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Synthesis of trisaccharides containing internal galactofuranose O-linked in *Trypanosoma cruzi* mucins

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ABSTRACT

The trisaccharides β-D-Gal*f*-(1→2)-β-D-Gal*f*-(1→4)-D-GlcNAc (**5**) and β-D-Gal*p*-(1→2)-β-D-Gal*f*-(1→4)-D-GlcNAc (**6**) constitute novel structures isolated as alditols when released by reductive β-elimination from mucins of *Trypanosoma cruzi* (Tulahuen strain). Trisaccharides **5** and **6** were synthesized employing the aldonolactone approach. Thus, a convenient D-galactono-1,4-lactone derivative was used for the introduction of the internal galactofuranose and the trichloroacetimidate method was employed for glycosylation reactions. Due to the lack of anchimeric assistance on O-2 of the galactofuranosyl precursor, glycosylation studies were performed under different conditions. The nature of the solvent strongly determined the stereochemical course of the glycosylation reactions when the galactofuranosyl donor was substituted either by 2-*O*-Gal*p* or 2-*O*-Gal*f*.

1. Introduction

Trypanosoma cruzi, the agent of Chagas' disease, the American trypanosomiasis, presents a surface dominated by carbohydrates.[1,2] The mucins of *T. cruzi* stand out, not only because of their crucial function but also from the structural point of view. They are acceptors of sialic acid in the well-studied trans-sialidase reaction,[3–5] closely related to the infectivity of the parasite. A puzzling fact is that the presence of galactofuranose is confined to strains of a sylvatic origin grouped as *T. cruzi* I[6] (Fig. 1). These are less infective than strains belonging to group II, from the domestic cycle,[7] that only contain galactopyranose. The oligosaccharides **1**–**4** and **8** (Fig. 1) were first described in mucins of the G-strain.[8,9] They present a unique core of β-D-Gal*f*-(1→4)-GlcNAc which is α-*O*-glycosidically linked to serine or threonine in the mucins. Later, the same structures were found in the Dm28c clon together with the novel oligosaccharide **7**.[10] More recently, in the Tulahuen strain, trisaccharides **5** and **6** as well as pentasaccharide **9** were also found.[6] In 2009, from drug-resistant Colombiana strain, isolated from a chronic human case in Colombia, oligosaccharides **1**–**4** and **8**, as in the G-strain, were found.[11] Among these oligosaccharides, **5**–**9**, present an internal Gal*f*, 2-O-substituted by Gal*p* or Gal*f*. Our laboratory has been involved in the synthesis of this family of compounds with the aim to use them for trans-sialidase studies.[12] To date, we have reported the synthesis of compounds **1**–**4** (Fig. 1).[13–16] Pentasaccharide **4**, the major oligosaccharide in the G-strain, has two terminal Gal*p* for possible sialylation. By preparative trans-sialylation of **4** and analysis of the product, we have demonstrated that selective monosialylation occurs on the (1→3)-linked Gal*p*.[16] Recently, the synthesis of a mucin oligosaccharide from the Y strain (group II), which lacks Gal*f*, has been also reported.[17]

In this work, we describe the synthesis of β-D-Gal*f*-(1→2)-β-D-Gal*f*-(1→4)-D-GlcNAc (**5**) and β-D-Gal*p*-(1→2)-β-D-Gal*f*-(1→4)-D-GlcNAc (**6**) and the corresponding alditols, employing the aldonolactone approach. Trisaccharide **5** is part of the higher oligosaccharides **7** and **9**, whereas trisaccharide **6** is included in **8**. Thus, intermediates of these syntheses are useful for the construction of the higher oligosaccharides. The two linear trisaccharides present an internal galactofuranose unit substituted at position 2 by β-Gal*f* or β-Gal*p*. Thus, stereoselective glycosidation was hampered by the lack of neighboring group assistance.

The biosynthetic pathways involved in the construction of these O-chains in the mucins have not been elucidated. Taking into account the various structures found in *T. cruzi*, the synthesis of these oligosaccharides would be useful for studies on their biosynthesis.

2. Results and discussion

The synthesis of Gal*f* containing oligosaccharides from other glycoconjugates of *T. cruzi* has been performed; however, in these cases, Gal*f* appeared as terminal non-reducing end.[18,19] The synthesis of oligosaccharides containing internal galactofuranose is

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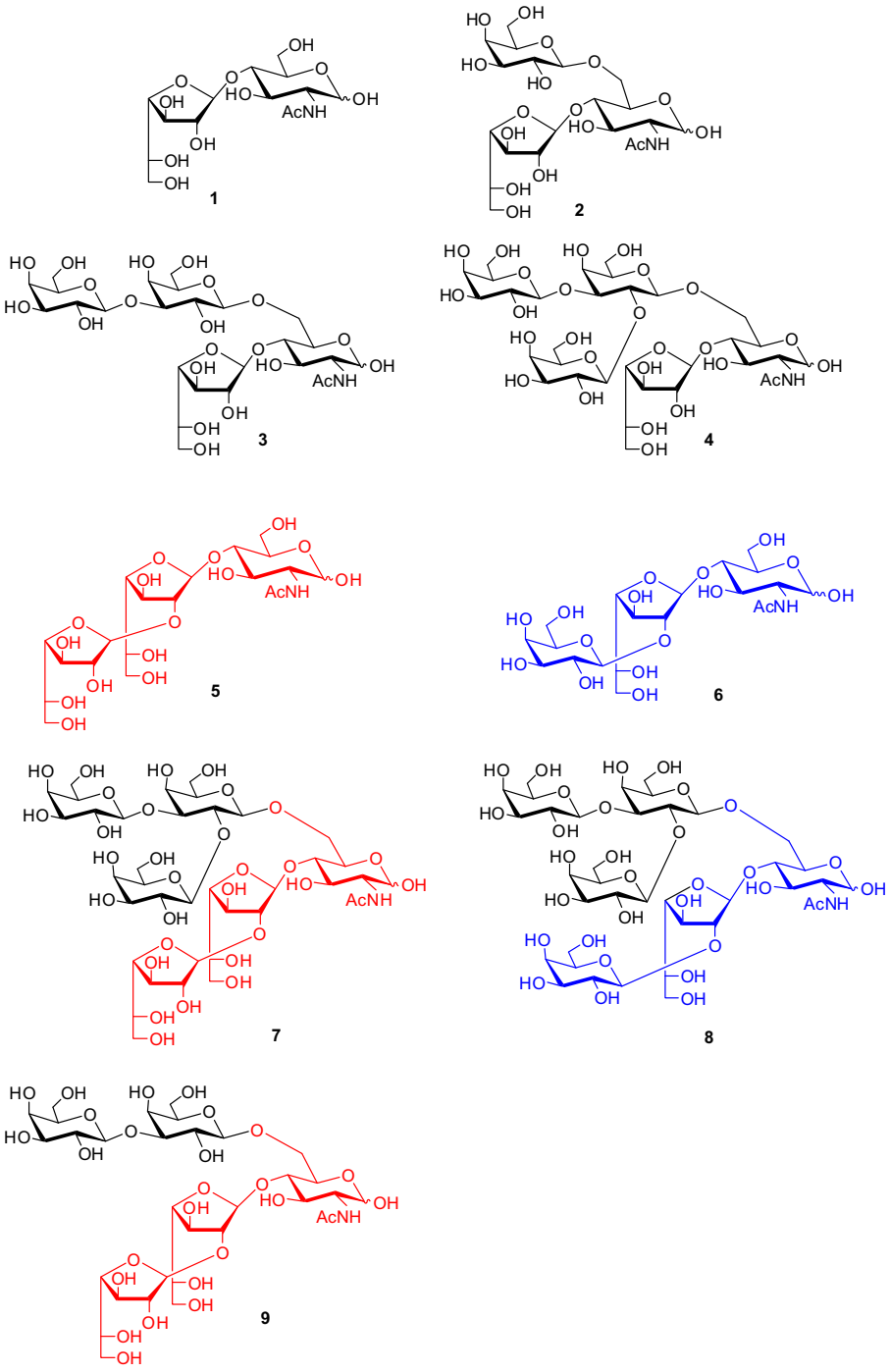


Figure 1. Oligosaccharides in mucins of *Trypanosoma cruzi* (group I).

more challenging, and involves the choice of convenient galactofuranosyl precursors as well as glycosylation methods. These aspects have been recently reviewed.[20,21] Few internal β-Gal*f* containing oligosaccharides have been synthesized to date.[22–29] The following methods were employed for the internal linkage construction: Koenings–Knörr,[22a] cyanoorthoester,[22b–d] anomeric acetate,[23] trichloroacetimidate,[24–26] and more recently, thioglycoside,[27] 2′-carboxybenzyl-glycosides[28,29] and glycosyl fluoride.[29]

Our strategy relied on the use of a convenient derivative of D-galactono-1,4-lactone as a common precursor of the internal Gal*f* of both trisaccharides, **5** and **6**. The glycosyl aldonolactone approach has been employed for the syntheses of a trisaccharide present in glycoinositolphospholipids from *Leishmania*[24] and oligosaccharide constituents of the arabinogalactan from *Mycobacterium tuberculosis*.[25] The stable lactone acts as a virtual-protected Gal*f* and may be also selectively substituted,[30,31] avoiding the tedious synthesis of the Gal*f* unit precursor from galactose, thus, providing a straightforward method for the oligosaccharide synthesis. On the other hand, in our previous work related to mucin oligosaccharides,[15,16] we have succeeded in glycosylating the 4-OH of GlcNAc derivative, benzyl 2-acetamido-3-*O*-benzoyl-6-*O*-*t*-butyldiphenylsilyl-2-deoxy-α-D-glucopyranoside (**18**) with a galactofuranosyl imidate donor with very good yield. For that reason, we decided to use the same derivative **18** considering, in addition, that

the orthogonal silyl group would allow further elongation to access to hexasaccharides **7** and **8**, and pentasaccharide **9**, our future targets. In that case, both oligosaccharides **5** and **6** have to be assembled from the non-reducing end.

The fact that selective acylation of 5,6-*O*-isopropylidene-D-galactono-1,4-lactone occurs on OH-2 due to stereoelectronic factors,[24,30] led us to evaluate the regioselectivity of trichloroacetimidate glycosylation employing 1.1 equiv of *O*-(2,3,5,6-tetra-*O*-benzoyl-β-D-galactofuranosyl) trichloroacetimidate. However, preliminary studies showed moderate regioselectivity toward the 1→2 linkage formation.

2.1. Synthesis of 3,4,5-tri-*O*-benzoyl-D-galactono-1,4-lactone (10), the common precursor of trisaccharides 5 and 6

Compound **10** was prepared from 5,6-*O*-isopropylidene-D-galactono-1,4-lactone (**11**)[32] temporarily protected with a bulky silyl group (Scheme 1). The fact that the isopropylidene group requires careful reaction conditions and cautious purification to avoid its partial hydrolysis along the synthesis[15] led us to its early replacement. Thus, *tert*-butyldiphenylsilyl was chosen as protecting group, taking into account its resistance to acids. Treatment of **11** with 1.2 equiv of *tert*-butyldiphenylchlorosilane gave the silyl derivative **12**, regioselectively in 83% yield; no 3-OH protection was detected (Scheme 1). Deprotection of the isopropylidene by HOAc–H_2O at 80 °C followed by benzoylation with benzoyl chloride in pyridine gave **13** in 68% yield. The ^{1}H NMR spectrum of **13** showed the H-5 and H-3 signals shifted downfield at 5.95 and 5.61 ppm, respectively, confirming the selective substitution at OH-2 by TBDPS in **12**. On the other hand, the TBDPS group resisted the hydrolysis conditions as demonstrated by the absence of tetra-*O*-benzoyl-D-galactono-1,4-lactone as byproduct. Upon aqueous acetic acid deprotection of the isopropylidene group of the TBDMS analog of **12**, partial undesired removal of the silyl group occurred.[33] Removal of the TBDPS group of **13** with 5% HF (48 wt % in water) in acetonitrile gave **10** in low yield with significant formation of more polar byproducts. A mixture of tetrabutylammonium fluoride buffered with acetic acid in THF–DMF solution[34] was then employed to give **10** in 81% yield.

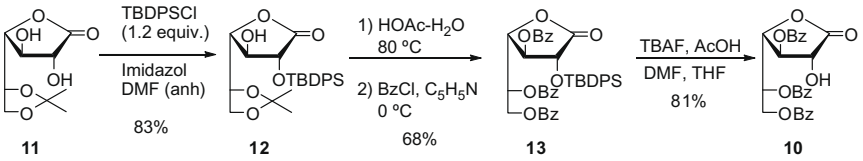
2.2. Synthesis of trisaccharide 5

Having **10** in hand, the next step was the introduction of the terminal Gal*f* (Scheme 2). The absence of acid labile-protecting groups in **10** allowed the use of the $SnCl_4$-promoted glycosylation method[13–15,35] with 1,2,3,5,6-penta-*O*-benzoyl-D-galactofuranose (**14**) as the donor, which was synthesized in one step from galactose.[14,36] Glycosyl lactone **15** crystallized from the crude mixture in 78% yield. Further purification from the mother liquors increased the yield to 92%. As expected, the new glycosidic linkage has the β-configuration as determined by ^{13}C NMR spectrum that showed only one anomeric signal at 104.9 ppm. The ^{1}H NMR spectrum showed the H-1′ as a singlet at 5.57 ppm and H-2′ as a doublet with a small coupling constant (J = 1.4 Hz), a characteristic pattern of the anomeric β stereochemistry for galactofuranosyl linkages. The H-2 of the lactone appeared as a doublet (J = 4.3 Hz) at 5.04 ppm, 0.23 ppm downfield shifted compared to **10** due to O-2 glycosylation. To obtain the furanose-reducing end for further activation, **15** was reduced with disiamylborane (DSB) to give disaccharide **16** in 82% yield as an anomeric mixture, as indicated by the ^{13}C NMR spectrum (104.7 and 97.7 ppm for the α anomer and 105.1 and 101.9 ppm for the β anomer). In spite of the superposition of signals in the ^{1}H NMR spectrum, HSQC and COSY experiments allowed full assignments. The integration of the H-2′ signals of both anomers (δ 5.45 and 5.37 ppm) showed a 7:3 β/α anomeric ratio of the mixture.

