

Synthesis of the *O*-linked hexasaccharide containing β -D-Galp-(1 $\rightarrow$ 2)- β -D-Galf in *Trypanosoma cruzi* mucins†

Gustavo A. Kashiwagi, Verónica M. Mendoza, Rosa M. de Lederkremer and Carola Gallo-Rodriguez*

Received 17th April 2012, Accepted 11th June 2012

DOI: 10.1039/c2ob25741f

The hexasaccharide β -D-Galp-(1 $\rightarrow$ 2)-[β -D-Galp-(1 $\rightarrow$ 3)]- β -D-Galp-(1 $\rightarrow$ 6)-[β -D-Galf(1 $\rightarrow$ 2)- β -D-Galf(1 $\rightarrow$ 4)]-D-GlcNAc (**1**) is the largest carbohydrate structure released as alditol by reductive β -elimination from mucins of some strains of *T. cruzi*. The terminal β -D-Galp units are sites of sialylation by *trans*-sialidase which transfers sialic acid from the host to the parasite. Hexasaccharide **1** was synthesized by a [3 + 3]-convergent strategy based on a nitrile assisted glycosylation, using the trichloroacetimidate method. The β -D-Galf-(1 $\rightarrow$ 2)- β -D-Galf-D-GlcNAc synthon was sequentially constructed from the reducing end to the non-reducing end employing benzyl α -D-galactofuranoside as starting material for the internal Galf unit. The choice of this novel precursor, obtained in one-reaction step from galactose, allowed the introduction of an orthogonal and participating levulinoyl group at O-2. Thus, the diastereoselective construction of the Galf- β (1 $\rightarrow$ 4)-GlcNAc linkage by the trichloroacetimidate method of glycosylation was achieved. The ^1H NMR spectrum of alditol **2** was identical to the product released by β -elimination from the parasite mucin.

Introduction

The protozoan *Trypanosoma cruzi* is the etiologic agent of Chagas disease, the American trypanosomiasis which is transmitted to animals, including humans, by insect vectors of the family Reduviidae.^{1–3} This neglected disease is a major public health issue in Latin America, and is also becoming an important health issue in the United States and Europe due to immigration and infection by blood transfusion or organ transplants.⁴ A dense glycocalyx covers the surface of the parasite and its composition is characteristic of each differentiation stage of the complex living cycle.^{5,6} The interplay between the enzymatically active *trans*-sialidase (TcTS) and the mucins in the surface of *T. cruzi* is crucial for pathogenesis.^{7–10} The *O*-linked oligosaccharides in the mucins account for up to 60% of the molecular mass.⁶ They play the function of being acceptors of sialic acid, which is not biosynthesized by the parasite but is needed to build a negatively charged coat. Stage variations and inter-strain differences in the epimastigote stage have been observed in the carbohydrates of mucins.⁵ The structures of the *O*-linked oligosaccharides have been elucidated for the mucins of the insect

stages of the parasite.⁵ The striking feature of the *O*-linked chains is that they are linked to the protein by α -GlcNAc instead of GalNAc common in vertebrate mucins. By comparing structural data on the mucins from different strains it followed that the *O*-linked oligosaccharides may be derived from two cores, β -Galp-(1 $\rightarrow$ 4)-GlcNAc or β -Galf-(1 $\rightarrow$ 4)-GlcNAc. The cores are further branched with various units of Galf and/or Galp. A puzzling fact is that, depending on the strain, galactose may be found in the furanose configuration together with the more common galactopyranose. Galactofuranose was first described in mucins of the G strain, classified as *T. cruzi* I, with a sylvatic origin.^{11,12} It was later found in other strains belonging to the same group,¹³ more recently from drug-resistant Colombiana strain, isolated from a chronic human case in Colombia.¹⁴ In the strain Y of group II, galactose is only present in the pyranose form,¹⁵ as well as in the mucins of the CL14 and CL Brener strains,^{16–18} previously included in group II and now reclassified as DTU VI.¹⁹ It is interesting that the Tulahuén strain, now included in the new hybrid type VI expresses mucins with substitution of GlcNAc by either Galp or Galf.²⁰ The biosynthetic steps for the incorporation of Galf in some mucins deserves further investigation since Galf is an antigenic epitope,²¹ which is not present in the mammalian host. In our laboratory we have undertaken the chemical synthesis of the mucin oligosaccharides, in particular of those containing Galf (Fig. 1),^{22–26} which should be useful tools for biosynthetic studies. *Trans*-sialidase studies have been performed on the substrates **3**, **4** and **6**.^{25,27} A tetrasaccharide from mucin of the Y strain which only contains galactose in the pyranose form, has been also synthesized.²⁸ In

CIHIDECAR, Departamento de Química Orgánica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Ciudad Universitaria, Pabellón II, 1428 Buenos Aires, Argentina.
E-mail: cgallo@qo.fcen.uba.ar; Fax: +54-11-4576-3352;
Tel: +54-11-4576-3346

† Electronic supplementary information: Experimental for **8** and **24**. ^1H and ^{13}C NMR spectra for **1**, **2**, **10**, **11**, **13–26** and bidimensional spectra for **1** and **26**. See DOI: 10.1039/c1ce05336a

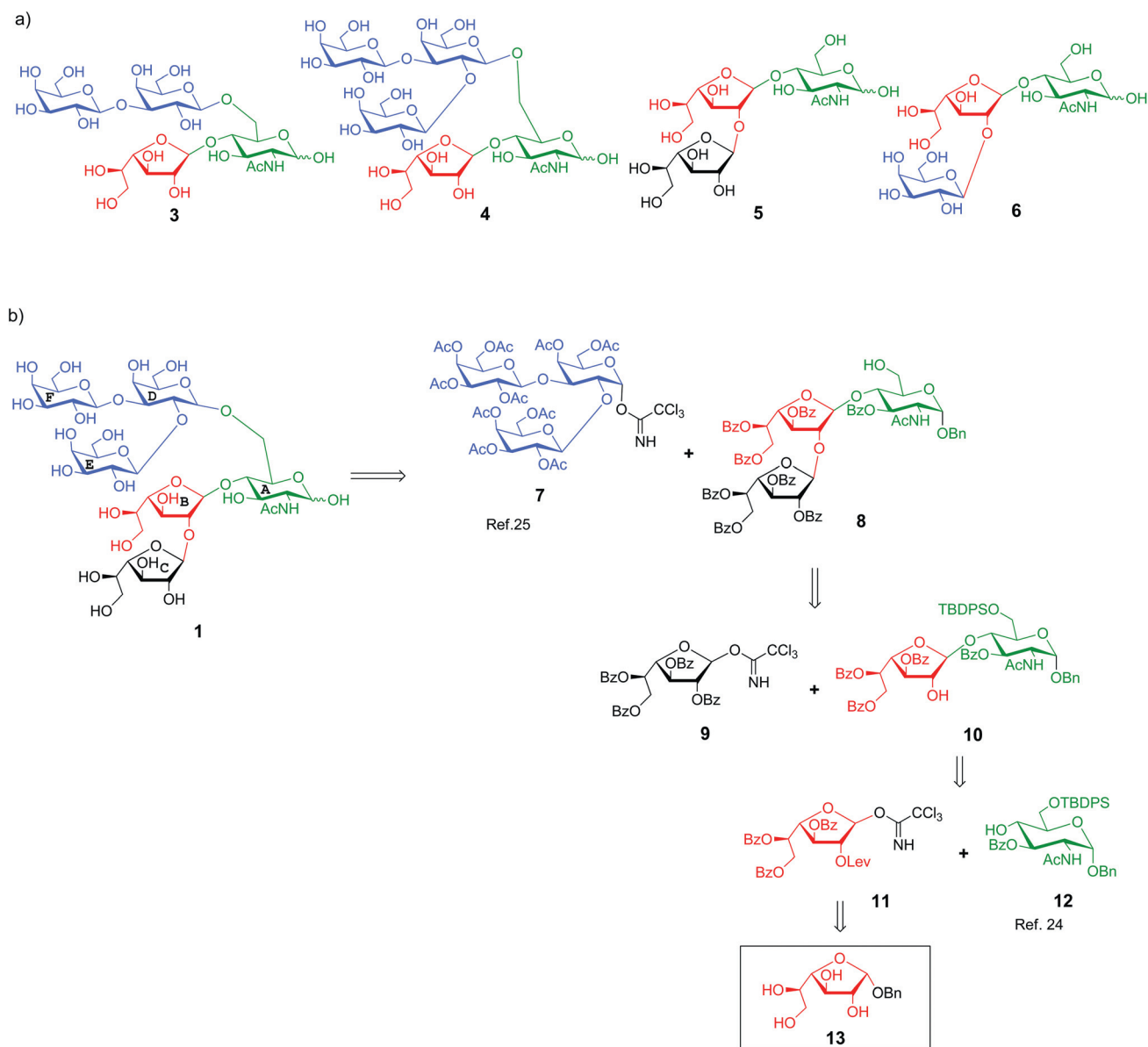


Fig. 1 (a) Representative oligosaccharides present in mucins of *T. cruzi* that have already been synthesized; (b) retrosynthesis of the target hexasaccharide 1.

this work we describe the first synthesis of the ramified hexasaccharide β -D-Galp-(1 $\rightarrow$ 2)-[β -D-Galp-(1 $\rightarrow$ 3)]- β -D-Galp-(1 $\rightarrow$ 6)-[β -D-Galp-(1 $\rightarrow$ 2)- β -D-Galp-(1 $\rightarrow$ 4)]-D-GlcNAc (**1**, Fig. 1) and the corresponding alditol **2**. The NMR spectra of **2** could be compared with the data for the alditol obtained by reductive β -elimination from the mucins of the Tulahuen strain. Hexasaccharide **1** and the related hexasaccharide β -D-Galp-(1 $\rightarrow$ 2)-[β -D-Galp-(1 $\rightarrow$ 3)]- β -D-Galp-(1 $\rightarrow$ 6)-[β -D-Galp-(1 $\rightarrow$ 2)- β -D-Galp-(1 $\rightarrow$ 4)]-D-GlcNAc constitute the largest *O*-linked oligosaccharides found in mucins of *T. cruzi*.

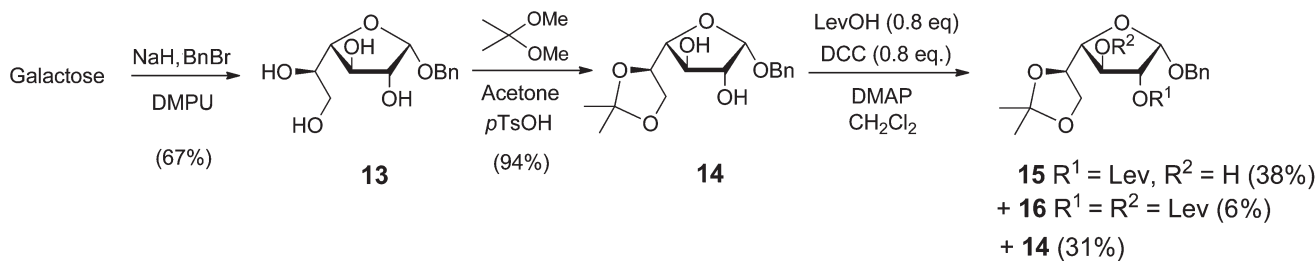
Results and discussion

Synthetic strategies

Based on our experience in the synthesis of several oligosaccharides present in mucins of *T. cruzi*, some aspects were initially

considered. Tetrasaccharide **3** and pentasaccharide **4** (Fig. 1) share a ramified GlcNAc at the reducing end substituted at the 4-O and 6-O positions. Due to the recognized low nucleophilicity of OH-4 in GlcNAc,²⁹ the Galp moiety was first introduced followed by glycosylation at O-6.^{24,25} Pentasaccharide **4** shares a 2,3-di-*O*-(β -D-Galp)- β -D-Galp unit with the target hexasaccharide **1** (Fig. 1). In the synthesis of **4**,²⁵ selective 1,2-*trans* β -glycosylation on O-6 of the GlcNAc derivative was achieved by the use of acetonitrile as solvent, taking advantage of the nitrile effect,^{30–34} and overcoming the lack of neighboring group participation in the Galp trisaccharide donor.

For that reason, retrosynthetic analysis for hexasaccharide **1** indicated a [3 + 3] *nitrilium*-based glycosylation between already synthesized Galp-containing trisaccharide imidate **7**²⁵ and the trisaccharide acceptor **8** (Fig. 1) which contains an internal Galp.



