 170.2, 170.1, 169.9, 169.7 (x 2), 169.3, 169.1 (11C, CH$_3$*C*O), 167.2, 165.4, 165.3, 164.8 (4C, Ph*C*O), 138.0, 137.7, 137.4, 136.9 (4C, C arom.), 133.3, 133.2, 133.0, 132.7 (4C, C arom.), 130.0–127.5 (C arom.), 107.7 (C–1^B), 102.0 (C-1^D), 100.2 (C–1^F), 99.6 (C-1^E), 99.3 (C–1^C), 96.0 (C-1^A), 85.3 (C–2^B), 80.2 (C–3^C), 79.8(C–4^B), 78.4 (C-2^D), 76.4 (C–3^B), 75.8 (C-4^A or C-3^D), 75.7 (C-4^A or C-3^D), 74.4 (*C*H$_2$Ph), 74.0 (C–5^C), 73.3 (*C*H$_2$Ph), 72.4 (C-3^A), 72.2 (C–4^C), 72.0 (*C*H$_2$Ph), 71.26 (C–2^C), 71.1 (C–3^F), 70.8 (C-5^E or C–5^F), 70.7 (C-5^A),

70.7 (C-5^{E} or C–5^{F}), 70.6 (C-3^{E}), 70.4 (C-5^{D}), 70.0 (C-2^{E}), 69.8 (C–5^{B}), 69.7 (C–6^{F}), 69.6 (*C*H$_2$Ph), 69.1 (C-4^{D}), 68.7 (C-6^{A}), 68.4 (C–6^{C}), 66.9 (C-4^{E}), 66.9 (C–4^{F}), 62.2 (C–6^{B}), 61.8 (C-6^{D}), 60.7 (C-6^{E} or C–6^{F}), 60.7 (C-6^{E} or C–6^{F}), 52.5 (C-2^{A}), 23.0, 21.0, 20.8, 20.7, 20.7, 20.6 (x 2), 20.6, 20.6, 20.5, 20.3 (11C, C*H*$_3$CO). HRMS (ESI/APCI) *m/z* calcd for $C_{121}H_{130}NO_{46}$ (M^+): 2332.7859. Found: 2332.7842.

*4.10. Benzyl β-D-galactopyranosyl-(1 → 2)-[β-D-galactopyranosyl-(1 → 3)]-β-D-galactopyranosyl-(1 → 6)-[3,5,6-tri-O-benzyl-β-D-galactopyranosyl-(1 → 2)-β-D-galactofuranosyl-(1 → 4)]-2-acetamido-2-deoxy-α-D-glucopyranoside (**32**)*

To a solution of **29** (250 mg, 0.107 mmol) in CH_3OH (2.0 mL) cooled to 0 °C, a solution of $NaOCH_3$ in CH_3OH (2.6 M, 1.6 mL) was added under nitrogen atmosphere. After 4 h of stirring at rt, a TLC analysis of an aliquot previously neutralized by the addition of an Amberlite resin particle showed one new product (R_f 0.27, 7:1:1 n-propanol – EtOH – H_2O). The mixture was passed through an ion exchange column (Amberlite resin IR-120 plus, H^+ form) eluting with 9:1 CH_3OH–H_2O. The eluate was concentrated under reduced pressure and coevaporated with H_2O (5 × 1 mL) under high vacuum to remove the methyl benzoate. The residue was dissolved in deionized H_2O (2 mL) and purified by passing the solution through a reverse phase C18 cartridge eluting with deionized water. The concentration of the eluate afforded **32** (119 mg, 80%) as a glassy solid: $[\alpha]_D$ +15.5 (*c* 1, H_2O); ^{1}H NMR (CD_3OD, 500 MHz): *δ* 7.37–7.35 (m, 2H, arom.), 7.29–7.10 (m, 18H, arom.), 5.60 (d, 1H, *J* = 2.1 Hz, H–1^{B}), 4.76 (d, 1H, *J* = 3.50 Hz, H-1^{A}), 4.71 (d, 1H, *J* = 12.1 Hz, C*H*$_2$Ph), 4.70 (d, 1H, *J* = 7.9 Hz, H-1^{E}), 4.68 (d, 1H, *J* = 10.8 Hz, C*H*$_2$Ph), 4.65 (d, 1H, *J* = 11.6 Hz, C*H*$_2$Ph), 4.60 (d, 1H, *J* = 12.1 Hz, C*H*$_2$Ph), 4.52 (d, 1H, *J* = 12.1 Hz, C*H*$_2$Ph), 4.48 (d, 1H, *J* = 7.9 Hz, H-1^{D}), 4.43 (d, 1H, *J* = 7.7 Hz, H–1^{C}), 4.40 (d, 1H, *J* = 7.8 Hz, H–1^{F}), 4.37 (d, 1H, *J* = 11.9 Hz, C*H*$_2$Ph), 4.33 (d, 1H, *J* = 12.0 Hz, C*H*$_2$Ph), 4.31 (d, 1H, *J* = 11.8 Hz, C*H*$_2$Ph), 4.21 (dd, 1H, *J* = 4.8, 2.1 Hz; H–2^{B}), 4.17 (dd, 1H, *J* = 7.9, 4.8 Hz; H–3^{B}), 4.01–3.98 (m, 1H, H-6a^{A}), 3.98 (dd, 1H, *J* = 7.9, 3.1 Hz, H–4^{B}), 3.92–3.78 (m, 8H, H-4^{A}, H-2^{A}, H–2^{C}, H-2^{D}, H-6b^{A}, H-6a*), 3.71–3.46 (m, 18H, H-6a^{B}, H-6b^{B}, H-6a*, H-6b*, H-6a*, H-6b*, H-6b*, H-6a^{C}, H–5^{B}, H-3^{D}, H-3^{A},H-5*, H-2^{E},H–2^{F}, H-4^{E}, H–4^{F}), 3.44–3.41 (m, 1H, H–3^{C}), 3.41–3.32 (m, 3H, H-3^{E}, H-5*, H-6b^{C}), 3,30–3,26 (m; 2H; H-5*; H–3^{F}), 1,97 (s, 3H, C*H*$_3$CONH). ^{13}C NMR (CD_3OD, 125.8 MHz): *δ* 173.8 (CH$_3$*C*ONH), 140.2, 140.2, 139.7; 139.0 (C-arom.), 130.0–128.9 (C-arom.), 109.3 (C–1^{B}), 106.2 (C–1^{F}), 104.8 (C-1^{E}), 103.6 (C–1^{C}), 103.4 (C-1^{D}), 97.9 (C-1^{A}), 89.1 (C–2^{B}), 85.9 (C-3^{D}), 83.8 (C–4^{B}), 83.7 (C–3^{C}), 80.6 (C-4^{A}), 77.2 (2C, C–3^{B}, C-5*), 76.8 (C-2^{D}), 76.5, 76.0 (C-5*), 75.7 (2C, *C*H$_2$Ph, C-3^{E}), 75.3, 75.1, 75.0, 74.53 (*C*H$_2$Ph), 74.45 (*C*H$_2$Ph), 73.4, 73.1, 72.6, 71.8 (C–5^{B}), 71.6, 71.4, 71.3, 71.1 (C–6^{C}), 70.6 (2C, *C*H$_2$Ph), 70.5, 69.0 (C-6^{A}), 64.5 (C–6^{B}), 62.8 (2C, C-6*, C-6*), 62.6 (C-6*), 55.5 (C-2^{A}), 22.7 (*C*H$_3$CONH). *could be D, E or F. HRMS (ESI/APCI) *m/z* calcd for $C_{66}H_{89}NNaO_{31}$ (M + Na^+): 1414.5311. Found: 1414.5329.