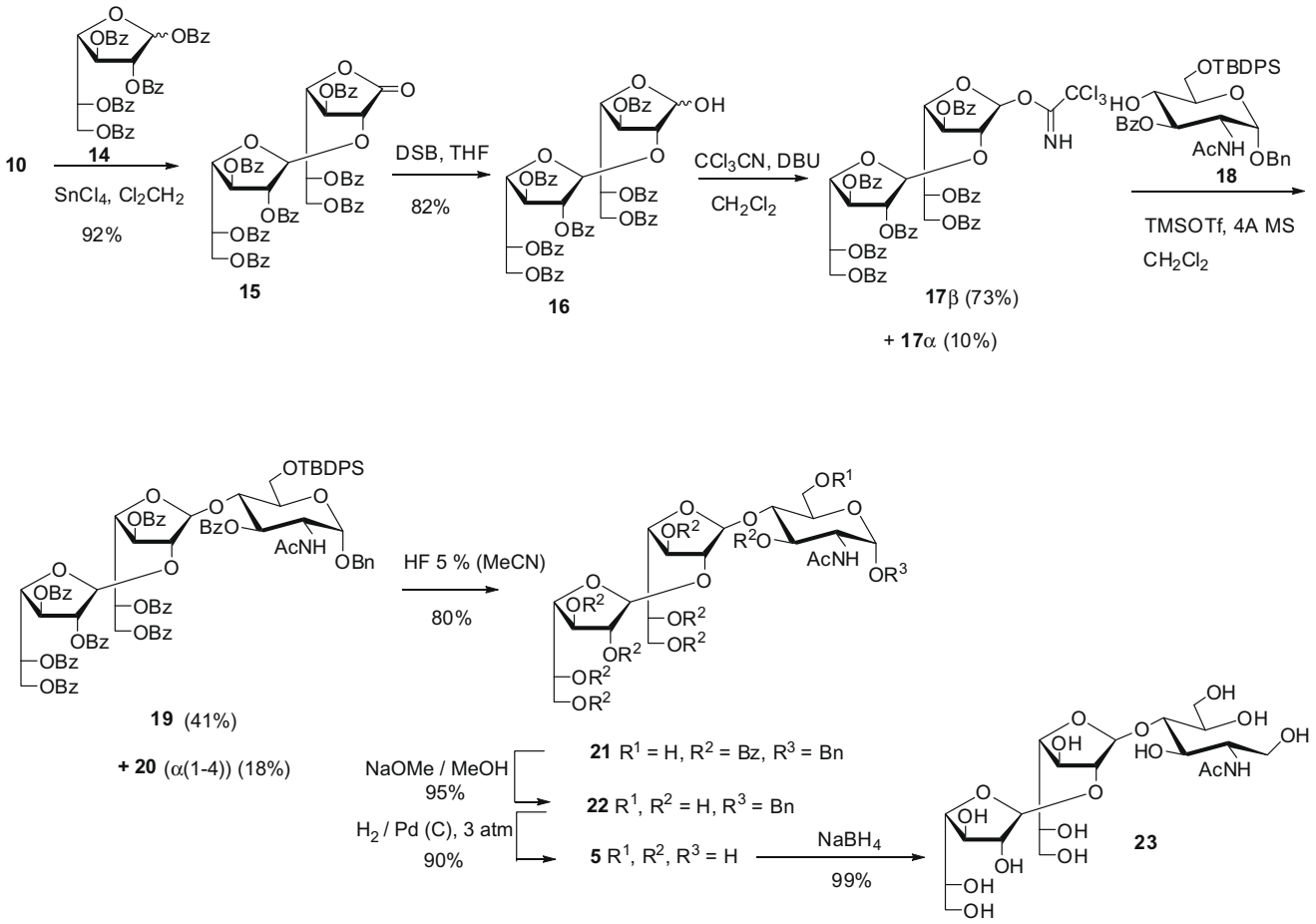
The next step was the activation of the anomeric center as the trichloroacetimidate derivative. This method has been widely used in the construction of β-Gal*f* linkages by anchimeric assistance.[15,24–26,27b,37] To our knowledge, there are no reports on the use of a Gal*f* donor with a 2-*O*-sugar substituent. Thus, treatment of **16** with trichloroacetonitrile and DBU at rt gave a mixture of imidates as shown by TLC, which was easily purified by column chromatography. The less polar β-imidate **17β** was obtained in 73% yield, and its stereochemistry was assigned on the basis of the H-1 signal that appeared as a downfield shifted singlet (δ 6.55 ppm) in the ^{1}H NMR spectrum, and confirmed by the ^{13}C NMR spectrum where the anomeric carbons appeared at 105.0 and 104.8 ppm for both galactofuranosyl moieties in the β-configuration. From the second fraction of the column, a sirupy α-imidate 17α was obtained (10%) and, in this case, the H-1 resonance appeared at 6.73 ppm as a doublet with a characteristic coupling constant (J = 4.5 Hz), whereas in the ^{13}C NMR spectrum the C-1 resonance appeared at 98.0 ppm confirming the α stereochemistry. It is interesting to point out that the sirupy α-imidate transformed partially in the β-imidate on standing in the freezer (−20 °C).

Considering the lack of anchimeric assistance in the imidate **17β**, we evaluated the influence of the solvent in the glycosylation reaction with GlcNAc acceptor **18**,[15] previously employed in the related synthesis of **3** and **4**. The difficulties in glycosylating the 4-OH group of GlcNAc encouraged many investigations in this area.[38] However, Gal*f* glycosylation of the 4-OH group of GlcNAc derivatives has been performed with very good yields in our synthesis of mucin oligosaccharides (**3** and **4**)[15,16], and by others,[39] employing 2,3,5,6-tetra-*O*-benzoyl-D-galactofuranose trichloroacetimidate[37b] as the donor. This reactivity could be attributed to the higher flexibility of the furanose ring. On reaction of glucosamine acceptor **18** with imidate **17β** in CH_2Cl_2 using TMSOTf as catalyst, the best results were obtained by maintaining the temperature at 5 °C for three days to give the desired trisaccharide **19** in 41% yield after column chromatography. The stereochemistry of the new glycosidic linkage was established as β as indicated by the ^{13}C NMR spectrum. The anomeric signals appeared at 96.2 (GlcNAc), 105.5 (C-1″) and 106.1 ppm (C-1′), whereas the ^{1}H NMR showed a doublet at 4.92 ppm (J = 3.7 ppm) for H-1 of GlcNAc, and two singlets (δ 5.58 and 5.44 ppm) assigned to the internal (H-1′) and terminal (H-1″) Gal*f* anomeric hydrogen by HETCOR and COSY experiments.

The α-(1→4) glycosylation product **20** was also obtained in 18% yield after column chromatography and crystallized from ethanol. The ^{13}C NMR spectrum showed the characteristic anomeric signals of β-Gal*f* and α-GlcNAc at 105.5 and 96.2 ppm, respectively, and a third signal at 102.9 ppm, which suggested a α-configuration for



Scheme 1. Synthesis of intermediate **10**.



Scheme 2. Synthesis of β-D-Gal*f*-(1→2)-β-D-Gal*f*-(1→4)-D-GlcNAc (5) and alditol **23**.

the new Gal*f* linkage. In a HETCOR experiment, this signal correlated to the corresponding H-1′, which appeared at 5.40 ppm as a doublet with a coupling constant of 3.2 Hz. This value was slightly smaller compared to other related α-Gal*f* compounds previously synthetized,[27a,28,40–42] particularly, compared to the pentasaccharide constituent of varianose,[27a] where the internal α-Gal*f* is 2-O-substituted by a glucopyranose. This small value was assigned to the α-D-Gal*f* stereochemistry considering that the H-2′ appeared as a doublet of doublets (J = 3.2, 2.3 Hz), whereas the H-5′appeared downfield shifted (δ 5.85 ppm) as expected for a benzoylated exocyclic-OH of the Gal*f* moiety. Moreover, C-2′ resonated at 81.5 ppm, as expected, due to glycosylation.

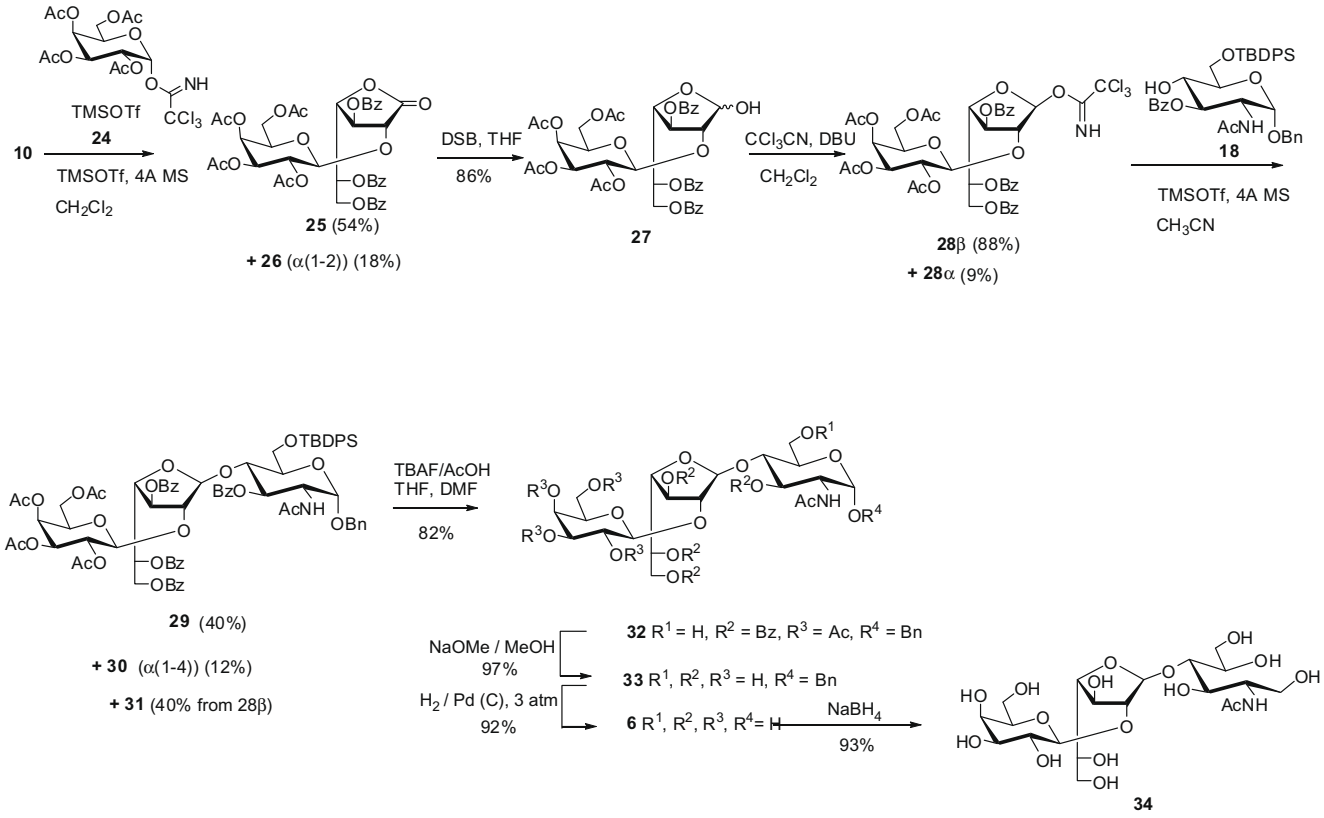
The global yield of the glycosylation step was 59% (β/α ratio of 2.3:1), with a moderate diastereoselectivity in favor of the β-(1→4) linked product **19**. This result prompted us to evaluate participating solvents, taking into account the strong influence of ether or acetonitrile in diastereoselective control of glycosylations with pyranose donors 2-O-substituted with non-participating protecting groups.[43] When acetonitrile was employed, the global yield of glycosylation was lower (37%), with a **19**:**20** diastereomeric ratio of 1:1 (after column chromatography). The use of ethyl ether increased the global glycosylation yield (57%), however slightly favoring the α anomer (**19**:**20** ratio 1:1.4).

With trisaccharide **19** in hand, the next step of the synthesis was the removal of the TBDPS group at O-6 with 5% HF (48 wt % in water) in acetonitrile to give **21** in 80% yield. No hydrolysis of the labile Gal*f* linkage was observed in the overnight acidic reaction conditions. Debenzoylation of **21** with sodium methoxide gave the benzyl glycoside **22** in 95% yield. Hydrogenolysis of **22** gave the free trisaccharide **5** (90% yield), as a mixture of anomers as indicated by the ^{13}C NMR spectrum. The anomeric carbon of the α-GlcNAc unit appeared at 91.3 ppm whereas the anomeric carbon of the β anomer appeared less intense at 95.6 ppm. The α/β anomeric ratio was established as 3:2 approximately based on the integration of the H-2′ signals of the internal Gal*f* units, which appeared at 4.17 and 4.14 ppm as doublet of doublets. Further reduction of **5** with $NaBH_4$ gave the corresponding alditol **23**. The chemical shifts in the ^{1}H NMR spectrum were in agreement with those reported for the novel alditol released by reductive β-elimination from mucins of *T. cruzi* (Tulahen strain).[6]

2.3. Synthesis of trisaccharide 6

With the aim to construct the β-Gal*f* linkage in **6**, we chose 2,3,4,6-tetra-*O*-acetyl-D-galactopyranosyl trichloroacetimidate[44] (**24**) as the donor, taking into account that the acetyl group in O-2 would provide the anchimeric assistance to afford the β linkage diastereoselectively. However, reaction of lactone acceptor **10** with imidate **24** afforded the β-(1→2) disaccharide lactone **25** in 54% yield together with the unexpected α(1→2)linked **26** in 18% yield (Scheme 3).

The ^{13}C NMR spectrum of **25** showed the anomeric carbon at 99.8 ppm indicating the β-configuration for the new glycosidic linkage, confirmed by the doublet at 4.63 ppm for the H-1′ (J = 7.9 Hz) in the ^{1}H NMR spectrum. On the other hand, the ^{13}C NMR spectrum of the minor product **26** showed a signal at 97.1 ppm in the anomeric region. This signal correlated, in a HSQC experiment, with a doublet at δ 5.58 ppm with J = 3.1 Hz, and



Scheme 3. Synthesis of β-D-Gal*p*-(1→2)-β-D-Gal*f*-(1→4)-D-GlcNAc (**6**) and alditol **34**.

confirmed the H-1′/H-2′ *cis* disposition, meaning a α-configuration for the anomeric center.