Scheme 1

Several methods have been employed for β -galactofuranosylation which led to the development of new Galf precursors.^{35–41} As Galf is thermodynamically less stable compared to galactopyranose, the synthesis of internal galactofuranosyl-containing oligosaccharides requires the choice of a convenient galactofuranosyl precursor in accordance with a proper glycosylation method. Examples of the synthesis of internal Galf-containing oligosaccharides have been reported and each synthesis has its advantages and drawbacks.^{26,35–37,42–49} The thioglycoside method was employed for the first time in the challenging synthesis of internal Galf-containing oligosaccharides from *Mycobacterium tuberculosis* as well as in the synthesis of the repeating unit of varianose.⁴⁴ More recently, a biotinylated tetra β (1–5)galactofuranoside was also synthesized by this method.⁴⁵ The precursor thiogalactofuranoside allowed the oligosaccharide construction from the reducing end to the non-reducing end; however, it required several reaction steps from galactose.

Very recently, regioselective one pot syntheses of trisaccharides with internal Galf were developed using partially protected thiogalactofuranosides.⁴⁶ A drawback is that the required stereoselective protection of Galf thioglycosides seems rather difficult. For instance, selective benzoylation at low temperature of *p*-tolyl 5,6-isopropylidene-1-thio- β -D-galactofuranoside afforded the 2-*O*- and 3-*O*-benzoyl derivatives in a 1.7 : 1 ratio.⁴⁶

Also, the carbobenzoxy method has been successfully employed in the challenging synthesis of agelagalastatin, which contains an internal β -D-Galf.⁴⁷ This method was also employed for the synthesis of cyclic galactooligosaccharides as well as in the synthesis of a tetrasaccharide constituent of the mycobacterial cell wall. However, this method failed in constructions of some internal-Galf linkages and was overcome by a fluoride replacement of the carbobenzoxy group in a later step.⁴⁸ On the other hand, the precursor carboxybenzyl galactofuranoside donor can be synthesized *via* the galactofuranosyl chloride in four reaction steps from pentaacetyl galactofuranose, involving the use of mercuric salts.⁴⁷

The trichloroacetimidate method³⁴ of glycosylation has been extensively applied for the synthesis of internal Galf-containing oligosaccharides,^{26,35–37,42,49} being pivotal in the aldolactone approach,⁴⁹ where D-galactono-1,4-lactone, which can be selectively substituted by protecting groups, acts as precursor of the Galf. By this methodology, oligosaccharides of *Leishmania*, and *Mycobacterium tuberculosis* were synthesized in high yields. Trisaccharide **8** (Fig. 1) was recently synthesized by the aldolactone approach as an intermediate in the synthesis of trisaccharide **5**²⁶ (Fig. 1). In this case, a derivative of D-galactono-1,4-lactone was employed for the introduction of the internal Galf, and the

trisaccharide was constructed from the non-reducing end to the reducing end. By this approach, only moderate yields were achieved in the glycosylation of the GlcNAc unit due to the absence of a participating group at O-2 of the β -D-Galf-(1→2)-Galf donor. In view of the low anomeric effect in furanoses,^{35,36} we had considered that the steric effect of the substituent in O-2 would prevail in the control of glycosylation. However, it was not as important as expected, and moderate diastereoselectivities were obtained, depending on the nature of the glycosylation solvent.²⁶

For that reason, in the present study, a sequential strategy from the reducing end to the non-reducing end, was designed for the synthesis of trisaccharide **8**. The terminal β -D-Galf of **8** would be installed in O-2 by glycosylation of partially protected β -Galf (1→4)GlcNAc **10** with known tetra-*O*-benzoyl- β -D-galactofuranosyl trichloroacetimidate (**9**, Fig. 1).⁵⁰ The internal Galf precursor **11** should possess a participating group at O-2 to ensure glycosylation diastereoselectivity and should be orthogonal to the benzoyl and silyl groups of the known GlcNAc acceptor **12**²⁴ successfully employed in the synthesis of the oligosaccharides of Fig. 1.^{24–26} A levulinoyl group was chosen in this case, and donor **11** could be prepared from benzyl α -D-galactofuranoside (**13**). The anomeric benzyl group, orthogonal to the benzoyl and the levulinoyl protecting-groups, could be easily removed by hydrogenolysis for further activation as trichloroacetimidate.

Synthesis of **11**, the precursor of the internal Galf

Based in our experience in the one-step anomeric *O*-alkylation of galactose,^{51,52} starting material benzyl α -D-galactofuranoside (**13**) was obtained crystalline in 67% yield by treatment of galactose with 1.2 equiv. of benzyl bromide and NaH, in DMPU as solvent (Scheme 1). The 2,3,5,6-tetra-*O*-acetyl derivative has been previously synthesized also using *O*-anomeric alkylation.⁵³ In the ¹H NMR spectrum of **13**, the anomeric hydrogen appeared at δ 5.09 as a doublet with $J_{1,2} = 4.6$ Hz, indicating the H-1–H-2 *cis* disposition of α -D-Galf. Also, the H-2 appeared as a doublet of doublets with $J = 8.0$ and 4.6 Hz. The ¹³C NMR spectrum showed the resonance of the C-1 at δ 100.9 confirming the α -configuration, and the downfield shifted signal of C-4 at δ 82.0 which is characteristic of the furanose form. In order to selectively introduce a levulinoyl group in O-2, the 5- and 6-*O* positions of **13** were temporarily protected. Thus reaction of **13** with dimethoxypropane in acetone and catalytic amounts of *p*-toluenesulfonic acid gave the 5,6-*O*-isopropylidene derivative **14** which was obtained crystalline from the crude mixture in

94% yield. Selective 2-*O*-benzoyl and 2-*O*-pivaloyl protection has been previously described for allyl^{52b} and pentenyl α -D-galactofuranoside⁵⁴ derivatives, respectively, probably as a consequence of the increment of the 2-*O*-nucleophilicity by the intramolecular hydrogen bond with the α -anomeric oxygen.⁵⁴ For that reason, and in order to introduce a levulinoyl group at that position, different conditions of levulinoylation were assayed. The best results were obtained by reaction of **14** with 0.8 equiv of levulinic acid, dicyclohexylcarbodiimide (0.8 equiv) and DMAP at -18°C in order to reduce the 2,3-di-*O*-levulinoyl byproduct. In this case, the reaction afforded the 2-*O*-Lev derivative **15** in 38% yield, the 2,3-di-*O*-Lev product **16** in 6% yield and the starting material **14** was recovered in 31% yield as described in the ESI† (Scheme 1). In the ^1H NMR spectrum of **15**, the H-2 signal appeared 0.82 ppm shifted downfield compared to the same signal of its precursor **14**, confirming the 2-*O*-acyl substitution.

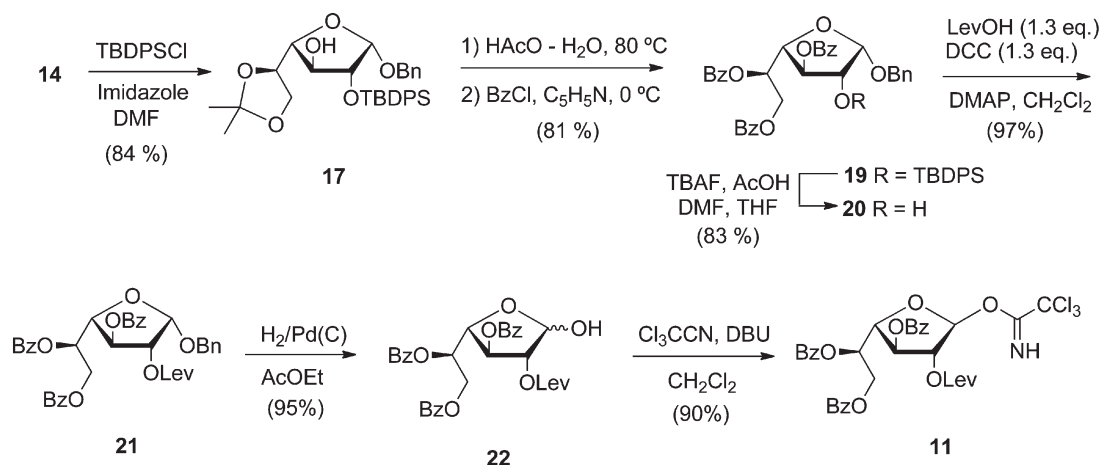
However, the moderate yield obtained for the 2-*O*-levulinoyl derivative **15** which included a tedious purification, prompted us to introduce this protecting group later. For that purpose, a bulky TBDPS group was chosen due to its resistance to acid hydrolysis. Thus, treatment of **14** with 1.25 equiv of *tert*-butyldiphenylchlorosilane and imidazole gave the silyl derivative **17** selectively (84%, Scheme 2). Interestingly, in the ^1H NMR spectrum, the signal of H-1 (δ 4.49) appeared shifted 0.5 ppm upfield compared to the same signal of **14**. Hydrolysis of the isopropylidene group of **17** with acetic acid–water at 80°C gave benzyl 2-*O*-*tert*-butyldiphenylsilyl- α -D-galactofuranoside (**18**). An analytical sample of **18** was purified for characterization. The crude product was treated with benzoyl chloride in pyridine to afford **19** (81% from **17**). The ^1H NMR spectrum of **19** showed the H-5 and H-3 signals shifted downfield at 5.71 and 6.07 ppm due to benzylation, compared to the same signals in **17** and **18**, confirming also the 2-OH substitution by TBDPS in **17** and **18**. The TBDPS group resisted the hydrolysis as demonstrated by the absence of benzyl tetra-*O*-benzoyl- α -D-galactofuranoside as byproduct.

In order to introduce the levulinoyl group, removal of the silyl group was performed by treatment of **19** with acetic acid–buffered tetrabutylammonium fluoride solution in THF to give the 2-OH free derivative **20** in 83% yield. Interestingly, in the ^1H

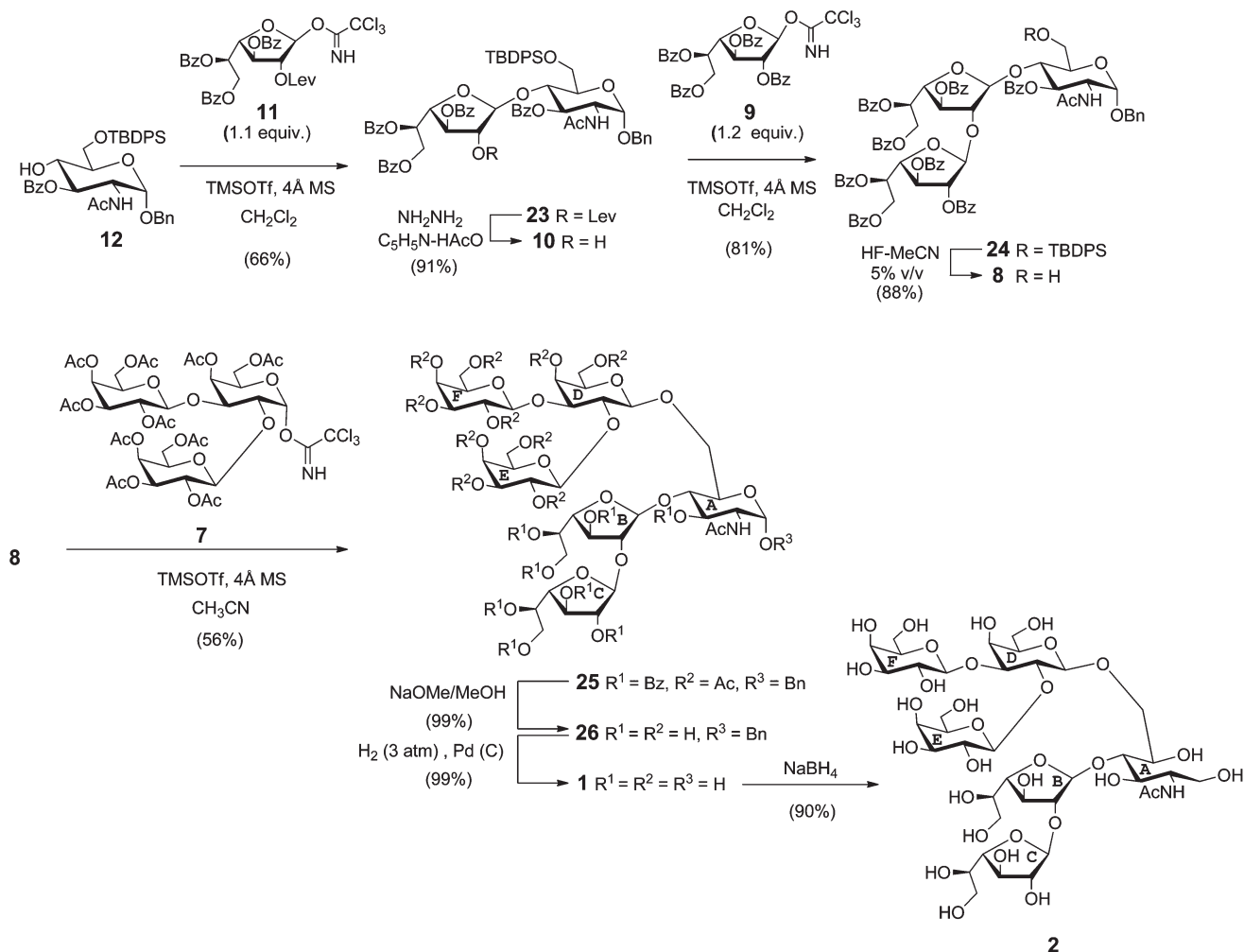
NMR spectrum of **20**, the signal for the anomeric hydrogen appeared 0.71 ppm downfield shifted compared to the same signal in **19**, whereas the signal for the H-2 remained almost at the same δ . The reaction of **20** with levulinic acid, DMAP and dicyclohexylcarbodiimide gave the 2-*O*-Lev derivative **21** (97%). Substitution at O-2 was confirmed by the signal for H-2 (δ 5.29) shifted 0.80 ppm downfield in comparison with the same signal in **20**, in the ^1H NMR spectrum. Hydrogenolysis of the anomeric benzyl group of **21** with $\text{H}_2/10\% \text{ Pd}(\text{C})$ afforded **22** in 95% yield as a 3 : 7 α : β anomeric mixture established by integration of the anomeric protons at δ 5.67 ($J_{1,2}$ 4 Hz, α anomer) and 5.56 (bs, β anomer) in the ^1H NMR spectrum. The anomeric center was further activated by treatment with trichloroacetonitrile and DBU to yield the precursor of the internal Galf, β -trichloroacetimidate **11**, in 90% yield.