*4.11. β-D-Galactopyranosyl-(1 → 2)-[β-D-galactopyranosyl-(1 → 3)]-β-D-galactopyranosyl-(1 → 6)-[β-D-galactopyranosyl-(1 → 2)-β-D-galactofuranosyl-(1 → 4)]-2-acetamido-2-deoxy-α,β-D-glucopyranose (**1**)*

A mixture of hexasaccharide **32** (39 mg, 28 μmol) and Pd/C 10% Deguzza type E101 NE/W (40 mg) in 9: 1 CH_3OH–H_2O (2.8 mL) was hydrogenated at 3 atm at rt. After 10 h, a TLC showed no **29** left and a new product (R_f 0.22, 7: 2:2.5 *n*-propanol - EtOH – H_2O, twice developed). The catalyst was filtered through a bed of Celite washing with H_2O (2 mL). The filtrate was concentrated at rt and the residue was purified by passing through C-18 reverse phase cartridge, to give **1** (29 mg, 99%) as a hygroscopic glassy solid, in an anomeric mixture of α/β 7:3: $[\alpha]_D$ +0.5 (*c* 1, H_2O), ^{1}H NMR (D_2O, 500 MHz): *δ* anomeric zone and diagnostic signals: 5.54 (m, 1H, H–1^{B} α and β anomers), 5.21 (d, 0.7H, *J* = 3.4 Hz, H-1^{A} α anomer), 4.83 (d, *J* = 7.7 Hz, Gal*p* H-1), 4.70 (d, 0.3H, *J* = 8.4 Hz, H-1^{A} β anomer), 4.67–4.58 (m, 3H, Gal*p* H-1, α and β anomers), 4.40 (dd, 0.7H, *J* = 4.1; 1.9 Hz, H–2^{B} α anomer), 4.37 (dd, 0.3H, *J* = 3.9; 1.7 Hz, H–2^{B} β anomer), 2.05 (s, 3H, C*H*$_3$CONH, α and β anomers). ^{13}C NMR (D_2O, 125.8 MHz): *δ* 175.4, 175.10 (CH$_3$*C*ONH, α and β anomers), 133.1, 129.4, 128.9 (C arom.), 107.6, 107.5 (C–1^{B}, α y β anomers), 104.7 (2C; C–1^{F}, α and β anomers), 103.5, 103.4 (2C, C-1^{E}, α and β anomers), 102.4, (2C, C-1^{D}, α and β anómers), 102.2 (2C, C–1^{C}, α and β anomers), 95.8 (C-1^{A}, β anomer), 91.4 (C-1^{A}, α anomer), 87.7 (C–2^{B}, β anomer), 87.6 (C–2^{B}, α anomer), 83.8, 83.2, 83.0, 78.7, 76.1, 76.1, 75.9, 75.8, 75.2, 73.7, 73.5, 73.2, 73.2, 72.1, 71.8, 71.6, 70.9, 70.0, 69.9, 69.7, 69.5, 69.4, 69.2, 63.4, 62.1, 61.8, 61.7, 61.5, 57.3 (C-2^{A}, β anomer), 54.8 (C-2^{A}, α anomer), 22.8 (*C*H$_3$CONH, β anomer), 22.5 (*C*H$_3$CONH, α anomer). The NMR spectra matched with already described [30].

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Appendix A. Supplementary data

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