The moderate selectivity obtained toward the β linkage in the latter glycosylation suggests that other factors are involved, and this could be related to the reciprocal donor acceptor selectivity (RDAS) concept introduced by Fraser-Reid.[45] In this sense, an unusual α-glycosylation was reported with imidate **24**, and other 2-*O*-acyl galactopyranose donors.[46]

Reduction of lactone **25** with diisoamylborane afforded the furanose **27** in 86% yield as a α/β mixture in 1:4 ratio as indicated by the integration of β anomer H-1 (δ = 5.61 ppm) and α anomer H-3 (δ = 5.69 ppm). Reaction of **27** with acetonitrile and DBU gave a mixture of imidates. After column chromatography, the less polar β-imidate **28β** was obtained in 88% yield. The ^{1}H NMR spectrum showed a downfield broad singlet (δ 6.66 ppm) for H-1 whereas H-2 resonated at 4.23 ppm as a singlet indicating a β stereochemistry for the imidate. On the other hand, the anomeric zone in the ^{13}C NMR showed two signals, 101.1 ppm for C-1′ and 104.3 ppm for C-1, in agreement with the β stereochemistry. Also, the α-imidate **28α** was recovered (9%), as indicated by the H-1 signal (δ = 6.58 ppm; J = 4.7 Hz) in the ^{1}H NMR spectrum.

The next step was the construction of the Gal*f*-(1→4)GlcNAc glycosidic linkage considering that furanosyl donor **28β**, with a β-Gal*p* substituent in O-2, lacks anchimeric assistance. On the other hand, in the synthesis of trisaccharide **5**, we used donor **17β** with a O-2 Gal*f* substituent. In that case, CH_2Cl_2 was the best solvent in terms of diastereoselectivity and obtained yields for the β linkage. Thus, we performed our first glycosylation attempt of acceptor **18** with donor **28β** in CH_2Cl_2 as solvent. The best results were obtained employing the same conditions as described before for glycosylation with **17β**, the galactofuranosyl equivalent of **28β**. Column chromatography purification gave the desired β(1→4) product **29** in 26% yield. The ^{13}C NMR spectrum of **29** showed three anomeric signals at 96.3 (C-1 GlcNAc), 99.5 (C-1″, Gal*p* unit), and 106.2 ppm, the latter indicating a β-Gal*f* linkage. The C-2′, C-4′, and C-3′ resonances of the internal Gal*f* unit appeared at 86.7, 80.4, and 76.9 ppm, the first downfield shifted by glycosylation. On the other hand, the broad singlet assigned to H-1′ (δ = 5.56 ppm) together with the doublet (δ = 4.21 ppm) with a coupling constant <1.5 Hz due to the H-2′ resonance, confirmed the β stereochemistry for the new linkage. A second fraction from the column afforded the α-(1→4) product **30** in 27% yield. In this case, the ^{13}C NMR spectrum showed three anomeric signals at 95.9 (C-1, GlcNAc), 99.8 (C-1″, Gal*p*), and 102.7 ppm, the latter is shifted upfield compared to the analogous signal in the β(1→4) product **29** (106.2 ppm), suggesting the α stereochemistry for the new Gal*f* linkage. The ^{1}H NMR spectrum showed a doublet (δ = 5.42 ppm) with a coupling constant of 4.8 Hz assigned to H-1′, and correlated with the signal at 102 ppm in the HSQC experiment, confirming the α stereochemistry. No diastereomeric preference was obtained by employing the low polar and non-participating solvent CH_2Cl_2 as the α and β glycosylation products **30** and **29** were obtained in 1:1 ratio and a total yield of 53%. Glycosidic substitution at *O*-2 of the Gal*f* donor plays a major influence in the stereochemistry of the glycosylation reaction. In fact, Gal*p* replacement by a Gal*f* unit in O-2 of the galactofuranosyl donor, under the same reaction conditions, afforded the β product **19** as major component in the synthesis of trisaccharide **5**.

To increase the diastereoselectivity to obtain the desired β linked product **29**, other solvents were assayed for the glycosylation reaction. When participating ethyl ether was used, the total glycosylation yield diminished to 35% and the diastereoselectivity slightly favored the undesired α product **30**, giving rise to a β/α ratio of 1:1.3 after purification. Similar diastereoselectivity was observed for the analogous glycosylation in the synthesis of trisaccharide **5**.

At this point, we decided to evaluate the glycosidic reaction in acetonitrile with low expectations because no diastereoselectivity preferences were obtained in the synthesis of trisaccharide **5**. However, to our surprise, the diastereoselectivity favored the desired β product **29** (3:1 β/α ratio) with a total glycosidation yield of 52%. This result confirmed that 2-O glycosyl substitution of the Gal*f* donor as well as the solvent play an important influence in the diastereoselectivity of the reaction. Glycosyl substitution would influence the conformation of the furanosyl oxocarbenium ion, considering the flexibility of the furanose ring.

It is interesting to note that all glycosylations required long reaction times, and in the case of acetonitrile as solvent, an imidate rearrangement byproduct with similar mobility of imidate **28β** was isolated.[41] In fact, an inseparable mixture of 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→2)-3,5,6-tri-*O*-benzoyl-*N*-trichloroacetyl-β-D-galactofuranosylamine and 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→2)-3,5,6-tri-*O*-benzoyl-*N*-trichloroacetyl-α-D-galactofuranosylamine (**31β** and **α**) was isolated in 40% yield from imidate **28β**, and in 1:1 ratio as indicated by ^{1}H NMR analysis. The H-1 of the α and β anomers appeared as a doublet of doublets at 5.95 ppm (J = 9.0, 4.7 Hz) and 5.85 ppm (J = 9.0, 2.3 Hz), respectively. The ^{13}C NMR spectrum showed the diagnostic signals at 87.4 and 81.9 ppm assigned to C-1 of β and α anomers respectively. COSY and HSQC experiments allowed complete assignment of the mixture.

The next step was the removal of the TPBPS group. When 5% HF (48 wt % in water) in acetonitrile was used, as in deprotection of **19**, after 22 h at rt TLC still showed the presence of starting material together with more polar compounds. Alternatively, treatment of **29** with TBAF buffered with AcOH in anhydrous THF–DMF[34] afforded crystalline desilylated product **32** in 82% yield. Deacylation with sodium methoxide in methanol gave the benzyl glycoside **33** in 98%, which crystallized from methanol. Debenzylation of **33** was accomplished by catalytic hydrogenation to give the free trisaccharide **6** with 92% yield. The α/β ratio of the anomeric mixture was established as 3:2 by integration of the α and β anomeric H-1 that appeared at 5.19 ppm (J = 3.4 Hz) and 4.70 ppm (J = 8.3 Hz), respectively. Upon reduction of **6** with sodium borohydride, alditol **34** was obtained and the chemical shifts in the ^{1}H NMR spectrum were in agreement with those reported for the novel alditol released from the *T. cruzi* mucins (Tulahen strain).[6]

In conclusion, two novel trisaccharide structures that contain an internal Gal*f*, β-D-Gal*f*-(1→2)-β-D-Gal*f*-(1→4)-D-GlcNAc (**5**) and β-D-Gal*p*-(1→2)-β-D-Gal*f*-(1→4)-D-GlcNAc (**6**), were synthesized and their alditols confirmed the structure of the natural oligosaccharides released from mucins of *T. cruzi* (Tulahuen strain). Intermediates **21** and **32** would allow the construction of the hexasaccharides **7** and **8** and the pentasaccharide **9**. The glycosyl aldonolactone approach was envisioned for the synthesis, a convenient D-galactono-1,4-lactone derivative was used for the introduction of the internal galactofuranose. The trisaccharides were assembled from the non-reducing to the reducing end, and the trichloroacetimidate method was employed for glycosylation reactions. The nature of the solvent strongly determined the stereochemical course of glycosylation reactions when the Gal*f* donor lacks anchimeric assistance by substitution either by 2-*O*-Gal*p* or 2-*O*-Gal*f*. Dichloromethane favored the β stereochemistry employing the Gal*f*-(1→2)-Gal*f* donor, whereas acetonitrile favored the β linkage when Gal*p*-(1→2)-Gal*f* was the donor.

3. Experimental

3.1. General methods

Thin layer chromatography (TLC) was performed on 0.2 mm Silica Gel 60 F254 aluminum-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 at 500 MHz (^{1}H) and 125.8 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. Assignments were supported by 2D ^{1}H-COSY and HSQC experiments. High resolution mass spectra (HRMS) were recorded on an Agilent LCTOF with Windows XP based OS and APCI/ESI ionization high resolution mass spectrometer analyzer or in a BRUKER micrOTOF-Q II spectrometer. Melting points were determined with a Fisher–Johns apparatus and are uncorrected. Optical rotations were measured with a path length of 1 dm at 25 °C.

3.2. 2-*O*-*tert*-Butyldiphenylsilyl-5,6-*O*-isopropylidene-D-galactono-1,4-lactone (12)

To a cooled solution (0 °C) of 5,6-*O*-isopropylidene-D-galactono-1,4-lactone[32] (**11**, 1.2 g, 5.61 mmol) and imidazole (0.65 g, 9.54 mmol, 1.7 equiv) in anhyd DMF (8.5 mL), was added *t*-butyldiphenylsilyl chloride (1.67 g, 1.56 mL, 1.2 equiv) with stirring. After stirring for 16 h at 5 °C, TLC revealed disappearance of starting material and the formation of only one product (R_f 0.4, hexane–EtOAc 2:1), the mixture was diluted with CH_2Cl_2 (200 mL), washed with H_2O (5 × 100 mL), dried (Na_2SO_4), and filtered. The filtrate was concentrated under vacuum to give a sirup that crystallized from *n*-hexane yielding 2.13 g of **12** (83%): mp 60–62 °C, $[\alpha]_D$ −11.2 (*c* 0.7, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.81–7.41 (m, 10H, arom.), 4.40 (d, 1H, J = 8.2 Hz, H-2), 4.31 (td, 1H, J = 8.1, 3.9 Hz, H-3), 4.25 (td, 1H, J = 6.8, 3.9 Hz, H-5), 4.04 (dd, 1H, J = 6.8, 8.6 Hz, H-6a), 3.92 (dd, 1H, J = 8.6, 6.9 Hz, H-6b), 3.87 (dd, 1H, J = 8.1, 3.9 Hz, H-4), 1.86 (d, 1H, J = 3.9 Hz, –OH), 1.42, 1.38 (2s, 6H, $(CH_3)_2C$), 1.13 (s, 9H, $(CH_3)_3CSi$). ^{13}C NMR ($CDCl_3$, 125.8 MHz,): δ 171.7 (CO), 136.0–128.0 (C-arom.), 110.2 ($(CH_3)_2C$), 78.0 (C-4), 76.2 (C-2), 75.3 (C-3), 73.9 (C-5), 64.9 (C-6), 26.7 ($(CH_3)_3CSi$), 26.1, 25.5 ($(CH_3)_2C$), 19.2 ($(CH_3)_3CSi$). Anal. Calcd for $C_{25}H_{32}O_6Si{\cdot}1/2H_2O$: C, 64.49; H, 7.14. Found: C, 64.25; H, 7.12.

3.3. 3,5,6-Tri-*O*-benzoyl-2-*O*-*tert*-butyldiphenylsilyl-D-galactono-1,4-lactone (13)

A suspension of **12** (2.0 g, 4.38 mmol) in a mixture of 2:1 HOAc–H_2O (13 mL) was warmed at 80 °C with stirring. After 30 min, TLC revealed disappearance of starting material, and the solution was cooled to rt, concentrated under vacuum and the residue was co-evaporated with toluene (5 × 20 mL) to give crude 2-*O*-*t*-butyldiphenylsilyl-D-galactono-1,4-lactone as an colorless sirup (1.91 g, 4.24 mmol). The crude was dissolved in dry pyridine (8 mL), cooled at 0 °C, and benzoyl chloride (1.92 mL, 16.5 mmol, 3.6 equiv) was slowly added with stirring. After stirring 30 min at 0 °C and 1 h at rt, the mixture was poured into ice-water (200 g). After 20 min, the mixture was extracted with CH_2Cl_2 (200 mL) and the organic phase was washed with H_2O (100 mL), HCl 10% (80 mL), H_2O (100 mL), $NaHCO_3$ 10% (80 mL), H_2O (2 × 150 mL) until pH 7, dried (Na_2SO_4) and filtered. The solution was concentrated and the sirupy residue was purified by column chromatography (6:1 hexane–EtOAc) to yield **13** (2.17 g, 68%). R_f 0.47 (3:1 hexane–EtOAc), $[\alpha]_D$ −18.2 (*c* 1, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.95–7.05 (m, 25H, arom.), 5.95 (td, 1H, J = 6.2, 2.5 Hz, H-5), 5.61 (t, 1H, J = 5.7 Hz, H-3), 4.77 (d, 1H, J = 6.0 Hz, H-2), 4.67 (m, 2H, H-6a, H-6b), 4.64 (dd, 1H, J = 5.5, 2.5 Hz, H-4), 0.95 (s, 9H, $(CH_3)_3CSi$); ^{13}C NMR ($CDCl_3$, 125.8 MHz): δ 171.4 (C-1), 165.9, 165.3, 165.2 (PhCO), 136.1–127.7 (C-arom.), 78.7 (C-4), 76.7 (C-3), 73.3 (C-2), 70.1 (C-5), 62.4 (C-6), 26.5 ($(CH_3)_3CSi$), 19.1 ($(CH_3)_3CSi$). Anal. Calcd for $C_{43}H_{40}O_9Si$: C, 70.86; H, 5.53. Found: C, 70.94; H, 5.39.

3.4. 3,5,6-Tri-*O*-benzoyl-D-galactono-1,4-lactone (10)

To a stirred cold solution (0 °C) of **13** (1.9 g, 2.61 mmol) in anhyd DMF (19 mL), was added glacial HOAc (0.31 mL, 2.11 equiv) and a solution of 1 M TBAF in anhyd THF (5.2 mL, 2 equiv), and the stirring continued at rt. After 2 h, TLC examination showed disappearance of the starting material and the mixture was diluted with CH_2Cl_2 (200 mL), washed with H_2O (5 × 100 mL), dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The resulting sirup was purified by silica gel column chromatography (4:1 hexane–EtOAc) to afford **10** (1.04 g, 81%), which crystallized from toluene: R_f 0.35 (3:2 hexane–EtOAc), mp 100–102 °C, $[\alpha]_D$ −54.8 (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz): δ 8.09 – 7.96 (m, 6H, arom.), 7.63–7.37 (m, 9H, arom.), 5.90 (ddd, 1H, *J* = 6.5, 5.2, 3.0 Hz, H-5), 5.54 (t, 1H, *J* = 6.6 Hz, H-3), 4.94 (dd, 1H, *J* = 6.6, 3.0 Hz, H-4), 4.81 (d, 1H, *J* = 6.5 Hz, H-2), 4.77 (dd, 1H, *J* = 11.8, 5.2 Hz, H-6a), 4.67 (dd, 1H, *J* = 11.8, 6.5 Hz, H-6b), 3.76 (br s, 1H, –OH); ^{13}C NMR ($CDCl_3$, 125.8 MHz): δ 171.9 (C-1), 166.4, 165.9, 165.4 (Ph*C*O), 134.1–128.0 (C-arom.), 77.8 (C-4), 76.9 (C-3), 72.9 (C-2), 69.4 (C-5), 62.4 (C-6). Anal. Calcd for $C_{27}H_{22}O_9$: C, 66.12; H, 4.52. Found: C, 66.18; H, 4.63.