Synthesis of β -D-Galf-(1 $\rightarrow$ 2)- β -D-Galf-(1 $\rightarrow$ 4)-D-GlcNAc fragment 8

With trichloroacetimidate **11** in hand, the construction of the β -Galf(1 $\rightarrow$ 4)GlcNAc linkage was attempted (Scheme 3). Reaction of benzyl 2-acetamido-3-*O*-benzoyl-6-*O*-*t*-butyldiphenylsilyl-2-deoxy- α -D-glucopyranoside **12**²⁴ with donor **11** in the presence of TMSOTf as catalyst gave, diastereoselectively, disaccharide **23** which crystallized from the crude reaction mixture in 66% yield. The signal at δ 105.5 for C-1' in the ^{13}C NMR spectrum indicated a β -configuration for the new anomeric center, which was in agreement with the H-1' and H-2' resonances that appeared as singlets at 5.60 and 5.03 ppm in the ^1H NMR spectrum. Delevulinoylation of **23** with hydrazine in pyridine–acetic acid⁵⁵ gave acceptor **10** in 91% yield, ready for glycosylation. Construction of the β -Galf(1 $\rightarrow$ 2)Galf linkage was achieved diastereoselectively by glycosylation of **10** with trichloroacetimidate **9**⁵⁰ and TMSOTf as catalyst to yield known trisaccharide **24** in 81% yield with the same physical properties compared to that obtained by the aldonolactone approach.²⁶ Compound **24** was synthesized in a total yield of 21% from benzyl α -D-galactofuranoside (**13**) in 11 steps diastereoselectively, while the shorter aldonolactone approach took 9 steps in 10% total yield from D-galactono-1,4-lactone requiring tedious purification of mixtures of anomers.



Scheme 2 Synthesis of internal galactofuranosyl building block **11**.

Scheme 3 Synthesis of hexasaccharide **1** and its alditol **2**.

Synthesis of hexasaccharide **1** and alditol **2**

Desilylation of **24** with 5% HF (48 wt% in H₂O) in acetonitrile as already described²⁶ gave acceptor **8** with the 6-OH free in 88% yield (as shown in the ESI[†]), ready for glycosylation. As mentioned above, pentasaccharide **4** was synthesized by reaction with *O*-[2,3-di-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-4,6-di-*O*-acetyl-α-D-galactopyranosyl] trichloroacetimidate²⁵ (**7**) taking advantage of the nitrile effect. In that case, only the desired β-isomer was obtained in acetonitrile despite the absence of anchimeric participation, whereas a mixture of β : α anomers in 1 : 2.5 ratio was obtained when the glycosylation was carried in CH₂Cl₂ as solvent.²⁵ Reaction of acceptor **8** with ramified Galp trichloroacetimidate **7** in acetonitrile as solvent in the presence of catalytic amounts of TMSOTf (0.16 equiv) gave hexasaccharide **25** diastereoselectively in 56% yield. No α-glycosylation product was detected and unreacted acceptor **8** was also recovered, raising the yield of **25** up to 66%. On the basis of the HSQC and COSY experiments, the ¹H and ¹³C NMR spectra assignments for **25** were performed. In the ¹³C NMR spectrum, the signal for the new β-D-Galp(1→6) linkage appeared at 101.8 ppm, more deshielded than for the anomeric

centers of the two terminal β-D-Galp (δ 99.3 and 99.1), showing similar δ values for these centers when compared to pentasaccharide **4**.²⁵ The signals for the other three anomeric centers of **25** appeared at 106.6 (internal Galp^B), 105.9 (terminal Galp^C) and 95.9 (GlcNAc), as expected. The anomeric β-configuration of the new glycosidic linkage was demonstrated by the coupling constant of *J*_{1,2} 7.5 Hz for the Galp(1→6) H-1^D signal at δ 4.70 in the ¹H NMR spectrum. On the other hand, H-2^D and H-3^D resonances for the internal Galp unit appeared shielded due to glycosylation, compared to the same signals of the benzoylated terminal Galp. The next step was the deacetylation of **25** with sodium methoxide to give benzyl glycoside **26** in 99% yield. Studies on **26** as substrate in the *trans*-sialidase reaction are currently being performed to establish if the reaction is selective for one of the two terminal Galp as in the case of the pentasaccharide **4**. For that reason full NMR assignments for compound **26** were necessary. ¹H and ¹³C NMR assignment were performed based on HSQC, COSY and ROESY experiments. Due to signal overlapping, the δ values of H-2^D and H-3^D of the internal Galp in the ¹H NMR spectrum were determined from the HSQC spectrum shown in the ESI[†]. Each terminal β-D-Galp was identified based on a ROESY experiment. The signal of Galp(β1→2) H-1^E

at δ 4.82 correlated with the Galp(β 1 $\rightarrow$ 6) H-2^D at δ 3.95. Moreover, the Galp(β 1 $\rightarrow$ 3) H-1^F at δ 4.66 correlated with Galp(β 1 $\rightarrow$ 6) H-3^D at δ 3.98.

The hexasaccharide alditol **2** was also prepared with the aim of comparing it with the alditol isolated from the natural source.²⁰ Hydrogenolysis of the benzyl glycoside of **26** was performed by treatment with H₂ and Pd(C). The free hexasaccharide **1** (99%) was obtained as a mixture of anomers in α : β ratio of 3:2, estimated by the integration of terminal Galf H-1^C of the α -anomer at δ 5.24 and GlcNAc H-1^A of the β -anomer at δ 4.70, in the ¹H NMR spectrum. Further reduction of **1** with NaBH₄ gave alditol **2** (90%), with the same ¹H NMR spectrum as previously depicted.²⁰

Conclusion

The novel ramified hexasaccharide β -D-Galp-(1 $\rightarrow$ 2)-[β -D-Galp-(1 $\rightarrow$ 3)]- β -D-Galp-(1 $\rightarrow$ 6)-[β -D-Galf-(1 $\rightarrow$ 2)- β -D-Galf-(1 $\rightarrow$ 4)]-D-GlcNAc (**1**) was synthesized and its alditol confirmed the structure of the natural oligosaccharide released from mucins of the Tulahuen strain. The nitrilium effect mediated glycosylation allowed the introduction of a ramified Galp trisaccharide donor that lacks anchimeric assistance demonstrating the convenient choice of the convergent [3 + 3] strategy. Benzyl α -D-galactofuranoside, synthesized in one step from galactose, proved to be a suitable precursor for the construction of internal Galf-containing oligosaccharides. This precursor together with the robust trichloroacetimidate method of glycosylation allowed the synthesis of the benzyl hexasaccharide **26**, useful also for studies on the unknown Galf transferases of *T. cruzi*.

Experimental

General

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) H₂SO₄ 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 (¹H) and 125.8 MHz (¹³C) or with a Bruker AC 200 at 200 MHz (¹H) and 50.3 MHz (¹³C). Chemical shifts are given relative to the signal of internal acetone standard at 2.22 and 30.89 ppm for ¹H NMR and ¹³C NMR spectra, respectively when recorded in D₂O. ¹H and ¹³C assignments were supported by 2D COSY, and HSQC experiments. ROESY experiment was performed when indicated. High resolution mass spectra (HRMS) were recorded on a BRUKER micrOTOF-Q II spectrometer. Melting points were determined with a Fisher–Johns apparatus and are uncorrected. Optical rotations were measured with a Perkin-Elmer 343 polarimeter with a path length of 1 dm at 25 °C.

Benzyl α -D-galactofuranoside (13). Dried ground D-galactose (5.5 g, 30.5 mmol) was partially dissolved in DMPU (50 mL) under argon atmosphere and was slowly cannula-added to 60% oil suspension of sodium hydride (1.82 g, 47.7 mmol) previously washed with hexane (2 $\times$ 40 mL). The remaining D-galactose with additional DMPU (12 mL) was added to the

reaction flask. After cooling to 0 °C, benzyl bromide (4.25 mL, 36.2 mmol) was added and the suspension was stirred at 25 °C for 16 h until TLC showed no D-galactose left. The resulting solution was cooled to 0 °C and CH₃OH (0.5 mL) was slowly added. The reaction mixture was extracted with hexane (12 $\times$ 130 mL) and the remaining syrup was dissolved in distilled water (70 mL) and extracted with EtOAc until TLC analysis showed no more product in the aqueous phase. The organic layers were combined, dried (Na₂SO₄) and concentrated under reduced pressure. The residue was purified by column chromatography (25:1 CH₂Cl₂–MeOH) to give a syrup that crystallized from EtOAc (20 mL) to yield 5.53 g of crystalline **13** (67%): *R*_f 0.45 (6:1 CH₂Cl₂–MeOH), mp 124–125 °C (needles), [α]_D +94.9° (c 1, H₂O). ¹H NMR (D₂O, 500 MHz): δ 7.45–7.38 (m, 5H, aromatic), 5.09 (d, 1H, *J* = 4.6 Hz, H-1); 4.82, 4.63 (2d, 2H, *J* = 12.0 Hz, CH₂Ph), 4.17 (t, 1H, *J* = 7.0 Hz, H-3), 4.12 (dd, 1H, *J* = 8.0, 4.6 Hz, H-2), 3.80 (dd, 1H, *J* = 7.0, 6.0 Hz, H-4), 3.73 (ddd, 1H, *J* = 7.0, 6.5, 4.0 Hz, H-5), 3.68 (dd, 1H, *J* = 11.5, 4.0 Hz, H-6a), 3.56 (dd, 1H, *J* = 11.5, 6.5 Hz, H-6b). ¹³C NMR (D₂O, 125.8 MHz): δ 137.8, 129.4, 129.1, 128.9 (C-arom.), 100.9 (C-1), 82.0 (C-4), 77.0 (C-2), 75.1 (C-3), 73.5 (C-5), 70.6 (CH₂Ph), 62.8 (C-6).

Anal. calcd for C₁₃H₁₈O₆: C 57.77, H 6.71. Found: C 57.67, H 6.52.

