Organic & Biomolecular Chemistry

PAPER

View Article Online
View Journal | View Issue

Cite this: *Org. Biomol. Chem.*, 2014, **12**, 3080

The versatile enzyme Araf51 allowed efficient synthesis of rare pathogen-related β-D-galactofuranosyl-pyranoside disaccharides

Ilona Chlubnová,[a,b,c] Blanka Králová,[c] Hana Dvořáková,[d] Petr Hošek,[c] Vojtěch Spiwok,[c] Dominik Filipp,[e] Caroline Nugier-Chauvin,*[a,b] Richard Daniellou†[a,b] and Vincent Ferrières*[a,b]

Received 17th December 2013,
Accepted 21st February 2014

DOI: 10.1039/c3ob42519c

www.rsc.org/obc

The preparation of galactofuranosyl-containing disaccharidic parts of natural glycoconjugates was performed according to a chemo-enzymatic synthesis. Our goals were firstly to develop an alternative approach to standard chemical strategies by limiting the number of reaction and purification steps, and secondly to evaluate the scope of the Araf51 biocatalyst to transfer a galactofuranosyl moiety to a set of pyranosidic acceptors differing from each other by the series, the anomeric configuration as well as the conformation. The study of binding mode of the resulting disaccharides was also performed by molecular modeling and showed significant differences between (1→2)- and (1→6)-linked disaccharides.

Introduction

The complex heterogeneity of carbohydrates in living systems is a direct result of several carbohydrate characteristics: the ability of different types and numbers of sugar residues to form glycosidic bonds with one another, the type of anomeric linkage, the position and the absence or presence of branching, the more or less flexible conformations of the resulting oligo-, polysaccharides or glycoconjugates,[1,2] and the size of the monosaccharidic ring.[3,4] Indeed, sugars exhibit significant differences depending on whether they are present as pyranosides or as furanosides. It is now well established that the importance of the furanose ring in biology can no longer be understated. A key characteristic of furanose ring systems is their higher flexibility compared to that of their pyranosidic counterparts,[5] and this profoundly influences their role in biological processes.[6] While furanosyl-containing oligosaccharides are crucial constituents of surface glycoconjugates in cell walls of bacteria,[7–9] fungi[10] and parasitic[11,12] microorganisms, including some clinically significant pathogens, with an exception of 2-deoxy-D-ribose and D-ribose, furanosides are completely absent from mammals. This makes them interesting targets for development of new therapeutics.

A major limitation preventing the use of oligosaccharides as therapeutics is the difficulty in producing sufficient amounts of these molecules in a desired purity. The isolation of these compounds from biological sources tends to be low yielding and presents the risk of contamination from infectious agents.[13] Although progress in the chemical synthesis of oligosaccharides has been made[14–20] and synthesis of docosana-arabinofuranoside realized by Lowary's research team[21] is impressive evidence, this approach still remains a challenge. The chemical synthesis requires stereo- and regioselective control of glycosidic bond formation, thus multiple protection and deprotection schemes are needed to achieve the required selectivity. Low yields of the desired products are also a result of the difficulty in purifying the deprotected compound along with product loss during each step of a multistep synthesis.[13,22] Nature's solution to the assembly of glycosidic bonds are enzymes belonging to the families of glycosyl transferases and of glycosidases. These enzymes have therefore enormous potential for the synthesis of biologically relevant carbohydrate structures.[23] These biocatalysts offer a significant advantage over their chemical counterparts in their ability to form a specific glycosidic linkage in the presence of other reactive functional groups.[22]

[a]*Ecole Nationale Supérieure de Chimie de Rennes, CNRS, UMR 6226, 11 Allée de Beaulieu, CS 50837, 35708 Rennes Cedex 7, France.*
E-mail: vincent.ferrieres@ensc-rennes.fr; Tel: +33 223238058
[b]*Université européenne de Bretagne, France*
[c]*Department of Biochemistry and Microbiology, Institute of Chemical Technology of Prague, Techniká 3, 166 28 Prague 6, Czech Republic*
[d]*Laboratory of NMR spectroscopy, Institute of Chemical Technology of Prague, Techniká 3, 166 28 Prague 6, Czech Republic*
[e]*Laboratory of Immunobiology, Institute of Molecular Genetics AS CR, Videnska 1083, 142 20 Prague 4, Czech Republic*
†Present address: Institut de Chimie Organique et Analytique (ICOA), UMR CNRS 7311, Université d'Orléans, BP 6759, Rue de Chartres, 45067 Orléans Cedex 2, France.