3.5. 2,3,5,6-Tetra-*O*-benzoyl-β-D-galactofuranosyl-(1→2)-3,5,6-tri-*O*-benzoyl-D-galactono-1,4-lactone (15)

To an externally cooled (0 °C) solution of 1,2,3,5,6-penta-*O*-benzoyl-α,β-D-galactofuranose[36] (**14**, 1.55 g, 2.21 mmol) in dry CH_2Cl_2 (45 mL), tin(IV) chloride (0.26 mL, 2.23 mmol) was slowly added with stirring. After 15 min at 0 °C, 3,5,6-tri-*O*-benzoyl-D-galactono-1,4-lactone (**10**, 0.98 g, 1.99 mmol) was added and the stirring continued for 2 h until consumption of **14**. The mixture was diluted with CH_2Cl_2 (350 mL), washed with 10% $NaHCO_3$ (2 × 100 mL) and H_2O (3 × 200 mL) until pH 6, and was dried (Na_2SO_4), filtered, and concentrated. The resulting sirup was washed with small amounts of cold ethanol and then CH_3OH to afford a solid. Recrystallization from isopropanol gave 1.66 g of **15** (78%). The combined mother liquors and the alcoholic washes were concentrated. Purification by column chromatography of the residue (95:5 toluene–EtOAc) gave additional 0.29 g of **15** (total yield 92%). Mp 82–85 °C (isopropanol), $[\alpha]_D$ −11.9 (*c* 1, $CHCl_3$), R_f 0.6 (9:1 toluene–EtOAc). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 8.07–7.15 (m, 35H, arom.), 6.01–5.98 (m, 2H, H-5, H-5′), 5.71 (t, 1H, *J* = 4.2 Hz, H-3), 5.60 (dd, 1H, *J* = 5.3, 1.4 Hz, H-3′), 5.57 (br s, 1H, H-1′), 5.04 (d, 1H, *J* = 1.4 Hz, H-2′), 5.01 (dd, 1H, *J* = 5.3, 3.6 Hz, H-4′), 4.98 (dd, 1H, *J* = 4.1, 2.8 Hz, H-4), 4.93 (d, 1H, *J* = 4.3 Hz, H-2), 4.79–4.68 (m, 4H, H-6a, H-6b, H-6a′, H-6b′), ^{13}C NMR ($CDCl_3$, 125.8 MHz): δ 170.6 (C-1), 166.0, 165.9, 165.6, 165.5, 165.4, 165.3, 164.9 (PhCO), 133.9–128.2 (C-arom.), 104.9 (C-1′), 82.2 (C-4′), 81.6 (C-2′), 80.1 (C-4), 76.9 (C-3′), 75.2 (C-3), 74.1 (C-2), 71.1, 70.0 (C-5, C-5′), 63.3, 62.5 (C-6, C-6′). Anal. Calcd $C_{61}H_{48}O_{18}$: C, 68.53; H, 4.53. Found: C, 68.52; H, 4.38.

3.6. 2,3,5,6-Tetra-*O*-benzoyl-β-D-galactofuranosyl-(1→2)-3,5,6-tri-*O*-benzoyl-α,β-D-galactofuranose (16)

A solution of bis(2-butyl-3-methyl)borane (6.5 mmol, 6 equiv) in anhyd THF (2.2 mL) cooled to 0 °C under an argon atmosphere was added to a flask containing dried **15** (1.16 g, 1.09 mmol). The resulting solution was stirred for 18 h at 5 °C and 3 h at rt and then processed as previously described.[47] The organic layer was washed with H_2O, dried (Na_2SO_4), and concentrated. Boric acid was eliminated by coevaporation with CH_3OH (6 × 10 mL) at rt. The residue was purified by column chromatography (95:5 toluene–EtOAc). Unreacted **15** (0.10 g, 8.8%) was recovered from the first fraction. The second fraction from the column gave 0.957 g (82%) of an amorphous solid **16** as a 3:7 α/β anomeric mixture: R_f 0.35 (9:1 toluene–EtOAc), $[\alpha]_D$ −11.1 (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz): δ for the β anomer, only the diagnostic signals are listed for the α anomer: 8.11–7.23 (m, 35H), 6.06 (ddd, 0.3H, *J* = 6.6, 4.9, 3.7 Hz, H-5 or H-5′ α anomer), 6.02 (ddd, 0.7H, *J* = 6.4, 4.8, 3.2 Hz, H-5′), 5.99 (ddd, 0.3H, *J* = 6.7, 6.2, 3.4 Hz, H-5 or H-5′α anomer), 5.93 (ddd, 0.7H, *J* = 6.6, 5.4, 4.1 Hz, H-5), 5.74 (br s, 0.3H, H-1′ α anomer), 5.70 (br s, 0.7H, H-1′), 5.62 (m, 1.4H, H-1 and H-3), 5.56 (dd, 0.3H, *J* = 10.0, 4.0 Hz, H-1 α anomer), 5.54 (dd, 0.7H, *J* = 4.1, 1.3 Hz, H-3), 5.37 (d, 0.7H, *J* = 1.5 Hz, H-2′), 4.86 (dd, 0.7H, *J* = 5.4, 4.1 Hz, H-4), 4.70 (dd, 0.7H, *J* = 5.2, 3.2 Hz, H-4′), 4.47 (d, 0.7H, *J* = 1.3 Hz, H-2), 4.10 (d, 0.3H, *J* = 10.0 Hz, –OH α anomer), 3.36 (d, 0.7H, *J* = 4.0 Hz, –OH); ^{13}C NMR ($CDCl_3$, 125.8 MHz): δ for β anomer 166.4–165.2 (*C*OPh), 133.6–128.2 (arom.), 105.1 (C-1′), 104.7 (C-1′ α anomer), 101.9 (C-1), 97.7 (C-1 α anomer), 84.8 (C-2), 82.3, 82.2 (C-2′, C-4′), 81.8 (C-4), 78.0 (C-3), 77.4 (C-3′), 71.0 (C-5), 70.2 (C-5′), 63.4, 63.2 (C-6, C-6′). Anal. Calcd for $C_{61}H_{50}O_{18}$: C, 68.40; H, 4.71. Found: C, 68.33; H, 4.62.

3.7. Preparation of *O*-(2,3,5,6-tetra-*O*-benzoyl-β-D-galactofuranosyl-(1→2)-3,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl) trichloroacetimidate (17β)

To a stirred solution of **16** (0.87 g, 0.81 mmol) and trichloroacetonitrile (0.4 mL, 4.03 mmol) in CH_2Cl_2 (8 mL), cooled to 0 °C, DBU (48 μL, 0.32 mmol, 0.4 equiv) was slowly added. After 1 h at rt the solution was concentrated at rt under reduced pressure, and the residue was purified by column chromatography (95:5:0.3 toluene–EtOAc–TEA). The first fraction from the column (R_f 0.6, toluene–EtOAc 9:1) gave a sirupy **17β** (0.72 g, 73%) and was stable for one day at –20 °C. $[\alpha]_D$ −22.6 (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.57 (s, 1H, N*H*), 8.15–7.84 (m, 14H, arom.), 7.59–7.15 (m, 21H, arom.), 6.55 (br s, 1H, H-1), 6.06 (m, 1H, H-5 or H-5′), 6.03 (ddd, 1H, *J* = 7.0, 4.0, 5.0 Hz, H-5 or H-5′), 5.79 (br s, 1H, H-1′), 5.67 (dd, 1H, *J* = 5.1, 1.3 Hz, H-3′), 5.63 (d, 1H, *J* = 3.5 Hz, H-3), 5.29 (d, 1H, *J* = 1.3 Hz, H-2′), 4.98 (dd, 1H, *J* = 5.0, 3.5 Hz, H-4 or H-4′), 4.84 (dd, 1H, *J* = 12.0, 4.0 Hz), 4.82 (dd, 1H, *J* = 5.1, 3.5 Hz, H-4 or H-4′), 4.81–4.76 (m, 2H), 4.73 (br s, 1H, H-2), 4.68 (dd, 1H, *J* = 12.0, 7.0 Hz); ^{13}C NMR ($CDCl_3$, 125.8 MHz) δ 166.1, 166.0, 165.7, 165.6, 165.2 (Ph*C*O), 160.3 (*C*NH), 133.6–128.2 (C-arom.), 105.0, 104.8 (C-1, C-1′), 90.8 (CCl_3), 84.6, 82.5, 82.3, 82.2 (C-2, C-2′, C-4, C-4′), 77.1, 76.9 (C-3, C-3′), 70.5, 70.2 (C-5, C-5′), 63.7, 63.4 (C-6, C-6′).

A second fraction from the column (R_f 0.5, 9:1 toluene–EtOAc), gave sirupy *O*-(2,3,5,6-tetra-*O*-benzoyl-β-D-galactofuranosyl-(1→2)-3,5,6-tri-*O*-benzoyl-α-D-galactofuranosyl) trichloroacetimidate (**17α**, 100 mg, 10%): $[\alpha]_D$ +11.7 (*c* 0.7, $CHCl_3$), ^{1}H NMR ($CDCl_3$, 500 MHz): δ 8.55 (s, 1H, N*H*), 8.13–7.14 (m, arom.), 6.73 (d, 1H, *J* = 4.5 Hz H-1), 6.16 (t, 1H, *J* = 7.1 Hz, H-3), 6.02 (m, 1H, H-5′), 5.78 (m, 1H, H-5), 5.59 (dd, 1H, *J* = 5.8, 1.4 Hz, H-3′), 5.50 (s, 1H, H-1′), 5.47 (d, 1H, *J* = 1.4 Hz, H-2′), 4.87 (dd, 1H, *J* = 12.3, 3.6 Hz, H-6a or H-6a′), 4.82 (dd, 1H, *J* = 7.5, 4.5 Hz, H-2), 4.80–4.65 (m, 5H). ^{13}C NMR ($CDCl_3$, 125.8 MHz): δ 166.1, 165.7, 165.6, 165.5, 165.3 × 2 (Ph*C*O), 161.2 (*C*NH), 133.6–128.2 (C-arom.), 106.8 (C-1′), 98.0 (C-1), 82.6, 81.9, 80.5, 79.3 (C-2, C-2′, C-4, C-4′), 77.5, 74.2, 71.6, 70.1, 63.5, 62.8 (C-6, C-6′).

Unreacted **16** was recovered from the last fraction (118 mg, 13%).

3.8. Benzyl 2,3,5,6-tetra-*O*-benzoyl-β-D-galactofuranosyl-(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 (19) and benzyl 2,3,5,6-tetra-*O*-benzoyl-β-D-galactofuranosyl-(1→2)-3,5,6-tri-*O*-benzoyl-α-D-galactofuranosyl-(1→4)-2-acetamido-3-*O*-benzoyl-6-*O*-*tert*-butyldiphenylsilyl-2-deoxy-α-D-glucopyranoside (20)

Benzyl 2-acetamido-3-*O*-benzoyl-6-*O*-*t*-butyldiphenylsilyl-2-deoxy-α-D-glucopyranoside **18**[15] (252 mg, 0.385 mmol) and

activated 4 Å powdered molecular sieves (300 mg) were dried in vacuo and a solution of trichloroacetimidate **17β** (560 mg, 0.46 mmol, 1.2 equiv) in anhyd CH_2Cl_2 (20 mL) was added under argon and the mixture was cooled to −10 °C with vigorous stirring. After 15 min, TMSOTf (0.116 mmol, 30 μL, 0.43 equiv) was added and the stirring continued for 1 h at −10 °C and 48 h at 5 °C. TLC monitoring showed the presence of **16** due to remaining trichloroacetimidate **17β**. The reaction was stirred for additional 24 h, then neutralized with TEA (23 μL, 0.43 equiv), filtered and concentrated under reduced pressure. The sirupy residue was purified by column chromatography (2:1 hexane–EtOAc).

The first fraction of the column (R_f 0.64 2:1 hexane–EtOAc, twice developed) was concentrated and after additional purification by column chromatography (4:1 hexane–EtOAc) gave an amorphous solid identified as 2,3,5,6-tetra-*O*-benzoyl-β-D-galactofuranosyl-(1→2)-3,5,6-tri-*O*-benzoyl-*N*-trichloroacetyl-β-D-galactofuranosylamine (75 mg, 13%): $[\alpha]_D$ −3.4 (*c* 0.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz): δ 8.16–7.78 (m, 14H, arom.), 7.75 (d, 1H, *J* = 9.3 Hz, N*H*), 7.63–7.23 (m, 21H, arom.), 6.18 (dd, 1H, *J* = 8.7, 5.9, 3.0 Hz, H-5), 6.13 (m, 1H, H-5′), 6.07 (dd, 1H, *J* = 9.3, 4.1 Hz, H-1), 5.86 (br s, 1H, H-1′), 5.72 (m, 2H, H-3, H-3′), 5.53 (d, 1H, *J* = 1.0 Hz, H-2′), 4.96 (dd, 1H, *J* = 12.5, 3.0 Hz, H-6a), 4.83 (dd, 1H, *J* = 12.5, 5.9 Hz, H-6b), 4.78–4.74 (m, 2H, H-4′, H-6a′), 4.69 (dd, 1H, *J* = 11.9, 4.1 Hz, H-6b′), 4.58 (dd, 1H, *J* = 8.7, 1.0 Hz, H-4), 4.56 (d, 1H, *J* = 4.1 Hz, H-2); ^{13}C NMR ($CDCl_3$, 125.8 MHz): δ 166.2, 166.1, 165.9, 165.8, 165.6, 165.5 (Ph*C*O), 161.5 (CCl_3*C*ONH), 133.9–128.3 (C-arom), 104.9 (C-1′), 92.0 (*C*Cl_3), 83.2 (C-2′), 83.0 (C-4′), 82.6 (C-1), 80.9 (C-4), 78.0 (C-2), 77.5, 76.8 (C-3, C-3′), 70.8 (C-5), 70.2 (C-5′), 63.6 (C-6), 63.5 (C-6′). HRMS (ESI/APCI) *m*/*z* calcd for $C_{63}H_{50}Cl_3NNaO_{18}$ (M+Na) 1236.1991, found 1236.1988.