Benzyl 5,6-O-isopropylidene- α -D-galactofuranoside (14). To a cooled solution (0 °C) of **13** (2.05 g, 7.58 mmol) in a mixture of 2,2-dimethoxypropane (3.4 mL, 27.2 mmol) and acetone (17 mL), *p*-toluenesulfonic acid (30 mg, 0.17 mmol) was added with stirring. After 45 min, 28% NH₄OH (0.2 mL) was added until pH = 10 and the mixture was concentrated under vacuum. Crystallization of the residue from hexane–EtOAc (13:10) gave 2.22 g of **14** (94%): *R*_f 0.30 (20:1 CH₂Cl₂–MeOH); mp 100–101 °C, [α]_D +65.7° (c 1, CHCl₃); ¹H NMR (CDCl₃, 500 MHz): δ 7.35–7.26 (m, 5H, arom.), 4.98 (d, 1H, *J* = 4.2 Hz, H-1), 4.85, 4.52 (2d, 2H, *J* = 11.6 Hz, CH₂Ph), 4.17 (apparent q, 1H, *J* = 6.7 Hz, H-5), 4.05–4.00 (m, 2H, H-2, H-3), 3.99 (dd, 1H, *J* = 8.6, 6.6 Hz, H-6a), 3.90 (dd, 1H, *J* = 7.0, 8.6 Hz, H-6b), 3.80 (t, 1H, *J* = 7.0 Hz, H-4), 3.68 (bs, 1H, –OH), 3.10 (d, 1H, *J* = 9.4 Hz, –OH), 1.43, 1.37 (2s, 6H, (CH₃)₂C). ¹³C NMR (CDCl₃, 125.8 MHz): δ 136.9–128.0 (C-arom.), 109.7 ((CH₃)₂C), 99.8 (C-1), 81.5 (C-4), 78.0 (C-2), 77.5 (C-5), 76.3 (C-3), 69.7 (CH₂Ph), 64.9 (C-6), 26.5, 25.3 ((CH₃)₂C).

Anal. calcd for C₁₆H₂₂O₆: C 61.92, H 7.15. Found: C 61.54, H 6.98.

Benzyl 2-O-*tert*-butyldiphenylsilyl-5,6-O-isopropylidene- α -D-galactofuranoside (17). To a cooled solution (0 °C) of **14** (1.9 g, 6.16 mmol) and imidazole (0.5 g, 7.4 mmol) in anhydrous DMF (15 mL), *t*-butyldiphenylsilylchloride (1.8 mL, 7.68 mmol, 1.25 equiv) was slowly added with stirring. After stirring for 20 h at 25 °C, the mixture was diluted with CH₂Cl₂ (250 mL), washed with H₂O (3 $\times$ 200 mL), dried (Na₂SO₄), filtered and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (6:1 hexane–EtOAc) to afford syrupy **17** (2.89 g, 84%): *R*_f 0.38 (4:1 hexane–EtOAc), [α]_D +60.0° (c 1, CHCl₃); ¹H NMR (CDCl₃, 200 MHz): δ 7.74–7.62 (m, 4H, arom.), 7.44–7.24 (m, 11H, arom.), 4.76, 4.33 (2d, 2H, *J* = 11.9 Hz, CH₂Ph), 4.49 (d, 1H, *J* = 4.2 Hz, H-1), 4.28–4.16

(m, 2H, H-5, H-3), 4.12 (dd, 1H, $J = 7.7, 4.2$ Hz, H-2), 3.95 (dd, 1H, $J = 8.5, 6.4$ Hz, H-6a), 3.84 (dd, 1H, $J = 8.5, 7.0$ Hz, H-6b), 3.70 (t, 1H, $J = 7.2$ Hz, H-4), 1.71 (d, 1H, $J = 4.2$ Hz, OH), 1.40, 1.35 (2s, 6H, $(\text{CH}_3)_2\text{C}$), 1.09 (s, 9H, $(\text{CH}_3)_3\text{CSi}$). ^{13}C NMR (CDCl_3 , 50.3 MHz): δ 137.7–127.5 (C-arom.), 109.6 ($(\text{CH}_3)_2\text{C}$), 100.4 (C-1), 81.4 (C-4), 79.0, 78.1, 76.2 (C-2, C-3, C-5), 69.0 (CH_2Ph), 64.9 (C-6), 26.8 ($(\text{CH}_3)_3\text{CSi}$), 26.6, 25.3 ($(\text{CH}_3)_2\text{C}$), 19.2 ($(\text{CH}_3)_3\text{CSi}$); HRMS (ESI/APCI) m/z calcd for $\text{C}_{32}\text{H}_{40}\text{NaO}_6\text{Si}$ ($\text{M} + \text{Na}$) $^+$: 571.2486. Found: 571.2508.

Benzyl 3,5,6-tri-*O*-benzoyl-2-*O*-tert-butylidiphenylsilyl- α -D-galactofuranoside (19). A suspension of **17** (1.86 g, 3.39 mmol) in a mixture of 3 : 2 HOAc–H₂O (11.6 mL) was warmed at 80 °C with stirring. After 10 min, the solution was cooled to rt and pyridine (11.5 mL) was added to avoid decomposition of the product during concentration. The residue was co-evaporated with toluene (8 $\times$ 4 mL) to give 1.72 g of crude benzyl 2-*O*-tert-butylidiphenylsilyl- α -D-galactofuranoside (**18**). Purification of an analytical sample gave pure **18** as a syrup: R_f 0.64 (EtOAc); $[\alpha]_D^{+57.9^\circ}$ (c 1, CHCl_3); ^1H NMR (CDCl_3 , 500 MHz): δ 7.71–7.65 (m, 4H, arom.), 7.43–7.29 (m, 11H, arom.), 4.57, 4.35 (2d, 2H, $J = 11.5$ Hz, CH_2Ph), 4.42 (d, 1H, $J = 4.0$ Hz, H-1), 4.42 (t, 1H, $J = 7.5$ Hz, H-3), 4.12 (dd, 1H, $J = 8.0, 4.5$ Hz, H-4), 3.71 (dd, 1H, $J = 7.0, 4.5$ Hz, H-2), 3.61 (apparent q, 1H, $J = 5.0$ Hz, H-5), 3.58 (dd, 1H, $J = 11.5, 5.5$ Hz, H-6a), 3.55 (dd, 1H, $J = 11.5, 5.0$ Hz, H-6b), 2.74 (bs, 3H, –OH), 1.08 (s, 9H, $(\text{CH}_3)_3\text{CSi}$). ^{13}C NMR (CDCl_3 , 125.8 MHz): δ 137.2–127.7 (C-arom.), 100.9 (C-1), 82.2 (C-2), 78.8 (C-4), 75.5 (C-3), 72.3 (C-5), 70.3 (CH_2Ph), 63.9 (C-6), 26.8 ($(\text{CH}_3)_3\text{CSi}$), 19.2 ($(\text{CH}_3)_3\text{CSi}$); HRMS (ESI/APCI) m/z calcd for $\text{C}_{29}\text{H}_{36}\text{NaO}_6\text{Si}$ ($\text{M} + \text{Na}$) $^+$: 531.2173. Found: 531.2173.

Crude **18** was dissolved in dry pyridine (9 mL), cooled to 0 °C and benzoyl chloride (1.45 mL, 12.5 mmol, 3.7 equiv) was slowly added with stirring. After stirring for 30 min at 0 °C and 16 h at rt, the mixture was poured into ice-water (150 g). After 20 min, the mixture was extracted with CH_2Cl_2 (150 mL) and the organic phase was washed with 10% HCl (3 $\times$ 100 mL), H₂O (100 mL), 10% NaHCO_3 (3 $\times$ 80 mL), H₂O (3 $\times$ 80 mL) until pH 7, dried (Na_2SO_4), and concentrated. Silica gel column chromatography (10 : 1 hexane–EtOAc) of the residue gave **19** (2.24 g, 80.5% from **17**) as a foamy solid: R_f 0.50 (20 : 1 toluene–EtOAc), $[\alpha]_D^{+43.8^\circ}$ (c 1, CHCl_3); ^1H NMR (CDCl_3 , 500 MHz): δ 8.05–7.52 (4m, 11H, arom.), 7.48–7.26 (m, 15H, arom.), 7.21–7.11 (2m, 4H, arom.), 6.07 (dd, 1H, $J = 7.8, 6.9$ Hz, H-3), 5.71 (dt, 1H, $J = 5.4, 4.5$ Hz, H-5), 4.87, 4.30 (2d, 2H, $J = 11.8$ Hz, CH_2Ph), 4.74 (dd, 1H, $J = 11.9, 4.3$ Hz, H-6a), 4.62 (dd, 1H, $J = 11.9, 5.7$ Hz, H-6b), 4.55 (dd, 1H, $J = 7.8, 4.6$ Hz, H-2), 4.49 (d, 1H, $J = 4.6$ Hz, H-1), 4.34 (dd, 1H, $J = 6.9, 5.2$ Hz, H-4), 1.00 (s, 9H, $(\text{CH}_3)_3\text{CSi}$); ^{13}C NMR (CDCl_3 , 125.8 MHz): δ 165.9, 165.8, 165.6 (PhCO), 137.3–127.6 (C-arom.), 100.3 (C-1), 78.3 (C-4), 76.9, 76.85 (C-2, C-3), 71.9 (C-5), 69.7 (CH_2Ph), 62.9 (C-6), 26.7 ($(\text{CH}_3)_3\text{CSi}$), 19.1 ($(\text{CH}_3)_3\text{CSi}$); HRMS (ESI/APCI) m/z calcd for $\text{C}_{50}\text{H}_{48}\text{NaO}_9\text{Si}$ ($\text{M} + \text{Na}$) $^+$: 843.2960. Found: 843.2978.

Benzyl 3,5,6-tri-*O*-benzoyl- α -D-galactofuranoside (20). To a stirred cold solution (0 °C) of **19** (2.22 g, 2.7 mmol) in anhydrous DMF (10 mL), was added glacial acetic acid (286 μL , 1.85 equiv) and a solution of 1 M TBAF in anhydrous THF (5.4 mL,

2 equiv), and the stirring continued at rt for 6 h. The mixture was diluted with CH_2Cl_2 (200 mL), washed with H₂O (5 $\times$ 150 mL), dried (Na_2SO_4), filtered and concentrated under reduced pressure. Column chromatography of the residue (15 : 4 hexane–EtOAc) gave **20** (1.3 g, 83%) as a foamy solid: R_f 0.49 (3 : 2 hexane–EtOAc), $[\alpha]_D^{+49.7^\circ}$ (c 0.5, CHCl_3); ^1H NMR (CDCl_3 , 500 MHz): δ 8.05–7.93 (m, 6H, arom.), 7.53–7.23 (m, 14H, arom.), 5.78 (dt, 1H, $J = 5.5, 4.0$ Hz, H-5), 5.71 (t, 1H, $J = 7.0$ Hz, H-3), 5.20 (d, 1H, $J = 4.5$ Hz, H-1), 4.98, 4.63 (2d, 2H, $J = 11.5$ Hz, CH_2Ph), 4.76 (dd, 1H, $J = 12.0, 4.0$ Hz, H-6a), 4.66 (dd, 1H, $J = 12.0, 6.0$ Hz, H-6b), 4.55 (dd, 1H, $J = 6.5, 5.5$ Hz, H-4), 4.49 (dd, 1H, $J = 7.0, 4.5$ Hz, H-2), 3.00 (bs, 1H, OH). ^{13}C NMR (CDCl_3 , 125.8 MHz): δ 166.0, 165.9, 165.7 (PhCO), 136.6–128.0 (C-arom.), 100.2 (C-1), 79.1 (C-4), 78.0 (C-3), 76.5 (C-2), 71.5 (C-5), 70.2 (CH_2Ph), 63.0 (C-6); HRMS (ESI/APCI) m/z calcd for $\text{C}_{34}\text{H}_{30}\text{NaO}_9$ ($\text{M} + \text{Na}$) $^+$: 605.1782, found: 605.1779.