The second fraction from the column (R_f 0.24 2:1 hexane–EtOAc, twice developed) afforded 118 mg of trisaccharide **20** (18%), upon recrystallization from CH_3OH gave: 100–104 °C, $[\alpha]_D$ +42.2 (*c* 1, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 8.10–7.01 (m, 55H, arom.), 6.91 (d, 1H, *J* = 9.9 Hz, N*H*), 6.04 (ddd, 1H, *J* = 9.8, 3.0, 1.7 Hz, H-5″), 5.85 (ddd, 1H, *J* = 7.5, 5.4, 3.0 Hz, H-5′), 5.59 (dd, 1H, *J* = 10.9, 8.5 Hz, H-3), 5.58 (br s, 1H, H-1″), 5.56 (d, 1H, *J* = 4.5 Hz, H-3″), 5.51 (dd, 1H, *J* = 3.5, 2.3 Hz, H-3′), 5.43 (br s, 1H, H-2″), 5.40 (d, 1H, *J* = 3.2 Hz, H-1′), 4.90 (d, 1H, *J* = 3.5 Hz, H-1), 4.88 (dd, 1H, *J* = 12.0, 9.8 Hz, H-6a″), 4.82 (dd, 1H, *J* = 12.0, 3.0 Hz, H-6b″), 4.70 (dd, 1H, *J* = 12.4, 3.0 Hz, H-6a′), 4.63 (dd, 1H, *J* = 12.4, 7.5 Hz, H-6b′), 4.60 (dd, 1H, *J* = 4.5, 1.7 Hz, H-4″), 4.51, 4.25 (2d, 2H, *J* = 11.3 Hz, C*H*$_2$Ph), 4.50 (ddd, 1H, *J* = 10.9, 9.9, 3.5 Hz, H-2), 4.43 (t, 1H, *J* = 9.0 Hz, H-4), 4.32 (dd, 1H, *J* = 5.4, 3.5 Hz, H-4′), 4.01 (dd, 1H, *J* = 3.2, 2.3 Hz, H-2′), 3.98 (dd, 1H, *J* = 11.2, 3.5 Hz, H-6a), 3.66 (ddd, 1H, *J* = 9.6, 3.5, 2.0 Hz, H-5), 3.56 (dd, 1H, *J* = 11.2, 2.0 Hz, H-6b), 1.92 (s, 3H, C*H*$_3$CO), 1.08 (s, 9H, (C*H*$_3$)$_3$CSi); ^{13}C NMR ($CDCl_3$, 125.8 MHz): δ 170.6 (CH_3*C*O), 166.2, 166.1, 166.0, 165.8, 165.7, 165.5, 165.2, 165.1 (Ph*C*O), 136.2–127.5 (C-arom.), 105.5 (C-1″), 102.9 (C-1′), 96.2 (C-1), 83.3 (C-4″), 81.5 (C-2′), 78.8 (C-2″), 78.3 (C-4′), 78.0 (C-3″), 76.1 (C-3′), 75.5, 75.4 (C-3, C-4), 71.0 (C-5′), 70.8 (C-5), 70.2 (C-5″), 69.6 (*C*H$_2$Ph), 65.6 (C-6″), 63.5 (C-6′), 61.9 (C-6), 51.5 (C-2), 26.8 ((*C*H$_3$)$_3$CSi), 22.9 (*C*H$_3$CO), 19.4 ((CH$_3$)$_3$CSi). HRMS (ESI/APCI) *m*/*z* calcd for $C_{99}H_{91}NNaO_{24}Si$ (M+Na) 1728.5598, found 1729.5593.

The last fraction of the column (R_f 0.17, 2:1 hexane–EtOAc, twice developed) gave **19** as an amorphous solid (271 mg, 41%), that crystallized from 4:1 hexane–toluene: mp 87–89 °C, $[\alpha]_D$ +14 (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz): δ 8.05–7.25 (m, 55H, arom.), 5.91 (ddd, 1H, *J* = 7.1, 4.0, 3.3 Hz, H-5″), 5.79 (d, 1H, *J* = 9.7 Hz, N*H*), 5.64 (ddd, 1H, *J* = 8.0, 5.7, 2.9 Hz, H-5′), 5.58 (br s, 1H, H-1′), 5.58 (dd, 1H, *J* = 5.6, 1.7 Hz, H-3″), 5.55 (dd, 1H, *J* = 10.9, 9.5 Hz, H-3), 5.44 (br s, 1H, H-1″), 5.38 (d, 1H, *J* = 3.4 Hz, H-3′), 5.32 (d, 1H, *J* = 1.7 Hz, H-2″), 4.92 (d, 1H, *J* = 3.7 Hz, H-1), 4.68, 4.44 (2d, 2H, *J* = 11.9 Hz, C*H*$_2$Ph), 4.54 (dd, 1H, *J* = 11.9, 7.1 Hz, H-6a″), 4.49 (dd, 1H, *J* = 5.6, 3.3 Hz, H-4″), 4.45 (dd, 1H, *J* = 12.3, 2.9 Hz, H-6a′), 4.42 (ddd, 1H, *J* = 10.9, 9.7, 3.7 Hz, H-2), 4.38 (dd, 1H, *J* = 11.9, 4.0 Hz, H-6b″), 4.36 (dd, 1H, *J* = 12.3, 8.0 Hz, H-6b′), 4.23 (dd, 1H, *J* = 5.7, 3.4 Hz, H-4′), 4.21 (br s, 1H, H-2′), 4.17 (t, 1H, *J* = 9.7 Hz, H-4), 3.93 (dd, 1H, *J* = 11.7, 4.1 Hz, H-6a), 3.86 (dd, 1H, *J* = 11.7, 1.3 Hz, H-6b), 3.84 (ddd, 1H, *J* = 9.8, 4.1, 1.3 Hz, H-5), 1.78 (s, 3H, C*H*$_3$CO), 0.99 (s, 9H, (C*H*$_3$)$_3$CSi); ^{13}C NMR ($CDCl_3$, 125.8 MHz): δ 166.9 (CH_3*C*O), 165.9, 165.7, 165.6, 165.5, 165.3 × 2 (Ph*C*O), 136.8–127.5 (C-arom.), 106.1 (C-1′), 105.4 (C-1″), 96.2 (C-1), 85.0 (C-2′), 82.6 (C-2″), 82.3 (C-4′), 81.9 (C-4″), 77.7 (C-3′), 77.3 (C-3″), 72.3 (C-3, C-4), 72.1 (C-5), 70.6 (C-5′), 70.2 (C-5″), 69.5 (*C*H$_2$Ph), 63.9 (C-6′), 63.5 (C-6″), 62.6 (C-6), 52.5 (C-2), 26.8 ((*C*H$_3$)$_3$CSi), 23.2 (*C*H$_3$CO), 19.4 ((CH$_3$)$_3$CSi). HRMS (ESI/APCI) *m*/*z* calcd for $C_{99}H_{91}NNaO_{24}Si$ (M+Na$^+$) 1728.5598, found 1729.5585.

3.9. Benzyl 2,3,5,6-tetra-*O*-benzoyl-β-D-galactofuranosyl-(1→2)-3,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→4)-2-acetamido-3-*O*-benzoyl-2-deoxy-α-D-glucopyranoside (21)

A solution of 5% HF (48 wt % in H_2O) in acetonitrile (2.7 mL) was added to **19** (88 mg, 0.052 mmol), and the resulting solution was stirred at 28 °C for 16 h. The reaction mixture was diluted with CH_2Cl_2 (30 mL), washed with H_2O (5 × 8 mL) until pH 7, dried (Na_2SO_4), filtered, and concentrated under reduced pressure. Column chromatography of the residue (3:2 hexane–EtOAc) gave sirupy **21** (61 mg, 80%): R_f 0.5 (5:7 hexane–EtOAc), $[\alpha]_D$ −1.7 (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz): δ 8.04–7.21 (m, 45H, arom.), 6.05 (m, 1H, H-5″), 5.78 (d, 1H, *J* = 9.6 Hz, N*H*), 5.68 (d, 1H, *J* = 4.8 Hz, H-3″), 5.62 (br s, 1H, H-1″), 5.56 (dd, 1H, *J* = 10.8, 9.5 Hz, H-3), 5.54 (br s, 1H, H-1′), 5.53 (m, 1H, H-5′), 5.40 (d, 1H, *J* = 3.6 Hz, H-3′), 5.25 (br s, 1H, H-2″), 4.96 (d, 1H, *J* = 3.7 Hz, H-1), 4.79 (dd, 1H, *J* = 12.0, 3.6 Hz, H-6a″), 4.74 (dd, 1H, *J* = 12.0, 7.3 Hz, H-6b″), 4.72, 4.49 (2d, 2H, *J* = 11.9 Hz, C*H*$_2$Ph), 4.71 (t, 1H, *J* = 4.4 Hz, H-4″), 4.45 (ddd, 1H, *J* = 10.8, 9.6, 3.7 Hz, H-2), 4.37 (br s, 1H, H-2′), 4.35 (dd, 1H, *J* = 12.2, 7.5 Hz, H-6a′), 4.29 (dd, 1H, *J* = 12.2, 3.1 Hz, H-6b′), 4.22 (dd, 1H, *J* = 4.8, 3.6 Hz, H-4′), 4.15 (t, 1H, *J* = 9.5 Hz, H-4), 3.90–3.83 (m, 3H, H-5, H-6a, H-6b), 2.37 (br s, 1H, –OH), 1.77 (s, 3H, C*H*$_3$CO), ^{13}C NMR ($CDCl_3$, 125.8 MHz): δ 169.9 (CH_3*C*ONH), 166.9, 166.4, 165.8 × 2, 165.7, 165.5, 165.4, 165.1 (Ph*C*O), 136.7–128.1 (C-arom.), 107.5 (C-1′), 105.8 (C-1″), 96.7 (C-1), 84.5 (C-2′), 82.2 (C-4′, C-4″), 81.9 (C-2″), 77.9 (C-3′), 76.9 (C-3″), 73.5 (C-4), 72.0 (C-3), 71.5 (C-5), 70.3 (C-5′, C-5″), 69.9 (*C*H$_2$Ph), 63.6 (C-6″), 63.4 (C-6′), 60.7 (C-6), 52.3 (C-2), 23.0 (*C*H$_3$CONH). Anal. Calcd for $C_{83}H_{73}NO_{24}Si{\cdot}1/2H_2O$: C, 67.47; H, 5.05; N, 0.95. Found: C, 67.45; H, 5.40; N, 0.98.

3.10. Benzyl β-D-galactofuranosyl-(1→2)-β-D-galactofuranosyl-(1→4)-2-acetamido-2-deoxy-α-D-glucopyranoside (22)

To a flask containing benzyl derivative **21** (39 mg, 0.027 mmol) was added 1.5 mL of cooled 0.5 M $NaOCH_3$ in CH_3OH with stirring and the resulting solution was stirred at rt. After 3 h, TLC examination showed only one compound (R_f 0.45, 14:1:1 *n*-propanol–EtOH–H_2O). The solution was passed through a column containing Amberlite IR-120 plus resin 200 mesh, H^+ form, and washed with CH_3OH. The solvent was evaporated and the methyl benzoate eliminated by successive coevaporation with H_2O (3 × 1 mL). Further purification through a C8 cartridge eluting with H_2O followed by concentration at 35 °C gave 16.3 mg of a glassy compound identified as **22** (95%): $[\alpha]_D$ −7.5 (*c* 0.8, H_2O); ^{1}H NMR (D_2O, 500 MHz): δ 7.46–7.38 (m, 5H, arom.), 5.25 (br s, 1H, H-1′), 5.19 (br s, 1H, H-1″), 4.94 (d, 1H, *J* = 3.0 Hz, H-1), 4.75, 4.55 (2d, 2H, *J* = 11.9 Hz, CH_2Ph), 4.23 (dd, 1H, *J* = 3.4, 6.6 Hz, H-3′), 4.16 (d, 1H, *J* = 3.4 Hz, H-2′), 4.12–4.07 (m, 3H, H-2″, H-3″, H-4′), 3.99 (dd, 1H, *J* = 5.9, 4.4 Hz, H-4″), 3.88 (dd, 1H, *J* = 3.0, 10.9 Hz, H-2), 3.86–3.82 (m, 6H), 3.72 (dd, 1H), 3.69–3.60 (m, 4H), 1.96 (s, 3H, CH_3CONH); ^{13}C

NMR (D_2O, 125.8 MHz): δ 174.9 (CH_3CONH), 137.6, 129.4, 129.2, 129.0 (C-arom.), 108.0 (C-1″), 107.1 (C-1′), 96.4 (C-1), 88.0 (C-2′), 84.0 (C-4″), 83.3, 81.9 (C-4′, C-2″), 77.5 (C-4), 77.3 (C-3″), 76.1 (C-3′), 71.7, 71.5, 71.0, 70.0 (C-5, C-5′, C-5″, C-3), 70.5 (CH_2Ph), 63.5, 63.3 (C-6′, C-6″), 60.6 (C-6), 54.3 (C-2), 22.4 (CH_3CONH). HRMS (ESI/APCI) *m*/*z* calcd. for $C_{27}H_{41}NNaO_{16}$ ($M+Na^+$) 658.2323, found 658.2308.

3.11. β-D-Galactofuranosyl-(1→2)-β-D-galactofuranosyl-(1→4)-2-acetamido-2-deoxy-α,β-D-glucopyranose (5)

A suspension of **22** (19.0 mg, 0.033 mmol) dissolved in 9:1 $CH_3OH–H_2O$ (2.5 mL) and 10% Pd/C (7 mg), was hydrogenated for 20 h at 45 psi (3 atm) at rt. The catalyst was filtered and the filtrate was concentrated. Evaporation of the solvent at rt afforded **5** (14.7 mg, 90%) as a glassy hygroscopic solid: $[\alpha]_D$ −77.5 (*c* 1, H_2O), R_f 0.35 and 0.30 (7:1:1 *n*-propanol–EtOH–H_2O), ^1H NMR (D_2O, 500 MHz): δ anomeric zone and diagnostic signals: 5.24, 5.25 (2 br s, 1H, α anomer H-1′, β anomer H-1′), 5.20–5.18 (m, 1.6H, anomer α H-1″, β anomer H-1″, α anomer H-1), 4.70 (d, 0.4H, *J* = 8.2 Hz, β anomer H-1), 4.17 (dd, 0.6H, *J* = 1.4, 3.4 Hz, α anomer H-2′), 4.15 (dd, 0.4H, *J* = 1.3, 3.2 Hz, β anomer H-2′), 2.04 (2s, 3H, α and β anomer CH_3CONH); ^{13}C NMR (D_2O, 125.8 MHz): δ 175.4, 175.1 (α and β anomers CH_3CONH), 108.0 (α and β anomers C-1″), 107.2 (α and β anomers C-1′), 95.6 (β anomer C-1), 91.3 (α anomer C-1), 88.0, 84.0, 83.4, 83.3, 81.9 × 2, 77.6, 77.3, 77.2, 76.1, 75.7, 73.1, 71.5, 71.2, 71.0 × 2, 69.9, 63.6, 63.5, 63.3, 60.9, 60.8, 57.4 (β anomer C-2), 54.7 (α anomer C-2), 22.8, 22.5 (α and β anomers CH_3CONH). HRMS (ESI) *m*/*z* calcd for $C_{20}H_{35}NNaO_{16}$ ($M+Na^+$) 568.1854, found 568.1851.

3.12. Preparation of β-D-Galactofuranosyl-(1→2)-β-D-galactofuranosyl-(1→4)-2-acetamido-2-deoxy-D-glucitol[6] (23)

To a cooled solution (0 °C) of **5** (10 mg, 0.018 mmol) in 9:1 $CH_3OH–H_2O$ (2 mL), sodium borohydride (21 mg, 0.53 mmol) was added, and the mixture was stirred at rt for 16 h. The solution was passed through a column of Amberlite IR-120 plus, and after washing the resin with 4:1 $CH_3OH–H_2O$, the combined solutions were concentrated to dryness. The residue was co-evaporated with CH_3OH (4 × 1 mL), dissolved in de-ionized H_2O and the solution filtrated through C8 cartridge. Evaporation of the filtrate under reduced pressure gave 10 mg of **23** (99%) as a glassy solid: R_f 0.14 (7:1:1 *n*-propanol–EtOH–H_2O), $[\alpha]_D$ −9.2 (*c* 0.6, H_2O); ^1H NMR (D_2O, 500 MHz): δ 5.29 (br s, 1H, H-1′), 5.20 (d, 1H, *J* = 1.5 Hz, H-1″), 4.24–4.22 (m, 1H, H-3′), 4.23 (br s, 1H, H-2′), 4.16–4.11 (m, 2H, H-2, H-4′), 4.10 (dd, 1H, *J* = 3.5, 1.5 Hz, H-2″), 4.07 (dd, 1H, *J* = 6.1, 3.5 Hz, H-3″), 4.00 (dd, 1H, *J* = 6.1, 4.3 Hz, H-4″), 3.93–3.90 (m, 2H), 3.86–3.80 (m, 3H), 3.77–3.61 (m, 8H), 2.05 (s, 3H, CH_3CONH), δ values matched lit.[6]; ^{13}C NMR (D_2O, 125.8 MHz): δ 175.1 (CH_3CONH), 107.6 (C-1′, C-1″), 87.7 (C-2′), 84.0 (C-4″), 83.9 (C-4′), 81.9 (C-2″), 78.3 (C-4), 77.3 (C-3″), 76.2 (C-3′), 71.7, 71.5, 71.2, 69.0 (C-3, C-5, C-5′, C-5″), 63.5, 63.3, 62.8, 61.4 (C-1, C-6, C-6′, C-6″), 53.4 (C-2), 22.8 (CH_3CONH). HRMS (ESI/APCI) *m*/*z* calcd. for $C_{20}H_{37}NNaO_{16}$ ($M+Na^+$) 570.2010, found 570.1997.

3.13. 2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl-(1→2)-3,5,6-tri-*O*-benzoyl-D-galactono-1,4-lactone (25) and 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl-(1→2)-3,5,6-tri-*O*-benzoyl-D-galactono-1,4-lactone (26)

A solution of **10** (1.5 g, 3.06 mmol) in anhyd CH_2Cl_2 (40 mL) was transferred under positive argon pressure over a mixture of dried *O*-(2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranoside) trichloroacetimidate[44] (**24**, 2 g, 4.01 mmol, 1.3 equiv) and activated 4 Å powdered molecular sieves, and the suspension was vigorously stirred at rt for 5 min. After cooling to −12° C during 15 min, TMSOTf (220 μL, 1.22 mmol, 0.4 equiv) was slowly added under an argon atmosphere, and the stirring continued for 45 min until consumption of imidate **24** showed by TLC. The reaction mixture was rapidly filtered, the molecular sieves were washed with CH_2Cl_2 (2 × 10 mL), and the filtrate was diluted with CH_2Cl_2 (250 mL). The resulting solution was washed with 5% $NaHCO_3$ (90 mL), H_2O (2 × 150 mL) until pH 7, dried (Na_2SO_4), filtered, and concentrated under reduced pressure at rt. Column chromatography of the residue (19:1 toluene–EtOAc, then 1:1) gave a first fraction of **26** (0.44 g, 18%, R_f 0.4, 3:2 hexane–EtOAc, twice developed) that crystallized from 1:2 Et_2O–hexane: mp 88–92 °C, $[\alpha]_D$ +67 (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$, 500 MHz): δ 8.07–7.39 (m, 15H, arom.), 5.92 (ddd, 1H, *J* = 3.4, 5.2, 6.3 Hz, H-5), 5.79 (t, 1H, *J* = 6.1 Hz, H-3), 5.58 (d, 1H, *J* = 3.1 Hz, H-1′), 5.30 (d, 1H, *J* = 2.5, 1.4 Hz, H-4′), 5.18–5.13 (m, 2H, H-2′, H-3′), 4.95 (dd, 1H, *J* = 5.9, 3.4 Hz, H-4), 4.77 (d, 1H, *J* = 6.2 Hz, H-2), 4.74 (dd, 1H, *J* = 11.8, 5.2 Hz, H-6a), 4.69 (dd, 1H, *J* = 11.8, 6.3 Hz, H-6b), 4.09 (td, 1H, *J* = 6.7, 1.4 Hz, H-5′), 3.87 (dd, 1H, *J* = 11.2, 6.7 Hz, H-6a′), 3.79 (dd, 1H, *J* = 11.2, 6.7 Hz, H-6b′), 2.12, 2.09, 1.98, 1.77 (4s, 12H, CH_3CO); ^{13}C NMR ($CDCl_3$, 125.8 MHz): δ 170.7, 170.2, 170.0, 169.8, 169.6 (C-1, CH_3CO), 165.8, 165.2, 165.1 (PhCO), 134.1–128.0 (C-arom.), 97.1 (C-1′), 78.5 (C-4), 76.8 (C-2), 74.0 (C-3), 69.9 (C-5), 67.5 (C-4′), 67.2, 67.1, 66.9 (C-2′, C-3′, C-5′), 62.2 (C-6), 61.4 (C-6′), 20.7, 20.6, 20.5, 20.3 (CH_3CO). Anal. Calcd for $C_{41}H_{40}O_{18}$: C, 60.00; H, 4.91. Found: C, 60.17; H, 4.94.

The next fraction from the column (R_f 0.3, 3:2 hexane–EtOAc, twice developed) afforded **25** (1.33 g, 54%) and after crystallization from isopropanol gave: mp 85–88 °C, $[\alpha]_D$ −29.4 (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$, 500 MHz): δ 8.07–7.38 (m, 15H, arom.), 5.94 (ddd, 1H, *J* = 6.4, 5.5, 2.8 Hz, H-5), 5.82 (dd, 1H, *J* = 6.4, 5.5 Hz, H-3), 5.19 (dd, 1H, *J* = 10.4, 7.9 Hz, H-2′), 5.17 (dd, 1H, *J* = 3.3, 1.1 Hz, H-4′), 5.02 (d, 1H, *J* = 6.4 Hz, H-2), 4.86 (dd, 1H, *J* = 5.5, 2.8 Hz, H-4), 4.82 (dd, 1H, *J* = 10.4, 3.3 Hz, H-3′), 4.73 (dd, 1H, *J* = 11.8, 5.5 Hz, H-6a), 4.67 (dd, 1H, *J* = 11.8, 6.4 Hz, H-6b), 4.63 (d, 1H, *J* = 7.9 Hz, H-1′), 3.92 (dd, 1H, *J* = 11.4, 6.5 Hz, H-6′a), 3.89 (dd, 1H, *J* = 11.4, 6.7 Hz, H-6b′), 3.43 (td, 1H, *J* = 6.6, 1.1 Hz, H-5′), 2.06, 2.01, 1.97, 1.94 (4s, 12H, CH_3CO); ^{13}C NMR ($CDCl_3$, 125.8 MHz): δ 170.4, 170.1, 169.9, 169.6, 168.7 (C-1, CH_3CO), 165.8, 165.1, 164.9 (PhCO), 134.1–128.4 (C-arom.), 99.8 (C-1′), 78.9 (C-4), 76.5 (C-2), 73.8 (C-3), 71.1 (C-5′), 70.6 (C-3′), 70.0 (C-5), 67.5 (C-2′), 66.7 (C-4′), 62.3 (C-6), 61.1 (C-6′), 20.5 (×4) (CH_3CO). Anal. Calcd for $C_{41}H_{40}O_{18}$: C, 60.00; H, 4.91. Found: C, 59.73; H, 5.15.

3.14. 2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl-(1→2)-3,5,6-tri-*O*-benzoyl-α,β-D-galactofuranose (27)

A freshly prepared solution of bis(2-butyl-3-methyl)borane (5.48 mmol, 6 equiv) in anhyd THF (1.6 mL) cooled to 0 °C under an argon atmosphere, was added to a flask containing dried **25** (758 mg, 0.914 mmol) with positive argon pressure and stirring. The resulting solution was allow to reach rt and stirred for 16 h when TLC showed small amount of lactone **25** (R_f 0.5, 2:1 toluene–EtOAc) and a new product (R_f 0.35, 2:1 toluene–EtOAc). The mixture was cooled to 0 °C, H_2O (0.5 mL) was slowly added and then processed as previously described.[47] The organic layer was washed with H_2O, dried (Na_2SO_4), and concentrated. Boric acid was eliminated by coevaporation with CH_3OH (4 × 25 mL) at rt. The residue was purified by column chromatography (7:1 toluene–EtOAc). Unreacted **25** (76 mg, 10%) was recovered from the first fraction (R_f 0.5, 2:1 toluene–EtOAc). Next fraction gave 646 mg of **27** (86%) as an amorphous solid: R_f 0.35 (2:1 toluene–

EtOAc), $[\alpha]_D$ −10.6 (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$, 500 MHz): δ for the β anomer, only the diagnostic signals are listed for the α anomer: 8.07–7.33 (m, 15H, arom.), 5.73 (ddd, 0.8H, *J* = 6.5, 5.8, 3.9 Hz, H-5), 5.69 (dd, 0.2H, *J* = 5.1, 4.0 Hz, H-3 α anomer), 5.61 (d, 0.8H, *J* = 1.5 Hz, H-1), 5.42 (dd, 0.8H, *J* = 5.9, 2.1 Hz, H-3), 5.39 (dd, 0.8H, *J* = 3.5, 1.1 Hz, H-4′), 5.25 (dd, 0.8H, *J* = 10.5, 7.9 Hz, H-2′), 5.03 (dd, 0.8H, *J* = 10.5, 3.5 Hz, H-3′), 4.84 (t, 0.8H, *J* = 5.8 Hz, H-4), 4.83 (d, 0.8H, *J* = 7.9 Hz, H-1′), 4.78 (d, 0.2H, *J* = 8.0 Hz, H-1′ α anomer), 4.73 (dd, 0.8H, *J* = 12.2, 3.9 Hz, H-6a), 4.63 (dd, 0.8H, *J* = 12.2, 6.5 Hz, H-6b), 4.45 (dd, 0.2 H, *J* = 6.5, 5.1 Hz, H-4 α anomer), 4.36 (t, 0.2H, *J* = 4.0 Hz, H-2 α anomer), 4.34 (dd, 0.8H, *J* = 2.1, 1.1 Hz, H-2), 4.15–4.09 (m, 1.6H, H-6′a, H-6′b), 3.93 (m, 0.8H, H-5′), 3.15 (d, 0.8H, *J* = 3.3 Hz, –OH), 2.15 (s, 2.4H, CH_3CO), 1.98 (3 s, 7.2H, CH_3CO); ^{13}C NMR ($CDCl_3$, 125.8 MHz): δ for the β anomer, only the diagnostic signals are listed for the α anomer: 170.4, 170.2, 170.0, 169.5 (CH_3CO), 166.0, 165.6, 165.5 (PhCO), 133.5–128.3 (C-arom.), 101.7 (C-1), 100.6 (C-1′ α anomer), 100.5 (C-1′), 96.7 (C-1 α anomer), 87.5 (C-2), 82.2 (C-2 α anomer), 79.7 (C-4), 77.9 (C-4 α anomer), 77.6 (C-3), 75.8 (C-3 α anomer), 71.1 (C-5′), 70.9, 70.8 (C-3′, C-5), 68.4 (C-2′), 67.1 (C-4′), 63.0 (C-6), 61.4 (C-6′), 20.6, 20.5 × 3 (CH_3CO). Anal. Calcd for $C_{41}H_{42}O_{18}$: C, 59.85; H, 5.15. Found: C, 59.96; H, 5.42.