Benzyl 3,5,6-tri-*O*-benzoyl-2-*O*-levulinoyl- α -D-galactofuranoside (21). To a cooled solution (0 °C) of **20** (1.19 g, 2.04 mmol) and DMAP (74.8 mg, 0.61 mmol) in CH_2Cl_2 (9 mL), levulinic acid (229 μL , 2.24 mmol) was added with stirring followed by DCC (462 mg, 2.24 mmol) and the mixture was allowed to reach room temperature. After 14 h of stirring, the solution was diluted with CH_2Cl_2 (100 mL), washed with 10% NaHCO_3 (3 $\times$ 80 mL), water (3 $\times$ 80 mL), dried and concentrated under reduced pressure. The residue was purified by column chromatography (5 : 2 hexane–EtOAc) to give 1.35 g of **21** (97%; R_f 0.41, 3 : 2 hexane–EtOAc) as a syrup: $[\alpha]_D^{+58.4^\circ}$ (c 1, CHCl_3); ^1H NMR (CDCl_3 , 500 MHz): δ 8.06–7.92 (m, 6H, arom.), 7.53–7.20 (m, 14H, arom.), 6.03 (t, 1H, $J = 6.8$ Hz, H-3), 5.80 (apparent q, 1H, $J = 5.0$ Hz, H-5), 5.44 (d, 1H, $J = 4.5$ Hz, H-1), 5.29 (dd, 1H, $J = 7.0, 4.5$ Hz, H-2), 4.92, 4.53 (2d, 2H, $J = 11.5$ Hz, CH_2Ph), 4.76 (dd, 1H, $J = 12.0, 4.5$ Hz, H-6a), 4.67 (dd, 1H, $J = 12.0, 6.0$ Hz, H-6b), 4.56 (t, 1H, $J = 5.7$ Hz, H-4), 2.70–2.56 (m, 4H, $(\text{CH}_2)_2$), 2.09 (s, 3H, CH_3CO). ^{13}C NMR (CDCl_3 , 125.8 MHz): δ 206.0 (CH_3CO), 171.9 (CH_2CO), 165.9, 165.6, 165.4 (PhCO), 137.0–127.5 (C-arom.), 99.6 (C-1), 78.7 (C-4), 76.9 (C-2), 74.5 (C-3), 71.4 (C-5), 70.4 (CH_2Ph), 62.9 (C-6), 37.6, 27.6 ($(\text{CH}_2)_2$), 29.6 (CH_3CO); HRMS (ESI/APCI) m/z calcd for $\text{C}_{39}\text{H}_{36}\text{NaO}_{11}$ ($\text{M} + \text{Na}$) $^+$: 703.2150. Found: 703.2143.

3,5,6-Tri-*O*-benzoyl-2-*O*-levulinoyl- α,β -D-galactofuranose (22). A mixture of **21** (1.45 g, 2.13 mmol) dissolved in EtOAc (45 mL) and 10% Pd(C) Degussa type (530 mg) was hydrogenated for 72 h at 45 psi (3 atm) at rt. The catalyst was filtered and the filtrate was concentrated under vacuum to give **22** (1.2 g, 95%) as an amorphous solid in a 3 : 7 α : β anomeric mixture: R_f 0.40 (1 : 1 hexane–EtOAc), $[\alpha]_D^{-10.1^\circ}$ (c 1, CHCl_3); ^1H NMR (CDCl_3 , 500 MHz): δ for the β -anomer 8.15–7.95 (m, 6H, arom.), 7.60–7.35 (m, 9H, arom.), 6.03 (dt, 0.7H, $J = 7.0, 4.3$ Hz, H-5), 5.91 (dt, 0.3H, $J = 6.5, 3.5$ Hz, H-5 α anomer), 5.84 (t, 0.3H, $J = 4.7$ Hz, H-3 α anomer), 5.67 (d, 0.3H, $J = 4.0$ Hz H-1 α anomer), 5.56 (bs, 0.7H, H-1), 5.44 (dd, 0.7H, $J = 4.5, 1.0$ Hz, H-3), 5.33 (dd, 0.3H, $J = 4.7, 4.0$ Hz, H-2 α anomer), 5.27 (d, 0.7H, $J = 1.0$ Hz, H-2), 4.81 (dd, 0.3H, $J = 12.0, 3.5$ Hz, H-6a α anomer), 4.78 (t, 0.7H, $J = 4.3$ Hz, H-4), 4.74 (dd, 0.7H, $J = 12.0, 4.5$ Hz, H-6a), 4.69 (dd, 0.3H, $J = 12.0, 6.5$ Hz,

H-6b α anomer), 4.68 (dd, 0.7H, $J = 12.0, 7.0$ Hz, H-6b), 4.47 (dd, 0.7H, $J = 6.5, 4.5$ Hz, H-4 α anomer), 3.31 (bs, 0.7H, HO-1), 2.77–2.68 (m, 1.3H, $(CH_2)_2$ α and β anomers), 2.62–2.44 (m, 2H, $(CH_2)_2$ α and β anomers), 2.33–2.27 (m, 0.7H, $(CH_2)_2$ Lev α and β anomers), 2.14, 2.13 (2s, 3H, CH_3CO α and β anomers); ^{13}C NMR ($CDCl_3$, 125.8 MHz): δ for the β -anomer 206.8, 206.3 (CH_3CO α and β anomers), 171.8 (CH_2CO), 171.7 (CH_2CO α anomer), 166.1–165.4 (PhCO), 133.5–128.3 (C-arom.), 100.9 (C-1), 96.1 (C-1 α anomer), 82.0 (C-4), 81.9 (C-2), 79.3 (C-4 α anomer), 77.7 (C-3), 77.0 (C-2 α anomer), 75.9 (C-3 α anomer), 71.9 (C-5 α anomer), 70.5 (C-5), 63.4 (C-6), 63.1 (C-6 α anomer), 37.9, 27.7 ($(CH_2)_2$ α anomer), 37.7, 27.5 ($(CH_2)_2$), 29.7 (CH_3CO α and β anomers); HRMS (ESI/APCI) m/z calcd for $C_{32}H_{30}NaO_{11}$ ($M + Na$) $^+$: 613.1680. Found: 613.1687.

O-(3,5,6-tri-*O*-benzoyl-2-*O*-levulinoyl- β -D-galactofuranosyl) trichloroacetimidate (11). To a stirred solution of **22** (1.79 g, 3.03 mmol) and trichloroacetonitrile (1.52 mL, 15.2 mmol) in anhydrous CH_2Cl_2 (20 mL), cooled to 0 $^{\circ}C$, DBU (128 μ L, 0.91 mmol, 0.3 equiv) was slowly added and the mixture was allowed to reach rt. After 25 min, the solution was concentrated under reduced pressure at room temperature, and the residue was purified by column chromatography (7 : 1 : 0.2 toluene–EtOAc–TEA) to afford syrupy **11** (2.01 g, 90%); R_f 0.5 (5 : 1 toluene–EtOAc); $[\alpha]_D^{25} -24.5^{\circ}$ (c 0.7, $CHCl_3$); 1H NMR ($CDCl_3$, 500 MHz): δ 8.70 (s, 1H, NH), 8.15–7.95 (m, 6H, arom.), 7.60–7.34 (m, 9H, arom.), 6.55 (bs, 1H, H-1), 6.10 (ddd, 1H, $J = 6.2, 5.4, 3.3$ Hz, H-5), 5.55 (dd, 1H, $J = 3.8, 0.6$ Hz, H-3), 5.51 (d, 1H, $J = 0.6$ Hz, H-2), 4.78 (t, 1H, $J = 3.5$ Hz, H-4), 4.74 (m, 2H, H-6a, H-6b), 2.69–2.52 (m, 2H, $(CH_2)_2$), 2.41–2.35 (m, 1H, $(CH_2)_2$), 2.22–2.14 (m, 1H, $(CH_2)_2$), 2.12 (s, 3H, CH_3CO); ^{13}C NMR ($CDCl_3$, 125.8 MHz): δ 205.8 (CH_3CO), 171.4 (CH_2CO); 165.6, 165.5, 164.1 (PhCO), 160.2 (CNH), 133.7–128.4 (C-arom.), 102.8 (C-1), 84.9 (C-4), 79.9 (C-2), 77.0 (C-3), 69.9 (C-5), 63.4 (C-6), 37.7, 27.4 ($(CH_2)_2$), 29.7 (CH_3CO); HRMS (ESI/APCI) m/z calcd for $C_{34}H_{30}Cl_3NNaO_{11}$ ($M + Na$) $^+$: 756.0777. Found: 756.0798.

Benzyl 3,5,6-tri-*O*-benzoyl-2-*O*-levulinoyl- β -D-galactofuranosyl-(1 $\rightarrow$ 4)-2-acetamido-3-*O*-benzoyl-6-*O*-tert-butylidiphenylsilyl-2-deoxy- α -D-glucopyranoside (23). A solution of trichloroacetimidate **11** (2.28 g, 3.10 mmol, 1.1 equiv) in anhydrous CH_2Cl_2 (30 mL) was added to a mixture of benzyl 2-acetamido-3-*O*-benzoyl-6-*O*-tert-butylidiphenylsilyl-2-deoxy- α -D-glucopyranoside (**12**)²⁴ (1.85 g, 2.83 mmol) and activated 4 $\text{\AA}$ powdered molecular sieves (3.4 g) with stirring under argon atmosphere. After 10 min of vigorous stirring at rt, the mixture was cooled to -20 $^{\circ}C$ and TMSOTf (110 μ L, 0.61 mmol, 0.2 equiv) was added, and stirring continued for 2 h at -20 $^{\circ}C$. TLC monitoring showed a small amount of unreacted **12** and the mixture was stirred for additional 16 h (-20 $^{\circ}C$), then neutralized with Et_3N (85 μ L, 0.61 mmol, 0.2 equiv), filtered and concentrated under reduced pressure to give an off-white residue (4.3 g). The crude product was recrystallized twice from CH_3OH (from 9.5 mL and 4 mL, respectively) yielding 2.3 g of pure **23** (66%); R_f 0.35 (3 : 2 : 0.2 hexane–EtOAc–TEA), mp 162–163 $^{\circ}C$ (CH_3OH); $[\alpha]_D^{25} +12.0^{\circ}$ (c 2, $CHCl_3$); 1H NMR ($CDCl_3$, 500 MHz): δ 7.99–7.93 (m, 6H, arom.), 7.83–7.77 (m, 4H, arom.), 7.68–7.61

(m, 3H, arom.), 7.52–7.48 (m, 4H, arom.), 7.44–7.24 (m, 18H, arom.), 5.74 (d, 1H, $J = 9.7$ Hz, NH), 5.66 (ddd, 1H, $J = 8.3, 3.5, 2.8$ Hz, H-5'), 5.60 (bs, 1H, H-1'), 5.57 (dd, 1H, $J = 10.7, 9.7$ Hz, H-3), 5.31 (d, 1H, $J = 3.8$ Hz, H-3'), 5.03 (s, 1H, H-2'), 4.91 (d, 1H, $J = 3.8$ Hz, H-1), 4.62, 4.44 (2d, 2H, $J = 11.9$ Hz, CH_2Ph), 4.49 (ddd, 1H, $J = 10.7, 9.7, 3.8$ Hz, H-2), 4.38 (t, 1H, $J = 9.5$ Hz, H-4), 4.38 (dd, 1H, $J = 12.3, 8.3$ Hz, H-6a'), 4.29 (dd, 1H, $J = 12.3, 2.8$ Hz, H-6b'), 4.09 (t, 1H, $J = 3.7$ Hz, H-4'), 4.07 (dd, 1H, $J = 12.3, 3.1$ Hz, H-6a), 3.84 (m, 2H, H-6b, H-5), 2.36 (t, 2H, $J = 7.2$ Hz, $(CH_2)_2$), 2.02 (t, 2H, $J = 7.2$ Hz, $(CH_2)_2$), 2.00 (s, 3H, CH_3CO Lev), 1.76 (s, 3H, CH_3CONH), 1.10 (s, 9H, $(CH_3)_3CSi$); ^{13}C NMR ($CDCl_3$, 125.8 MHz): δ 205.6 (CH_3CO Lev), 171.6 (CH_2COO), 169.9 (CH_3CONH), 166.7, 165.9, 165.4 (PhCO), 136.8–127.5 (C-arom.), 105.5 (C-1'), 96.4 (C-1), 83.1 (C-4'), 81.3 (C-2'), 77.5 (C-3'), 72.2 (C-3), 71.9 (C-4), 71.8 (C-5), 70.3 (C-5'), 69.7 (CH_2Ph), 63.8 (C-6'), 62.4 (C-6), 52.4 (C-2), 37.6, 27.2 ($(CH_2)_2$), 29.5 (CH_3CO Lev), 26.7 ($(CH_3)_3CSi$), 23.1 (CH_3CONH), 19.5 ($(CH_3)_3CSi$).

Anal. calcd for $C_{70}H_{71}NO_{17}Si$: C 68.55, H 5.84. Found: C 68.55, H 5.55.