3.15. Preparation of *O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galacto pyranosyl-(1→2)-3,5,6-tri-*O*-benzoyl-β-D-galactofuranoside) trichloroacetimidate (28β)

To a solution of dry **27** (0.27 g, 0.324 mmol), trichloroacetonitrile (0.2 mL, 1.99 mmol, 6 equiv) in anhyd CH_2Cl_2 (3.5 mL) cooled to 0 °C, DBU (20 μL, 0.133 mmol, 0.4 equiv) was added with stirring. After 1 h at rt, TLC showed consumption of **19** and the mixture was concentrated under reduced pressure at rt. Purification of the sirupy residue by column chromatography (5:1 toluene–EtOAc, 0.3% TEA) gave a first fraction of **28β** (0.274 g, 88%) as a sirup: R_f 0.55 (2:1 toluene–EtOAc), $[\alpha]_D$ −12.8 (*c* 1, $CHCl_3$), ^1H NMR ($CDCl_3$, 500 MHz): δ 8.60 (s, 1H, NH), 8.06–7.15 (m, 15H, arom.), 6.66 (br s, 1H, H-1), 5.81 (ddd, 1H, *J* = 6.7, 5.2, 4.0 Hz, H-5), 5.51 (d, 1H, *J* = 4.8 Hz, H-3), 5.40 (d, 1H, *J* = 3.5 Hz, H-4′), 5.23 (dd. 1H. *J* = 10.4, 8.1 Hz, H-2′), 5.03 (dd, 1H, *J* = 10.4, 3.5 Hz, H-3′), 4.92 (t, 1H, *J* = 5.0 Hz, H-4), 4.88 (d, 1H, *J* = 8.1 Hz, H-1), 4.76 (dd, 1H, *J* = 12.2, 4.0 Hz, H-6a), 4.68 (dd, 1H, *J* = 12.2, 6.7 Hz, H-6b), 4.53 (br, 1H, H-2), 4.15 (dd, 1H, *J* = 11.3, 6.8 Hz, H-6a′), 4.13 (dd, 1H, *J* = 11.3, 6.6 Hz, H-6b′), 3.97 (t, 1H, *J* = 6.7 Hz, H-5′), 2.18, 2.05, 1.98, 1.95 (4s, 12H, CH_3CO); ^{13}C NMR ($CDCl_3$, 125.8 MHz): δ 170.3, 170.2, 170.0, 169.4 (CH_3CO), 165.9, 165.6, 165.4 (PhCO), 160.4 (CNH), 133.7–128.4 (C-arom), 104.3 (C-1), 101.0 (C-1′), 90.9 (CCl_3), 86.8 (C-2), 82.8 (C-4), 77.0 (C-3), 71.1 (C-5′), 70.8 (C-3′), 70.4 (C-5), 68.4 (C-2′), 67.0 (C-4′), 62.9 (C-6), 61.1 (C-6′), 20.6, 20.5 (CH_3CO).

The next fraction from the column gave 29 mg of a sirup identified as *O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→2)-3,5,6-tri-*O*-benzoyl-α-D-galactofuranoside) trichloroacetimidate (**28α**, 9%): R_f 0.50 (2:1 toluene–EtOAc), $[\alpha]_D$ +5.8 (*c* 0.7, $CHCl_3$); ^1H NMR ($CDCl_3$, 500 MHz): δ 8.51 ppm (s, 1H, NH), 7.98–7.30 (m, 15H, arom.), 6.57 (d, 1H, *J* = 4.7 Hz, H-1), 6.18 (t, 1H, *J* = 7.6 Hz, H-3), 5.71 (ddd, 1H, *J* = 6.5, 5.5, 3.4 Hz, H-5), 5.31 (dd, 1H, *J* = 3.4, 1.0 Hz, H-4′), 5.18 (dd, 1H, *J* = 10.6, 7.9 Hz, H-2′), 4.93 (dd, 1H, *J* = 10.6, 3.4 Hz, H-3′), 4.82 (dd, 1H, *J* = 12.3, 3.4 Hz, H-6a), 4.69 (dd, 1H, *J* = 7.2, 6.5 Hz, H-4), 4.66 (dd, 1H, *J* = 12.4, 5.5 Hz, H-6b), 4.61 (d, 1H, *J* = 7.9 Hz, H-1′), 4.57 (dd, 1H, *J* = 8.0, 4.7 Hz, H-2), 4.02 (dd, 1H, *J* = 11.3, 7.0 Hz, H-6a′), 3.94 (dd, 1H, *J* = 11.3, 5.8 Hz, H-6b′), 3.86 (ddd, 1H, *J* = 7.0, 5.8, 1.0 Hz, H-5′), 2.09, 2.06, 1.94, 1.81 (4s, 12H, CH_3CO); ^{13}C NMR ($CDCl_3$, 125.8 MHz): δ 170.5, 170.1, 170.0, 169.0 (CH_3CO), 165.8, 165.5, 164.7 (PhCO), 161.2 (CNH), 133.5–128.4 (C-arom.), 101.2 (C-1′), 97.8 (C-1), 91.0 (CCl_3), 82.6 (C-2), 78.5 (C-4), 73.9, 71.7, 70.9, 70.5, 68.2, 66.9, 62.5 (C-6), 61.6 (C-6′), 20.7, 20.6, 20.5, 20.3 (CH_3CO).

3.16. Benzyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→2)-3,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→4)-2-acetamido-3-*O*-benzoyl-6-*O*-*t*-butyldiphenylsilyl-2-deoxy-α-D-glucopy ranoside (29) and benzyl 2,3,4,6-tetra-*O*-acetyl-β-D-galacto pyranosyl-(1→2)-3,5,6-tri-*O*-benzoyl-α-D-galactofuranosyl-(1→4)-2-acetamido-3-*O*-benzoyl-6-*O*-t-butyldiphenylsilyl-2-deoxy-α-D-glucopyranoside (30)

To a flask containing dry benzyl 2-acetamido-3-*O*-benzoyl-6-*O*-*t*-butyldiphenylsilyl-2-deoxy-α-D-glucopyranoside[15] (**18**, 162 mg, 0.25 mmol, 1 equiv) and activated 4 Å powdered molecular sieves was added a solution of imidate **28β** 270 mg, 0.28 mmol, 1.2 equiv) in anhyd CH_3CN (10 mL) under argon pressure and the mixture was cooled to 0 °C with vigorous stirring. After 15 min, TMSOTf (20 μL, 0.011 mmol, 0.44 equiv) was added and the stirring continued for 1 h at 0 °C and for additional 48 h at 5 °C until disappearance of imidate **28β** shown by TLC. The mixture was filtered, the molecular sieves were washed with CH_2Cl_2 (2 × 8 mL), and the combined filtrate was diluted with CH_2Cl_2 (100 mL), washed with 5% $NaHCO_3$ (50 mL) and H_2O (2 × 50 mL) until pH 6, dried (Na_2SO_4), filtered, and concentrated in vacuo at rt. The residue was purified by column chromatography (5:1 toluene–EtOAc). The first fraction from the column (R_f 0.5, 2:0.6 toluene–EtOAc, twice developed) afforded 109 mg of an inseparable mixture of imidate transposition byproducts: 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→2)-3,5,6-tri-*O*-benzoyl-*N*-trichloroacetyl-β-D-galactofuranosylamine and 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→2)-3,5,6-tri-*O*-benzoyl-*N*-trichloroacetyl-α-D-galactofuranosylamine (**31β** and **α**) in 1:1 ratio as indicated by ^1H NMR analysis (40% yield from imidate **28β**): $[\alpha]_D$ +2.5 (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$, 500 MHz): δ 8.09–7.94 (m, 12H, arom.), 7.90 (d, 1H, *J* = 9.0 Hz, NH α anomer), 7.72 6–7.30 (m, 19H, H-arom. and NH β anomer), 5.95 (dd, 1H, *J* = 9.0, 4.7 Hz, H-1α anomer), 5.85 (dd, 1H, *J* = 7.0, 2.3 Hz, H-1 β anomer), 5.76 (m, 1H, H-5 β anomer), 5.73 (ddd, 1H, *J* = 7.5, 6.3, 3.3 Hz, H-5 α anomer), 5.60 (dd, 1H, *J* = 4.3, 2.7 Hz, H-3 β anomer), 5.57 (dd, 1H, *J* = 3.7, 1.6 Hz, H-3 α anomer), 5.39–5.37 (m, 2H, H-4′ α and β anomers), 5.25 (dd, 1H, *J* = 7.9, 10.5 Hz, H-2′β anomer), 5.23 (dd, 1H, *J* = 10.5, 8.0 Hz, H-2′ α anomer), 5.05 (dd, 1H, *J* = 10.5, 3.5 Hz, H-3′ β anomer), 4.98 (dd, 1H, *J* = 10.5, 3.5 Hz, H-3′ α anomer), 4.94 (d, 1H, *J* = 7.9 Hz, H-1′ β anomer), 4.84 (dd, 1H, *J* = 5.7, 4.3 Hz, H-4 β anomer), 4.79 (dd, 1H, *J* = 12.4, 3.3 Hz, H-6a α anomer), 4.77 (dd, 1H, *J* = 12.3, 3.8 Hz, H-6a β anomer), 4.71 (d, 1H, *J* = 8.0 Hz, H-1′ anomer α), 4.70 (t, 1H, *J* = 2.5 Hz, H-2 anomer β), 4.68 (dd, 1H, *J* = 12.3, 6.3 Hz, H-6b β anomer), 4.63 (dd, 1H, *J* = 12.4, 6.3 Hz, H-6b α anomer), 4.56 (dd, 1H, *J* = 4.7, 1.6 Hz, H-2 α anomer), 4.47 (dd, 1H, *J* = 7.5, 3.7 Hz, H-4 α anomer), 4.24 (dd, 1H, *J* = 11.4, 6.5 Hz, H-6a′α or β anomer), 4.13–4.06 (m, 3H, H-6b′α or β anomer, H-6b′α anomer , H-6b′β anomer), 3.98–3.89(m, 2H, H-5′α and β anomers), 2.14 × 2, 2.02, 1.98, 1.97, 1.96, 1.95, 1.93 (8s, 24H, CH_3CO); ^{13}C NMR ($CDCl_3$, 125.8 MHz): δ 170.5, 170.3, 170.2, 170.0 × 3, 169.9 × 2, 169.5 (CH_3CO), 166.0, 165.8, 165.5, 165.1 (PhCO), 161.6, 161.2 (CH_3CONH), 135.6–127.8 (C-arom.), 100.2, 99.7 (C-1′ α and β anomers), 87.4 (C-1 β anomer), 85.1 (C-2 β anomer), 81.9 (C-1 α anomer, C-4 β anomer), 79.9 (C-2 α anomer), 79.2 (C-4 α anomer), 77.5 (C-3 β anomer), 76.5 (C-3 α anomer), 71.2 × 2 (C-5′ α and β anomers), 71.0 (C-5 β anomer), 70.6 × 3 (C-5 α anomer, C-3′ α and β anomers), 68.5 (C-2′ β anomer), 68.1 (C-2′ α anomer), 67.0, 66.0 (C-4′ α and β anomers), 63.1 × 2 (C-6 α and β anomers), 61.2, 61.0 (C-6′α and β anomers), 20.6, 20.5 × 3 (CH_3CO). HRMS (ESI/APCI) *m*/*z* calcd. for $C_{43}H_{42}Cl_3NNaO_{18}$ ($M+Na^+$) 988.1365, found 988.1389.

Next fraction from the column (R_f 0.35, 2:0.6 toluene–EtOAc, twice developed) gave 144 mg of an amorphous solid identified as **29** (40%): $[\alpha]_D$ +3.5 (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$, 500 MHz): δ 8.07–7.25 (m, 35H, arom.), 5.87 (d, 1H, *J* = 9.7 Hz, NH), 5.60 (dd, 1H, *J* = 10.8, 9.3 Hz, H-3), 5.56 (s, 1H, H-1′), 5.39 (ddd, 1H, *J* = 7.4, 6.2, 3.3 Hz, H-5′), 5.33 (dd, 1H, *J* = 5.1, 1.2 Hz, H-3′), 5.27 (dd, 1H, *J* = 3.5, 1.2 Hz, H-4″), 5.17 (dd, 1H, *J* = 10.4, 8.0 Hz, H-2″), 4.97 (d, 1H, *J* = 3.8 Hz, H-1), 4.87 (dd, 1H, *J* = 10.4, 3.5 Hz, H-3″), 4.70 (d, 1H, *J* = 8.0 Hz, H-1″), 4.68, 4.47 (2d, 2H, *J* = 11.5 Hz, CH_2Ph), 4.50 (ddd, 1H, *J* = 10.8, 9.7, 3.8 Hz, H-2), 4.31 (dd, 1H, *J* = 6.2, 5.1 Hz, H-4′), 4.30 (dd, 1H, *J* = 9.3, 8.6 Hz, H-4), 4.27 (dd, 1H, *J* = 12.1, 3.3 Hz, H-6a′), 4.21 (d, 1H, *J* = 1.2 Hz, H-2′), 4.13 (dd, 1H, *J* = 12.1, 7.4 Hz, H-6b′), 3.97 (dd, 1H, *J* = 11.3, 6.7 Hz, H-6a″), 3.92 (dd, 1H, *J* = 11.3, 6.5 Hz, H-6b″), 3.92–3.88 (m, 2H, H-5, H-6a), 3.81 (d, 1H, *J* = 10.5 Hz, H-6b), 3.69 (td, 1H, *J* = 6.6, 1.2 Hz, H-5″), 2.11, 1.96, 1.95, 1.91 (4s, 12H, CH_3CO), 1.81 (s, 3H, CH_3CONH), 1.01 (s, 9H, $(CH_3)_3CSi$); ^{13}C NMR ($CDCl_3$, 125.8 MHz): δ 170.2, 170.1, 169.9, 169.3 (CH_3CO), 167.0 (CH_3CONH), 165.7, 165.5, 165.2 (PhCO), 136.7–127.7 (C-arom.), 106.2 (C-1′), 99.5 (C-1″), 96.3 (C-1), 86.7 (C-2′), 80.4 (C-4′), 76.9 (C-3′), 72.6 (C-4), 72.4 (C-3), 71.7 (C-5), 70.9 (C-3″), 70.8 (C-5″), 70.5 (C-5′), 69.6 (CH_2Ph), 68.1 (C-2″), 67.2 (C-4″), 62.9 (C-6′), 62.5 (C-6), 61.1 (C-6″), 52.4 (C-2), 26.6 ($(CH_3)_3CSi$), 23.1 (CH_3CONH), 20.6 × 2, 20.5, 20.4 (CH_3CO), 19.2 ($(CH_3)_3CSi$). Anal. Calcd for $C_{79}H_{83}NO_{24}Si$: C, 65.05; H, 5.74. Found: C, 64.82; H, 5.63.

The last fraction from the column (R_f 0.1, 2:0.6 toluene–EtOAc, twice developed) gave 44 mg of **30** as an amorphous solid (12%): $[\alpha]_D$ +15.3 (*c* 1, $CHCl_3$). ^1H NMR ($CDCl_3$, 500 MHz): δ 8.03–7.22 (m, 35H, arom.), 6.21 (d, 1H, *J* = 9.4 Hz, NH), 5.69 (dd, 1H, *J* = 10.8, 8.5 Hz, H-3), 5.62 (t, 1H, *J* = 6.4 Hz, H-3′), 5.50 (dt, 1H, *J* = 6.0, 4.7 Hz, H-5′), 5.42 (d, 1H, *J* = 4.8 Hz, H-1′), 5.18 (dd, 1H, *J* = 3.6, 1.3 Hz, H-4″), 5.13 (dd, 1H, *J* = 10.4, 8.0 Hz, H-2″), 4.94 (d, 1H, *J* = 3.7 Hz, H-1); 4.80, 4.49 (2d, 2H, *J* = 12.1 Hz, CH_2Ph), 4.67 (dd, 1H, *J* = 10.4, 3.6 Hz, H-3″), 4.50 (dd, 1H, *J* = 12.0, 4.8 Hz, H-6a′), 4.38 (ddd, 1H, *J* = 10.8, 9.4, 3.7 Hz, H-2), 4.36 (dd, 1H, *J* = 12.0, 6.0 Hz, H-6b′), 4.35 (dd, 1H, *J* = 6.5, 4.8 Hz, H-2′), 4.26 (dd, 1H, *J* = 6.3, 4.6 Hz, H-4′), 4.22 (d, 1H, *J* = 8.0 Hz, H-1″), 4.17–4.02 (m, 3H, H-4, H-5, H-6a), 3.77 (d, 1H, *J* = 11.0 Hz, H-6b), 3.74 (dd, 1H, *J* = 11.1, 6.2 Hz, H-6a″), 3.66 (dd, 1H, *J* = 11.1, 7.8 Hz, H-6b″), 3.52 (ddd, 1H, *J* = 7.8, 6.2, 1.3 Hz, H-5″); 2.13, 2.04, 1.95, 1.92 (4s, 12H, CH_3CO), 1.84 (s, 3H, CH_3CONH), 1.01 (s, 9H, $(CH_3)_3CSi$); ^{13}C NMR ($CDCl_3$, 125.8 MHz): δ 170.2 × 2, 170.1, 170.0, 169.0 (CH_3CO), 166.6 (CH_3CONH), 165.7, 165.5, 164.8 (PhCO), 137.1 – 127.5 (C-arom.), 102.7 (C-1′), 99.8 (C-1″), 95.9 (C-1), 79.1 (C-2′), 78.7 (C-4′), 77.3 (C-4), 75.6 (C-3′), 74.2 (C-3), 71.2 (C-5), 71.0 (C-3″), 70.9 (C-5′), 70.7 (C-5″), 69.1 (CH_2Ph), 67.9 (C-2″), 66.6 (C-4″), 63.0 (C-6), 62.6 (C-6′), 60.4 (C-6″), 52.7 (C-2), 26.9 ($(CH_3)_3CSi$), 23.1 (CH_3CONH), 20.6, 20.5 × 2, 20.4 (CH_3CO), 19.4 ($(CH_3)_3CSi$).

HRMS (ESI/APCI) *m*/*z* calcd for $C_{79}H_{83}NNaO_{24}Si$ ($M+Na^+$) 1480.4972, found 1480.4931.

3.17. Benzyl 2,3,4,6-tetra-*O*-acetyl-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 (32)

To a solution of **29** (163 mg, 0.134 mmol) in anhyd DMF (1.7 mL) cooled at 0 °C, was added a freshly prepared solution of 1 M TBAF in anhyd THF (223 μL, 2 equiv) with vigorous stirring, followed by glacial HOAc (14.8 μL, 2.1 equiv). The solution was stirred at 25 °C for 2 h and left at −18 °C overnight. The solution was diluted with CH_2Cl_2 (50 mL), washed with H_2O (5 × 10 mL), dried (Na_2SO_4), and concentrated under reduced pressure. Column chromatography of the crude (1.1 to 1:2 *n*-hexane–EtOAc) gave **32**, (134 mg, 82%) that crystallized from THF–hexane (1:2): R_f 0.21 (1:2 hexane–EtOAc), mp 103–105 °C, $[\alpha]_D$ −4.4 (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$, 500 MHz): δ 8.03–7.28 (m, 25H, arom.), 5.90 (d, 1H, *J* = 9.6 Hz, -NH), 5.59 (dd, 1H, *J* = 10.7, 9.5 Hz, H-3), 5.44 (s, 1H, H-1′), 5.39 (ddd, 1H, *J* = 7.3, 5.2, 3.6 Hz, H-5′), 5.36 (d, 1H, *J* = 3.5 Hz, H-4″), 5.27 (dd, 1H, *J* = 5.2, 1.7 Hz, H-3′), 5.16 (dd, 1H, *J* = 10.5, 8.0 Hz, H-2″), 4.98 (d, 1H, *J* = 3.5 Hz, H-1), 4.97 (dd, 1H, *J* = 10.5, 3.5 Hz, H-3″), 4.76 (d, 1H, *J* = 8.0 Hz, H-1″); 4.76, 4.52 (2d, 2H, *J* = 12.0 Hz, CH_2Ph), 4.47 (ddd, 1H, *J* = 10.7, 9.6, 3.5 Hz, H-2), 4.32 (t, 1H, *J* = 5.2 Hz, H-4′), 4.26 (d, 1H, *J* = 1.7 Hz, H-2′), 4.26–4.10 (m, 3H, H-6a′, H-6b′, H-6a″), 4.14 (t, 1H, *J* = 9.5 Hz, H-4), 4.01 (dd, 1H, *J* = 11.2, 7.5 Hz, H-6b″), 3.92–3.86 (m, 4H, H-5, H-5″, H-6a, H-6b), 2.16, 1.98, 1.96, 1.93 (4s, 12H, CH_3CO), 1.78 (s, 3H, CH_3CONH); ^{13}C NMR ($CDCl_3$, 125.8 MHz): δ 170.5, 170.2 × 2, 170.0 (CH_3CO), 167.0 (CH_3CONH), 165.8, 165.4, 165.3 (PhCO), 136.7–128.1 (C-arom.), 106.9 (C-1′), 100.7 (C-1″), 96.7 (C-1), 87.0 (C-2′), 80.4 (C-4′), 77.5 (C-3′), 73.9 (C-4), 72.1 (C-3), 71.3 (C-5 or C-5″), 70.8 (C-3″, C-5′ or C-5″), 70.3 (C-5′), 70.0 (CH_2Ph), 68.3 (C-2″), 66.8 (C-4″), 62.9 (C-6′), 61.0 (C-6), 60.8 (C-6″), 52.3 (C-2), 23.0 (CH_3CONH), 20.6, 20.5, 20.4 (CH_3CO).

Anal. Calcd for $C_{63}H_{65}NO_{24}\cdot H_2O$: C, 61.11; H, 5.45; N, 1.13. Found: C, 61.50; H, 5.16; N, 1.39.

3.18. Benzyl β-D-galactopyranosyl-(1→2)-β-D-galactofuranosyl-(1→4)-2-acetamido-2-deoxy-α-D-glucopyranoside (33)

To a solution of **32** (38 mg, 0.031 mmol) in anhyd CH_3OH (1 mL) cooled to 0 °C, was added a solution of 1.15 M $NaOCH_3$ in CH_3OH (0.72 mL) and the mixture was stirred at rt. After 3 h, TLC examination showed only one compound (R_f 0.5, 7:1:1 *n*-propanol–EtOH–H_2O) and the solution was processed as described for **22**. Evaporation of the solvent gave a white solid that after crystallization from CH_3OH yielded **33** (19 mg, 97%): mp 233–235 °C; $[\alpha]_D$ +31 (*c* 0.4, H_2O); ^1H NMR (D_2O, 500 MHz): δ 7.46–7.38 (m, 5H, arom.), 5.30 (br s, 1H, H-1′), 4.94 (d, 1H, *J* = 3.5 Hz, H-1); 4.73; 4.55 (2d, 2H, *J* = 11.9 Hz, CH_2Ph), 4.57 (d, 1H, *J* = 7.8 Hz, H-1″), 4.29 (d, 1H, *J* = 3.0 Hz, H-2′), 4.26 (dd, 1H, *J* = 6.4, 3.0 Hz, H-3′), 4.12 (dd, 1H, *J* = 6.4, 3.7 Hz, H-4′), 3.94 (dd, 1H, *J* = 3.4, 1.0 Hz, H-4″), 3.89 (dd, 1H, *J* = 10.7, 3.5 Hz, H-2), 3.84 (dd, 1H, *J* = 10.7, 8.5 Hz, H-3), 3.85–3.80 (m, 4H, H-5′, H-5, H-6a or H-6a″, H-6b or H-6b″), 3.79 (dd, 1H, *J* = 11.6, 7.4 Hz, H-6a or H-6a″), 3.76 (dd, 1H, *J* = 11.6, 4.8 Hz, H-6b or H-6b″), 3.71 (ddd, 1H, *J* = 7.4, 4.8, 1.0 Hz, H-5″), 3.69 (dd, 1H, *J* = 9.6, 8.5 Hz, H-4), 3.68 (dd, 1H, *J* = 11.8, 4.3 Hz, H-6a′), 3.66 (dd, 1H, *J* = 10.0, 3.4 Hz, H-3″), 3.63 (dd, 1H, *J* = 11.8, 7.4 Hz, H-6b′), 3.53 (dd, 1H, *J* = 10.0, 7.8 Hz, H-2″), 1.96 (s, 3H, CH_3CONH); ^{13}C NMR (D_2O, 125.8 MHz): δ 174.9 (CH_3CONH), 137.6, 129.4, 129.2, 129.0 (C-arom.); 107.2 (C-1′), 103.2 (C-1″), 96.5 (C-1), 89.2 (C-2′), 83.6 (C-4′), 77.1 (C-4), 76.3 (C-3′), 75.9 (C-5″), 73.1 (C-3″), 71.7 (C-5 or C-5′), 71.4 (C-2″), 71.1 (C-5 or C-5′), 70.5 (CH_2Ph), 70.0 (C-3), 69.2 (C-4″), 63.6 (C-6′); 61.6, 60.6 (C-6, C-6″), 54.4 (C-2), 22.4 (CH_3CONH). Anal. Calcd for $C_{27}H_{41}NO_{16}\cdot 2H_2O$: C, 48.28; H, 6.75; N, 2.09. Found: C, 48.10; H, 6.52; N, 2.58.

3.19. β-D-Galactopyranosyl-(1→2)-β-D-galactofuranosyl-(1→4)-2-acetamido-2-deoxy-α,β-D-glucopyranose (6)

Compound **33** (12.8 mg, 0.020 mmol) dissolved in 8:1 CH_3OH–H_2O (1.2 mL) was hydrogenated and purified as described for **5** to yield **6** (10 mg, 92%) as an amorphous solid: R_f 0.25–0.11 (7:1:1 *n*-propanol–EtOH–H_2O); $[\alpha]_D$ −22.5 (*c* 0.8, H_2O); ^1H NMR (D_2O, 500 MHz) δ anomeric zone and other diagnostic signals: 5.31 (m, 1H, H-1′α anomer and H-1′ β anomer), 5.19 (d, 0.6H, *J* = 3.4 Hz, H-1 α anomer), 4.70 (d, 0.4H, *J* = 8.3 Hz, H-1 β anomer), 4.57 (d, 0.6H, *J* = 7.8 Hz, H-1″ α anomer), 4.56 (d, 0.4H, *J* = 7.8 Hz, H-1″ β anomer), 4.31 (d, 0.6H, *J* = 3 Hz, H-2′ α anomer), 4.29 (d, 0.4H, *J* = 2.8 Hz, H-2′ β anomer), 2.04 (2s, 3H, CH_3CONH α and β anomers); ^{13}C NMR (D_2O, 125.8 MHz): δ 175.4, 175.2 (CH_3CONH α and β anomers), 107.3, 107.2 (C-1′ α and β anomers), 103.2 (C-1″ α and β anomers), 95.6 (C-1 β anomer), 91.3 (C-1 α anomer),

89.1 × 2 (C-2′ α and β anomers); 83.8, 83.7 (C-4′ α and β anomers); 77.2, 76.9, 76.4, 75.9, 75.7, 73.1, 73.0, 71.4, 71.2 × 2, 69.9, 69.2, 63.6, 61.6, 60.9, 60.8; 57.4 (C-2 β anomer), 54.8 (C-2 α anomer); 22.8, 22.5 (CH_3CONH anomers α and β). HRMS (ESI/APCI) m/z calcd for $C_{20}H_{35}NNaO_{16}$ ($M+Na^+$) 568.1854, found 568.1840.

3.20. Preparation of β-D-galactopyranosyl-(1→2)-β-D-galactofuranosyl-(1→4)-2-acetamido-2-deoxy-D-glucitol[6] (34)

Compound **6** (8.0 mg; 0.015 mmol) was reduced with $NaBH_4$ (14 mg, 0.37 mmol) in CH_3OH (0.5 mL) and then processed and purified as described for **23** to yield 7.5 mg of **34** as a very hygroscopic solid (93%): R_f 0.27 (7:1:1.5 *n*-propanol–EtOH–H_2O), $[\alpha]_D$ −46 (*c* 0.5, H_2O); ^1H NMR (D_2O, 500 MHz) δ anomeric zone and diagnostic signals: 5.36 (br s, 1H, H-1′), 4.59 (d, 1H, *J* = 7.9 Hz, H-1″), 4.37 (d, 1H, *J* = 2.9 Hz, H-2′), 4.26 (dd, 1H, *J* = 6.2, 2.9 Hz, H-3′), 4.14 (dd, 1H, *J* = 6.2, 3.8 Hz, H-4′), 4.13 (m, 1H, H-2), 3.53 (dd, 1H, *J* = 10.0, 7.9 Hz, H-2″), 2.05 (s, 3H, C*H*$_3$CONH). δ values matched those described for the natural compound;[6] ^{13}C NMR (D_2O, 125.8 MHz): δ 175.1 (CH_3CONH), 107.8 (C-1′), 102.9 (C-1″), 88.9 (C-2′), 84.2 (C-4′), 78.5 (C-4), 76.5 (C-3′), 76.0 (C-5″), 73.2 (C-3″), 71.7 (C-4″), 71.4, 71.3 (C-5′, C-2″); 69.2, 69.0 (C-3, C-5), 63.6 (C-6′), 62.7 (C-6″); 61.6, 61.4 (C-1, C-6), 53.3 (C-2), 22.8 (*C*H$_3$CONH). HRMS (ESI/APCI) m/z calcd for $C_{20}H_{37}NNaO_{16}$ ($M+Na^+$) 570.2010, found 570.1997.

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Supplementary data

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