Benzyl 3,5,6-tri-*O*-benzoyl- β -D-galactofuranosyl-(1 $\rightarrow$ 4)-2-acetamido-3-*O*-benzoyl-6-*O*-tert-butylidiphenylsilyl-2-deoxy- α -D-glucopyranoside (10). To a solution of **23** (826 mg, 0.68 mmol) in a mixture of pyridine–HOAc (4 : 1 v/v, 7 mL), cooled to 0 $^{\circ}C$, hydrazine monohydrate (212 μ L, 4.4 mmol, 6 equiv) was added with stirring. After 20 min at rt, TLC analysis showed complete conversion of **23** (R_f 0.43, 3 : 2 hexane–EtOAc, twice developed) into a new product (R_f 0.48), and the mixture was diluted with CH_2Cl_2 (180 mL) and subsequently washed with 5% HCl (3 $\times$ 70 mL), 10% $NaHCO_3$ (2 $\times$ 70 mL) and H_2O (70 mL), dried with Na_2SO_4 and concentrated. The crude product was purified by column chromatography (3 : 2 hexane–EtOAc) to give glassy **10** (689 mg, 91%); R_f 0.5 (3 : 2 hexane–EtOAc, twice developed), $[\alpha]_D^{25} +15.5^{\circ}$ (c 1, $CHCl_3$); 1H NMR ($CDCl_3$, 500 MHz): δ 8.03–7.57 (m, 13H, arom.), 7.52–7.18 (m, 22H, arom.), 5.79 (d, 1H, $J = 9.7$ Hz, NH), 5.55 (dd, 1H, $J = 10.8, 9.4$ Hz, H-3), 5.48 (dt, 1H, $J = 7.4, 3.9$ Hz, H-5'), 5.38 (bs, 1H, H-1'), 4.98 (d, 1H, $J = 3.7$ Hz, H-1), 4.90 (dd, 1H, $J = 5.6, 2.4$ Hz, H-3'), 4.67, 4.48 (2d, 2H, $J = 11.9$ Hz, CH_2Ph), 4.48 (ddd, 1H, $J = 10.8, 9.7, 3.7$ Hz, H-2), 4.24 (t, 1H, $J = 10.0$ Hz, H-4), 4.22 (dd, 1H, $J = 12.0, 7.4$ Hz, H-6a'), 4.22 (dd, 1H, $J = 5.6, 3.8$ Hz, H-4'), 4.19 (dd, 1H, $J = 12.0, 4.0$ Hz, H-6b'), 4.09 (ddd, 1H, $J = 5.1, 2.4, 1.0$ Hz, H-2'), 3.88 (dd, 1H, $J = 11.8, 3.6$ Hz, H-6a), 3.82 (dd, 1H, $J = 11.8, 1.6$ Hz, H-6a), 3.80 (m, 1H, H-5), 2.79 (d, 1H, $J = 5.1$ Hz, 2-OH), 1.79 (s, 3H, CH_3CONH), 1.07 (s, 9H, $(CH_3)_3CSi$); ^{13}C NMR ($CDCl_3$, 125.8 MHz): δ 170.0 (CH_3CONH), 166.9, 165.8, 165.6 (PhCO), 135.9–127.5 (C-arom.), 108.6 (C-1'), 96.4 (C-1), 81.3 (C-3'), 81.0 (C-2'), 80.5 (C-4'), 74.1 (C-4), 72.5 (C-3), 71.8 (C-5), 70.3 (C-5'), 69.6 (CH_2Ph), 63.4 (C-6'), 62.3 (C-6), 52.3 (C-2), 26.8 ($(CH_3)_3CSi$), 23.1 (CH_3CONH), 19.3 ($(CH_3)_3CSi$).

Anal. calcd for $C_{65}H_{65}NO_{15}Si$: C 69.19, H 5.81, N 1.24. Found: C 69.08, H 5.55, N 1.37.

Benzyl 2,3,5,6-tetra-*O*-benzoyl- β -D-galactofuranosyl-(1 $\rightarrow$ 2)-3,5,6-tri-*O*-benzoyl- β -D-galactofuranosyl-(1 $\rightarrow$ 4)-2-acetamido-3-*O*-benzoyl-6-*O*-tert-butylidiphenylsilyl-2-deoxy- α -D-glucopy-

ranoside (24).²⁶ To a solution of dried acceptor **10** (956 mg, 0.85 mmol) and *O*-(2,3,5,6-tetra-*O*-benzoyl- β -D-galactofuranosyl) trichloroacetimidate³⁰ (**9**, 780 mg, 1.05 mmol, 1.2 equiv) in freshly distilled anhydrous CH_2Cl_2 (22 mL), activated 4 Å powdered molecular sieves (1.6 g) were added, and the suspension was stirred under argon atmosphere for 10 min. The mixture was then cooled to -20°C and after 15 min of vigorous stirring, TMSOTf (18.5 μL , 0.10 mmol, 0.12 equiv) was added and the stirring continued for 23 h at -20°C until TLC examination showed total consumption of acceptor **10** (R_f 0.41, 1.0:0.9 hexane–EtOAc). The reaction was quenched by addition of triethylamine (15 μL , 0.11 mmol) and the mixture was allowed to reach room temperature and then filtered over Celite. The filtrate was concentrated under vacuum and the residue was purified by column chromatography (1.9:1 hexane–EtOAc) to give 1.18 g of **24** (81%) as an amorphous solid (R_f 0.32 (1:0.9 hexane–EtOAc)). Physical properties and ^1H and ^{13}C NMR spectra matched lit.²⁶ mp $87\text{--}89^\circ\text{C}$ (hexane–toluene);²⁶ $[\alpha]_{\text{D}}^{25} +11^\circ$ (c 1, CHCl_3);²⁶ ^1H NMR (CDCl_3 , 500 MHz): δ 8.06–7.59 (m, 21H), 7.53–7.23 (m, 34H), 5.90 (m, 1H), 5.81 (d, 1H, $J = 9.7$ Hz), 5.63 (ddd, 1H, $J = 8.0, 5.7, 2.9$ Hz), 5.58 (bs, 1H), 5.58–5.56 (m, 1H), 5.54 (dd, 1H, $J = 10.9, 9.6$ Hz), 5.43 (bs, 1H), 5.37 (d, 1H, $J = 3.4$ Hz), 5.31 (d, 1H, $J = 1.7$ Hz), 4.92 (d, 1H, $J = 3.8$ Hz), 4.67, 4.44 (2d, 2H, $J = 11.9$ Hz), 4.53 (dd, 1H, $J = 12.0, 7.0$ Hz), 4.49 (dd, 1H, $J = 5.6, 3.2$ Hz), 4.44 (dd, 1H, $J = 12.3, 2.9$ Hz), 4.42 (ddd, 1H, $J = 10.9, 9.7, 3.8$ Hz), 4.37 (dd, 1H, $J = 12.0, 4.0$ Hz), 4.35 (dd, 1H, $J = 12.3, 8.0$ Hz), 4.23 (dd, 1H, $J = 5.7, 3.4$ Hz), 4.20 (bs, 1H), 4.16 (t, 1H, $J = 9.6$ Hz), 3.92 (dd, 1H, $J = 11.7, 4.1$ Hz), 3.87–3.82 (m, 2H), 1.78 (s, 3H), 0.99 (s, 9H); ^{13}C NMR (CDCl_3 , 50.3 MHz): δ 169.9, 167.0, 166.0, 165.8, 165.73, 165.65, 165.6, 165.3, 136.8–127.5 (C-arom.), 106.1, 105.4, 96.2, 84.7, 82.6, 82.3, 81.9, 77.6, 77.2, 72.3, 72.1, 70.5, 70.2, 69.4, 63.8, 63.4, 62.5, 52.5, 26.8, 23.1, 19.2.

Benzyl (2,3,4,6-tetra-*O*-acetyl- β -D-galactopyranosyl)-(1 $\rightarrow$ 2)-[2,3,4,6-tetra-*O*-acetyl- β -D-galactopyranosyl-(1 $\rightarrow$ 3)]-4,6-di-*O*-acetyl- β -D-galactopyranosyl-(1 $\rightarrow$ 6)-[2,3,5,6-tetra-*O*-benzoyl- β -D-galactofuranosyl-(1 $\rightarrow$ 2)-3,5,6-tri-*O*-benzoyl- β -D-galactofuranosyl-(1 $\rightarrow$ 4)]-2-acetamido-3-*O*-benzoyl-2-deoxy- α -D-glucopyranoside (25**).** To a flask containing *O*-[2,3-di-*O*-(2,3,4,6-tetra-*O*-acetyl- β -D-galactopyranosyl)-4,6-di-*O*-acetyl- α -D-galactopyranosyl] trichloroacetimidate²⁵ **7** (320 mg, 0.30 mmol, 1.1 equiv) and activated 4 Å powdered molecular sieves (210 mg), a solution of acceptor **8** (400 mg, 0.27 mmol) in freshly distilled anhydrous CH_3CN (15 mL) was added, and the suspension was cooled to -20°C under argon atmosphere. After 10 min of vigorous stirring, TMSOTf (8 μL , 44 μmol , 0.16 equiv) was added and the stirring continued for 24 h at -20°C . TLC analysis showed the presence of unreacted acceptor **8** (R_f 0.65; 2:3:0.2 toluene–EtOAc–TEA), trisaccharide *O*-(2,3,4,6-tetra-*O*-acetyl- β -D-galactopyranosyl)-(1 $\rightarrow$ 2)-[2,3,4,6-tetra-*O*-acetyl- β -D-galactopyranosyl-(1 $\rightarrow$ 3)]-4,6-di-*O*-acetyl- α , β -D-galactopyranose (R_f 0.3) due to remaining trichloroacetimidate **7**, and a new spot (R_f 0.38). The mixture was allowed to reach 0°C and was stirred for an additional 20 h. The reaction was stopped by the addition of Et_3N (6.3 μL , 45 μmol), filtered and the molecular sieves were washed with CH_3CN . The filtrate was concentrated under reduced pressure and the residue was purified by column chromatography (1:1 toluene–EtOAc). The first fraction gave

unreacted acceptor **8** (60 mg, 15%, R_f 0.60; 1:2 toluene–EtOAc). The next fraction afforded 360 mg of hexasaccharide **25** as a glassy solid (56%); R_f 0.37 (1:2 toluene–EtOAc), $[\alpha]_{\text{D}}^{25} +6.9^\circ$ (c 1.5, CHCl_3); ^1H NMR (CDCl_3 , 500 MHz): δ 8.07–7.82 (m, 16H, arom.), 7.59–7.32 (m, 29H, arom.), 6.05 (dt, 1H, $J = 7.5, 4.0$ Hz, H-5^C), 5.81 (d, 1H, $J = 9.5$ Hz, NH), 5.70 (dd, 1H, $J = 5.5, 1.0$ Hz, H-3^C), 5.65 (ddd, 1H, $J = 8.5, 6.0, 2.5$ Hz, H-5^B), 5.63 (bs, 1H, H-1^C), 5.57 (dd, 1H, $J = 10.5, 9.5$ Hz, H-3^A), 5.47 (d, 1H, $J = 3.5$ Hz, H-3^B), 5.45 (bs, 1H, H-1^B), 5.41 (d, 1H, $J = 1.0$ Hz, H-2^C), 5.36 (d, 1H, $J = 3.0$ Hz, H-4^F), 5.34 (d, 1H, $J = 3.5$ Hz, H-4^E), 5.26 (d, 1H, $J = 3.5$ Hz, H-4^D), 5.18 (dd, 1H, $J = 10.0, 8.0$ Hz, H-2^F), 5.16 (dd, 1H, $J = 10.5, 3.5$ Hz, H-3^E), 5.08 (d, 1H, $J = 8.0$ Hz, H-1^F), 5.05 (dd, 1H, $J = 10.5, 8.0$ Hz, H-2^E), 5.01 (dd, 1H, $J = 10.0, 3.5$ Hz, H-3^F), 4.91 (d, 1H, $J = 3.5$ Hz, H-1^A), 4.85, 4.52 (2d, 2H, $J = 11.5$ Hz, CH_2Ph), 4.83 (d, 1H, $J = 8.0$ Hz, H-1^E), 4.77 (dd, 1H, $J = 5.5, 4.0$ Hz, H-4^C), 4.74 (dd, 1H, $J = 11.5, 7.5$ Hz, H-6a^C), 4.70 (d, 1H, $J = 7.5$ Hz, H-1^D), 4.62 (dd, 1H, $J = 11.5, 4.0$ Hz, H-6b^C), 4.47 (dd, 1H, $J = 12.0, 2.5$ Hz, H-6a^B), 4.38 (bs, 1H, H-2^B), 4.38–4.31 (m, 3H, H-2^A; H-4^B and H-6b^B), 4.32–4.24 (m, 3H, H-5^A, H-6a^A, H-6a^F), 4.17–4.10 (m, 2H, H-6b^F, H-6a^E), 4.09–4.03 (m, 3H, H-6b^E, H-3^D, H-5^F), 3.99 (dd, 1H, $J = 12.0, 7.0$ Hz, H-6a^D), 3.96–3.90 (m, 2H, H-6b^D, H-5^E), 3.88 (dd, 1H, $J = 9.0, 7.5$ Hz, H-2^D), 3.80–3.74 (m, 2H, H-6b^A, H-5^D), 3.70 (t, 1H, $J = 9.5$ Hz, H-4^A), 2.15, 2.08, 2.03, 1.98, 1.97, 1.96, 1.84 (8s, 27H, CH_3CO), 1.75 (s, 3H, CH_3CONH); ^{13}C NMR (CDCl_3 , 125.8 MHz): δ 170.3, 170.2 ($\times 2$), 170.1, 169.9 ($\times 2$), 169.8, 169.7, 169.6, 168.8 (CH_3CO), 166.8, 166.1, 165.8, 165.6 ($\times 2$), 165.5, 165.3, 165.2 (PhCO), 136.6, 133.4–132.8 (C-arom.), 129.9–128.2 (C-arom.), 106.6 (C-1^B), 105.9 (C-1^C), 101.8 (C-1^D), 99.3 (C-1^F), 99.1 (C-1^E), 95.9 (C-1^A), 85.3 (C-2^B), 82.6 (C-2^C), 82.2 (C-4^B), 81.7 (C-4^C), 79.5 (C-2^D), 77.3 (C-3^B), 76.9 (C-3^C), 75.7 (C-4^A), 74.7 (C-3^D), 71.7 (C-3^A), 71.1 (C-3^F), 70.7 (C-5^E), 70.6 (C-5^A), 70.5 (C-5^D, C-5^F), 70.3 (C-5, C-5^C), 70.0 (C-3^E, C-2^F), 69.8 (C-2^E), 69.6 (CH_2Ph), 69.0 (C-6^A), 68.2 (C-4^D), 67.0, 66.7 (C-4^E, C-4^F), 63.4 (C-6^B), 63.3 (C-6^C), 61.2 (C-6^D), 60.8 (C-6^E), 60.3 (C-6^F), 52.1 (C-2^A), 23.0 (CH_3CONH), 20.9, 20.8, 20.7, 20.6, 20.5 ($\times 2$), 20.4 ($\times 2$) (CH_3CO). The assignments for the Galp- β -(1 $\rightarrow$ 2) (E) and Galp- β -(1 $\rightarrow$ 3) (F) could be interchanged. HRMS (ESI/APCI) m/z calcd for $\text{C}_{121}\text{H}_{123}\text{NNaO}_{49}$ ($\text{M} + \text{Na}$)⁺: 2396.7056. Found: 2396.6966.

Benzyl β -D-galactopyranosyl-(1 $\rightarrow$ 2)-[β -D-galactopyranosyl-(1 $\rightarrow$ 3)]- β -D-galactopyranosyl-(1 $\rightarrow$ 6)-[β -D-galactofuranosyl-(1 $\rightarrow$ 2)- β -D-galactofuranosyl-(1 $\rightarrow$ 4)]-2-acetamido-2-deoxy- α -D-glucopyranoside (26**).** Compound **25** (270 mg, 0.11 mmol) was treated with 2.5 mL of cooled 0.6 M $\text{NaOCH}_3\text{--CH}_3\text{OH}$ and the mixture was stirred at rt. After 2.5 h the resulting solution was diluted with water (0.25 mL), and purified by cationic interchange column chromatography (Amberlite IR-120 plus resin 200 mesh, H^+ form) eluting with 9:1 $\text{CH}_3\text{OH--H}_2\text{O}$ (2×8 mL). The eluate was concentrated under reduced pressure and the methyl benzoate was co-evaporated with water (3×2.5 mL). Further purification through a C18 cartridge, eluting with 95:5 $\text{H}_2\text{O--CH}_3\text{OH}$ followed by concentration at 30°C gave 126 mg of **26** (99%), as an amorphous hygroscopic solid: R_f 0.34 (7:1:2 *n*-propanol–EtOH– H_2O); $[\alpha]_{\text{D}}^{25} +9.6^\circ$ (c 1, CHCl_3); ^1H NMR (D_2O , 500 MHz): δ 7.54–7.38 (m, 5H, arom.), 5.47 (d, 1H, $J = 1.0$ Hz, H-1^B), 5.24 (d, 1H, $J = 1.5$ Hz, H-1^C), 4.96 (d, 1H, $J = 3.5$ Hz,

H-1^A), 4.82 (d, 1H, $J = 7.5$ Hz, H-1^E), 4.74, 4.57 (2d, 2H, $J = 12.0$ Hz, CH₂Ph), 4.66 (d, 1H, $J = 7.0$ Hz, H-1^F), 4.53 (d, 1H, $J = 7.5$ Hz, H-1^D), 4.26–4.23 (m, 2H, H-2^B and H-3^B), 4.20 (dd, 1H, $J = 11.5$, 1.5 Hz, H-6a^A), 4.19 (d, 1H, $J = 3.5$ Hz, H-4^D), 4.14 (dd, 1H, $J = 3.5$, 1.5 Hz, H-2^C), 4.12–4.08 (m, 2H, H-3^C, H-4^B), 4.02 (dd, 1H, $J = 6.5$, 4.0 Hz, H-4^C), 4.00–3.59 (m, 28H), 1.95 (s, 3H, CH₃CONH); ¹³C NMR (D₂O, 125.8 MHz): δ 174.9 (CH₃CONH), 137.6, 129.4, 129.0 (C-arom.), 107.1 (C-1^B), 106.5 (C-1^C), 104.6 (C-1^F), 103.6 (C-1^E), 102.6 (C-1^D), 96.5 (C-1^A), 86.0 (C-2^B), 84.1 (C-4^C), 83.6 (C-3^C), 83.2 (C-4^B), 82.3 (C-2^C), 78.4 (C-4^A), 77.5 (C-3^C), 76.1 (C-2^D, and undetermined Galp C-5), 75.8 (undetermined Galp C-5); 75.4, 75.3 (C-3^B and undetermined Galp C-5), 73.6, 73.5 (C-3^E, C-3^F); 72.1, 71.8 (C-2^E, C-2^F), 70.7 (CH₂Ph), 71.4, 70.9, 70.4; 69.8 (C-5^A, C-5^B, C-5^C, C-3^A); 69.9, 69.3 (C-4^E, C-4^F), 69.5 (C-4^D), 68.6 (C-6^A); 63.32, 63.27 (C-6^B, C-6^C), 62.0, 61.7, 61.5 (C-6^E, C-6^F, C-6^D), 54.4 (C-2^A), 22.4 (CH₃CONH). HRMS (ESI/APCI) m/z calcd for C₄₅H₇₁NNaO₃₁ (M + Na)⁺: 1144.3902. Found: 1144.3878.

β -D-Galactopyranosyl-(1 $\rightarrow$ 2)-[β -D-galactopyranosyl-(1 $\rightarrow$ 3)]- β -D-galactopyranosyl-(1 $\rightarrow$ 6)-[β -D-galactofuranosyl-(1 $\rightarrow$ 2)]- β -D-galactofuranosyl-(1 $\rightarrow$ 4)]-2-acetamido-2-deoxy- α , β -D-glucopyranose (1). A solution of **26** (33 mg, 0.029 mmol) dissolved in 9 : 1 CH₃OH–H₂O (2 mL) and 10% Pd(C) (12 mg), was hydrogenated for 20 h at 40 psi (3 atm) and rt. The catalyst was filtered over Celite and the filtrate was concentrated at 25 °C, affording **1** (30 mg, 99%) as a glassy hygroscopic solid, in a 3 : 2 α : β anomeric mixture: [α]_D +12.7° (c 0.5, H₂O), R_f 0.15 (7 : 1 : 2 n -propanol–EtOH–H₂O); ¹H NMR (D₂O, 500 MHz): δ anomeric zone and diagnostic signals 5.47 (m, 1H, H-1^B in α and β anomer), 5.24 (d, 0.6H, $J = 1.5$ Hz, H-1^C in α anomer), 5.22 (d, 0.4H, $J = 1.5$ Hz, H-1^C in β anomer), 5.21 (d, 0.6H, $J = 3.5$ Hz, H-1^A α anomer), 4.82 (Galp H-1 superimposed with DHO signal), 4.70 (d, 0.4H, $J = 8.0$ Hz, H-1^A β anomer), 4.65 (d, 1H, $J = 7.0$ Hz, Galp H-1), 4.55 (d, 0.4H, $J = 7.5$ Hz, Galp H-1), 4.53 (d, 0.6H, $J = 7.5$ Hz, Galp H-1), 2.04 (s, 3H, α and β anomers CH₃CONH); ¹³C NMR (D₂O, 50.3 MHz): δ 107.1 (α and β anomers C-1^B), 106.6, (C-1^C in β anomer) 106.5 (C-1^C in α anomer); 104.6, 103.5, 102.6; 95.8 (β anomer C-1^A), 91.4 (α anomer C-1^A); 86.2, 85.9 (C-2^B in α and β anomers), 84.1, 83.5, 83.4, 83.3, 82.3, 81.9, 78.5, 78.0, 77.5, 77.3, 76.4, 76.2, 76.1, 75.8, 75.4, 75.3, 74.3, 73.6, 73.5, 72.9, 72.0, 71.8, 71.4, 71.0, 70.0, 69.9, 69.7, 69.5, 69.3, 68.8 (C-6^A α and β anomers), 63.3, 63.2, 62.1, 61.9, 61.7, 61.5, 57.2 (β anomer C-2^A), 54.7 (α anomer C-2^A), 22.8, 22.5 (α and β anomers CH₃CONH). HRMS (ESI/APCI) m/z calcd for C₃₈H₆₅NNaO₃₁ (M + Na)⁺: 1054.3433. Found: 1054.3402.

β -D-Galactopyranosyl-(1 $\rightarrow$ 2)-[β -D-galactopyranosyl-(1 $\rightarrow$ 3)]- β -D-galactopyranosyl-(1 $\rightarrow$ 6)-[β -D-galactofuranosyl-(1 $\rightarrow$ 2)]- β -D-galactofuranosyl-(1 $\rightarrow$ 4)]-2-acetamido-2-deoxy-D-glucitol.²⁰ (2). To a cooled solution (0 °C) of **1** (16 mg, 0.016 mmol) in 9 : 1 methanol–water (1.5 mL), sodium borohydride (39 mg, 0.98 mmol) was added, and the suspension was gently stirred at rt for 17 h. After further addition of water (0.6 mL) and sodium borohydride (20 mg, 0.5 mmol) and stirring at 25 °C for 5 h, TLC analysis showed disappearance of the starting compound (R_f 0.35, 7 : 1 : 3 n -propanol–EtOH–H₂O) and a new spot (R_f 0.27) was detected. The solution was passed through a

column of Amberlite IR-120 plus, the resin was washed with 2 : 1 CH₃OH–H₂O and the combined solutions were concentrated to dryness. The residue was co-evaporated with methanol (4 $\times$ 1 mL), dissolved in deionized water and the solution was filtered through a C18 cartridge. Evaporation of the filtrate under reduced pressure gave 14.4 mg of **2** (90%) as a glassy solid: [α]_D +24.1° (c 1, H₂O); ¹H NMR (D₂O, 500 MHz): δ 5.43 (bs, 1H, H-1^B), 5.21 (d, 1H, $J = 1.7$ Hz, H-1^C), 4.84 (d, 1H, $J = 7.8$ Hz), 4.65 (d, 1H, $J = 7.5$ Hz), 4.57 (d, 1H, $J = 7.7$ Hz, H-1^D), 4.25 (dd, 1H, $J = 3.5$, 1.5 Hz, H-2^B), 4.22 (dd, 1H, $J = 6.5$, 3.5 Hz, H-3^B), 4.19 (d, 1H, $J = 3.0$ Hz, H-4^D), 4.18 (dd, 1H, $J = 8.0$, 3.0 Hz, H-6a^A), 4.15–4.12 (m, 1H, H-2^A), 4.13 (dd, 1H, $J = 3.5$, 1.5 Hz, H-2^C), 4.11 (t, 1H, $J = 6.0$ Hz, H-4^B), 4.09 (dd, 1H, $J = 6.5$, 3.5 Hz, H-3^C), 4.08–4.04 (m, 1H), 4.02 (dd, 1H, $J = 6.5$, 4.0 Hz, H-4^C), 4.00 (dd, 1H, $J = 10.0$, 3.0 Hz, H-3^D), 3.94 (dd, 1H, $J = 10.0$, 7.7 Hz, H-2^D), 3.93–3.90 (m, 2H), 3.86–3.82 (m, 3H), 3.87–3.62 (m, 21H), 3.56 (dd, 1H, $J = 10.0$, 7.8 Hz), 3.60 (dd, 1H, $J = 10.0$, 7.5 Hz), 2.05 (s, 3H, CH₃CONH), δ values matched lit.;²⁰ ¹³C NMR (D₂O, 125.8 MHz): δ 175.1 (CH₃CONH), 107.4 (C-1^B), 107.0 (C-1^C), 104.7, 103.5; 102.5 (C-1^D), 87.1 (C-2^B), 83.9 (C-4^C), 83.8 (C-4^B), 83.4 (C-3^D), 82.1 (C-2^C), 77.9 (C-4^A), 77.3 (C-3^C), 76.7 (C-2^D), 76.04 (C-3^B); 75.99, 75.8, 75.4, 73.5, 72.2, 71.8, 71.4; 71.3 (C-6^A); 71.2, 70.8, 70.4, 70.0, 69.6; 69.4 (C-4^D); 69.3, 69.0, 63.9, 63.5, 63.4, 61.8, 61.6, 61.5; 53.2 (C-2^A), 22.8 (CH₃CONH). HRMS (ESI/APCI) m/z calcd for C₃₈H₆₇NNaO₃₁ (M + Na)⁺: 1056.3589. Found: 1056.3550.

Acknowledgements

This work was supported by grants from Agencia Nacional de Promoción Científica y Tecnológica, Universidad de Buenos Aires and CONICET. R. M. de Lederkremer and C. Gallo-Rodriguez are research members of CONICET.

References

- 1 Z. Brener, *Annu. Rev. Microbiol.*, 1973, **27**, 347–382.
- 2 A. Moncayo, *Mem. Inst. Oswaldo Cruz*, 2003, **98**, 577–591.
- 3 World Health Organization, Sixty-third World Health Assembly, A63/17, 2010. Chagas disease: control and elimination. http://apps.who.int/gb/ebwha/pdf_files/WHA63/A63_17-en.pdf
- 4 R. L. Tarleton, R. Reithinger, J. A. Urbina, U. Kitron and R. E. Gürtler, *PLoS Med.*, 2007, **4**, 1852–1857.
- 5 R. M. de Lederkremer and R. Agusti, *Adv. Carbohydr. Chem. Biochem.*, 2009, **62**, 311–366.
- 6 A. C. Buscaglia, V. A. Campo, A. C. C. Frasch and J. M. Di Noia, *Nat. Rev. Microbiol.*, 2006, **4**, 229–236.
- 7 M. E. Giorgi and R. M. de Lederkremer, *Carbohydr. Res.*, 2011, **346**, 1389–1393.
- 8 S. Schenkman, M. S. Jiang, G. W. Hart and V. Nussenzweig, *Cell*, 1991, **65**, 1117–1125.
- 9 A. C. C. Frasch, *Parasitol. Today*, 2000, **16**, 282–286.
- 10 S. Schenkman, D. Eichinger, M. E. Pereira and V. Nussenzweig, *Annu. Rev. Microbiol.*, 1994, **48**, 499–523.
- 11 J. O. Previato, C. Jones, L. B. P. Gonçalves, R. Wait, L. R. Travassos and L. Mendonça-Previato, *Biochem. J.*, 1994, **301**, 151–159.
- 12 A. Acosta Serrano, S. Schenkman, N. Yoshida, A. Mehlert, J. M. Richardson and M. A. J. Ferguson, *J. Biol. Chem.*, 1995, **270**, 27244–27253.
- 13 O. A. Agrellos, C. Jones, A. R. Todeschini, J. O. Previato and L. Mendonça-Previato, *Mol. Biochem. Parasitol.*, 2003, **126**, 93–96.

- 14 A. R. Todeschini, E. G. de Almeida, O. A. Agrellos, C. Jones, J. O. Previato and L. Mendonça-Previato, *Mem. Inst. Oswaldo Cruz*, 2009, **104**, 270–274.
- 15 J. O. Previato, C. Jones, P. B. Gonçalves, R. Wait, L. R. Travassos, A. J. Parodi and L. Mendonça-Previato, *J. Biol. Chem.*, 1995, **270**, 7241–7250.
- 16 M. L. Salto, C. Gallo-Rodriguez, C. Lima and R. M. de Lederkremer, *Anal. Biochem.*, 2000, **279**, 79–84.
- 17 G. D. Pollevick, J. M. Di Noia, M. L. Salto, C. Lima, M. S. Leguizamon, R. M. de Lederkremer and A. C. C. Frasch, *J. Biol. Chem.*, 2000, **275**, 27671–27680.
- 18 A. R. Todeschini, E. X. da Silveira, C. Jones, R. Wait, J. O. Previato and L. Mendonça-Previato, *Glycobiology*, 2001, **11**, 47–55.
- 19 B. Zingales, S. G. Andrade, M. R. Briones, D. A. Campbell, E. Chiari, O. Fernandes, F. Guhl, E. Lages-Silva, A. M. Macedo, C. R. Machado, M. A. Miles, A. J. Romanha, N. R. Sturm, M. Tibayrenc and A. G. Schijman, *Mem. Inst. Oswaldo Cruz*, 2009, **104**, 1051–1054.
- 20 C. Jones, A. R. Todeschini, O. A. Agrellos, J. O. Previato and L. Mendonça-Previato, *Biochemistry*, 2004, **43**, 11889–11897.
- 21 M. V. de Arruda, W. Colli and B. Zingales, *Eur. J. Biochem.*, 1989, **182**, 413–421.
- 22 C. Gallo-Rodriguez, O. Varela and R. M. de Lederkremer, *J. Org. Chem.*, 1996, **61**, 1886–1889.
- 23 C. Gallo-Rodriguez, O. Varela and R. M. de Lederkremer, *Carbohydr. Res.*, 1998, **305**, 163–170.
- 24 C. Gallo-Rodriguez, M. A. Gil-Libarona, V. M. Mendoza and R. M. de Lederkremer, *Tetrahedron*, 2002, **58**, 9373–9380.
- 25 V. M. Mendoza, R. Agusti, C. Gallo-Rodriguez and R. M. de Lederkremer, *Carbohydr. Res.*, 2006, **341**, 1488–1497.
- 26 V. M. Mendoza, G. A. Kashiwagi, R. M. de Lederkremer and C. Gallo-Rodriguez, *Carbohydr. Res.*, 2010, **345**, 385–396.
- 27 R. Agusti, M. E. Giorgi, V. M. Mendoza, C. Gallo-Rodriguez and R. M. de Lederkremer, *Bioorg. Med. Chem.*, 2007, **15**, 2611–2616.
- 28 R. M. van Well, B. Y. M. Collet and R. A. Field, *Synlett*, 2008, 2175–2177.
- 29 D. Crich and V. J. Dudkin, *J. Am. Chem. Soc.*, 2001, **123**, 6819–6825, and references therein.
- 30 J.-R. Pougny and P. Sinaÿ, *Tetrahedron Lett.*, 1976, **17**, 4073–4076.
- 31 R. R. Schmidt, M. Behrendt and A. Toepfer, *Synlett*, 1990, 694–696.
- 32 A. J. Ratcliffe and B. Fraser-Reid, *J. Chem. Soc., Perkin Trans. 1*, 1990, 747–750.
- 33 L. Bohé and D. Crich, *C. R. Chim.*, 2011, **14**, 3–16.
- 34 (a) R. R. Schmidt and X. Zhu, Glycosyl Trichloroacetimidates, in *Glycoscience*, ed. B. Fraser-Reid, K. Tatsuta and J. Thiem Springer-Verlag, Berlin Heidelberg, 2008, pp. 451–453; (b) R. R. Schmidt and W. Kinzy, *Adv. Carbohydr. Chem. Biochem.*, 1994, **50**, 21–123.
- 35 P. Peltier, R. Euzen, R. Daniellou, C. Nugier-Chauvin and V. Ferrières, *Carbohydr. Res.*, 2008, **343**, 1897–1923.
- 36 M. R. Richards and T. L. Lowary, *ChemBioChem*, 2009, **10**, 1920–1938.
- 37 C. Marino, C. Gallo-Rodriguez and R. M. de Lederkremer, Galactofuranosyl-containing glycans: occurrence, synthesis and biochemistry, in *Glycans: Biochemistry, Characterization and Applications*, ed. H. M. Mora Montes, Nova Science Publishers, 2012, chapter X, pp. 207–268.
- 38 A. Imamura and T. Lowary, *Trends Glycosci. Glycotechnol.*, 2011, **23**, 134–152.
- 39 L. Baldoni and C. Marino, *J. Org. Chem.*, 2009, **74**, 1994–2003.
- 40 R. Pragani and P. H. Seeberger, *J. Am. Chem. Soc.*, 2011, **133**, 102–107.
- 41 R. Dureau, L. Legentil, R. Daniellou and V. Ferrières, *J. Org. Chem.*, 2011, **77**, 1301–1307.
- 42 L. Gandolfi-Donadio, M. Santos, R. M. de Lederkremer and C. Gallo-Rodriguez, *Org. Biomol. Chem.*, 2011, **9**, 2085–2097.
- 43 I. Chlubnova, D. Filipp, V. Spiwok, H. Dvorakova, R. Daniellou, C. Nugier-Chauvin, B. Kralova and V. Ferrières, *Org. Biomol. Chem.*, 2010, **8**, 2092–2102.
- 44 (a) Y. Bai and T. L. Lowary, *J. Org. Chem.*, 2006, **71**, 9672–9680; (b) G. C. Completo and T. L. Lowary, *J. Org. Chem.*, 2008, **73**, 4513–4525.
- 45 L. Cattiaux, B. Sendid, M. Collot, E. Machez, D. Poulain and J.-M. Mallet, *Bioorg. Med. Chem.*, 2011, **19**, 547–555.
- 46 L.-M. Deng, X. Liu, X.-Y. Liang and J.-S. Yang, *J. Org. Chem.*, 2012, **77**, 3025–3037.
- 47 Y. J. Lee, B.-Y. Lee, H. B. Jeon and K. S. Kim, *Org. Lett.*, 2006, **8**, 3971–3974.
- 48 (a) Y. J. Lee, D. B. Fulse and K. S. Kim, *Carbohydr. Res.*, 2008, **343**, 1574–1584; (b) K. S. Kim, B.-Y. Lee, S. H. Yoon, H. J. Jeon, J. Y. Baek and K.-S. Jeong, *Org. Lett.*, 2008, **10**, 2373–2376.
- 49 (a) L. Gandolfi-Donadio, C. Gallo-Rodriguez and R. M. de Lederkremer, *J. Org. Chem.*, 2002, **67**, 4430–4435; (b) L. Gandolfi-Donadio, C. Gallo-Rodriguez and R. M. de Lederkremer, *J. Org. Chem.*, 2003, **68**, 6928–6934; (c) L. Gandolfi-Donadio, C. Gallo-Rodriguez and R. M. de Lederkremer, *Carbohydr. Res.*, 2008, **343**, 1870–1875.
- 50 C. Gallo-Rodriguez, L. Gandolfi and R. M. de Lederkremer, *Org. Lett.*, 1999, **1**, 245–248.
- 51 G. Gola, P. Libenson, L. Gandolfi-Donadio and C. Gallo-Rodriguez, *ARKIVOC*, 2005, **xii**, 234–242.
- 52 (a) G. Gola, M. J. Tilve and C. Gallo-Rodriguez, *Carbohydr. Res.*, 2011, **346**, 1495–1502; (b) M. J. Tilve and C. Gallo-Rodriguez, *Carbohydr. Res.*, 2011, **346**, 2838–2848.
- 53 W. Klotz and R. R. Schmidt, *Liebigs Ann. Chem.*, 1993, 683–690.
- 54 M. Gelin, V. Ferrières, M. Lefeuvre and D. Plusquellec, *Eur. J. Org. Chem.*, 2003, 1285–1293.
- 55 J. D. C. Codée, B. Stubba, M. Schiattarella, H. S. Overkleef, C. A. A. van Boeckel, J. H. van Boom and G. A. van der Marel, *J. Am. Chem. Soc.*, 2005, **127**, 3767–3773.