In this context, only few groups have been interested in the chemo-enzymatic synthesis of furanosyl-containing conjugates.[3,24–27] Furanosyl transferases are however rare and require both nucleotide-diphospho (NDP)-sugars as donors and suitable acceptors.[28–30] Another approach, recently proposed by Thorson, is based on the glycosyl transferase reversibility, that uses simple glycosides as precursors of NDP-sugars for further transfer onto more complex natural products.[31,32] To the best of our knowledge, this strategy was not applied for the synthesis of furanosides. Recently, our team developed an enzyme-based protocol for the preparation of di- and oligofuranosides using the thermophilic Araf51 as biocatalyst.[33] Considering the structural similarity between L-arabino- and D-galactofuranosyl (D-Galf) entities, and the wide presence of D-Galf residues in natural glycoconjugates and polysaccharides, we now propose to expand the chemo-enzymatic methodology to transglycosylation reactions in order to transfer a galactofuranosyl entity to a variety of pyranosidic acceptors (Fig. 1). These substrates were chosen so as to perform the synthesis of furanosyl-pyranoside sequences relevant to pathogenic microorganisms[3] as well as to estimate the specificity of this enzyme towards carbohydrate acceptors. As we expected that such pyranosides are resistant to hydrolysis by the furanosidase Araf51, the presence of the *p*NP group in the anomeric position was envisaged to enable simple UV detection of reaction products. Moreover, the D-gluco- (D-Glcp), D-galacto- (D-Galp) and D-mannopyranosidic (D-Manp) epimers, as well as their anomeric configurations, offer stereochemical variations that may influence the fate of the coupling process. Finally, we made a focus on the L-rhamnopyranoside (L-Rhap) **8** for structural and biological reasons but also because it displays a $^1C_4$ conformation instead a $^4C_1$ one observed for other acceptors **2–7**.

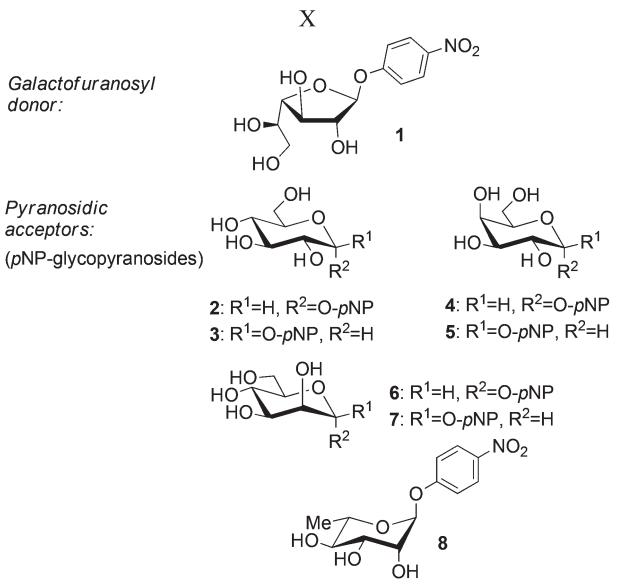


Fig. 1 Structure of glycosyl donor **1** and acceptors **2–8**.

Results

In the first place, unavailability of the −1 subsite of Araf51 to pyranosidic acceptors was confirmed spectrophotometrically (405 nm) by incubation of individual *p*NP pyranosides **2–8** with the enzyme for 2 h at 60 °C in a phosphate buffer at pH 7.4. No release of *p*-nitrophenolate was recorded, indicating that these compounds cannot act as donors, thus confirming our first hypothesis. Subsequently, to screen enzyme readiness to catalyze transglycosylation, individual analytical-scale reactions were performed starting from donor **1** and each of *p*NP glycopyranoside, and in the presence of Araf51. Monitoring by thin layer chromatography showed the formation of disaccharides when the donor **1** was incubated with an equal molar quantity or with a 5-fold molar excess of pyranosidic acceptor, after only short incubation periods (5–20 min). These compounds resulted from both self-condensation of **1** and desired transglycosylation reactions.

As a model of biocatalyzed coupling, the progress of the reaction of **1** with **2** (*p*NP α-D-Glcp) was monitored by HPLC (Fig. 2). Between 5 and 20 minutes, digalactofuranosides constituted the main reaction products.[33] At the same time, these products of self-condensation were hydrolyzed more rapidly than furanosyl-pyranoside disaccharides, which resisted to hydrolysis for more than 3 h. This could be explained by the structures of the resulting disaccharides which significantly differ from those of natural substrates of the enzyme. In the present model reaction, traces of trisaccharides were observed from the early stage of the reaction (5–10 min) and were confirmed by mass spectrum analysis, but precise structures could not be elucidated. Importantly, when the ratio donor/acceptor was increased to 1 : 10, no products of self-condensation were detected. Moreover, synthesis of furano-pyrano-disaccharides was strongly favored over hydrolysis of **1**. After careful chromatographic purification, two main disaccharides **9** and **10** were isolated and their structures were elucidated by NMR spectroscopy. The major product **9** was obtained in the yield of 44%. It exhibited an intense three-bond coupling in the ^{13}C–^1H (HMBC) spectrum between H-1′ (5.09 ppm) and C-2 (79.2 ppm) as well as C-1′ (109.4 ppm) and H-2 (3.69 ppm) and was identified as *p*NP β-D-galactofuranosyl-(1→2)-α-D-glucopyranoside **9** (Fig. 2). The second regioisomer, isolated in 34% yield, presented a correlation between H-1′ (5.24 ppm) and C-3 (79.4 ppm) as well as between C-1′ (108.3 ppm) and H-3 (3.96 ppm). The compound was identified as (1→3)-linked disaccharide **10**. The time-course analysis monitored by HPLC revealed that the (1→3)-disaccharide **10** was kinetically synthesized, followed by the formation of the (1→2)-isomer **9**. This monitoring also showed that these compounds disappeared with time in favor of a third regioisomer. The yield of the latter reached its maximum after 4 hours of reaction and it was still present after 24 hours. The precise nature of the corresponding glycosidic linkage could however not be clearly elucidated. As a result, the overall yield of transglycosylation reached a maximum, slightly greater than 80%, between 5 and 20 minutes. In the present model reaction, traces of trisacchar-

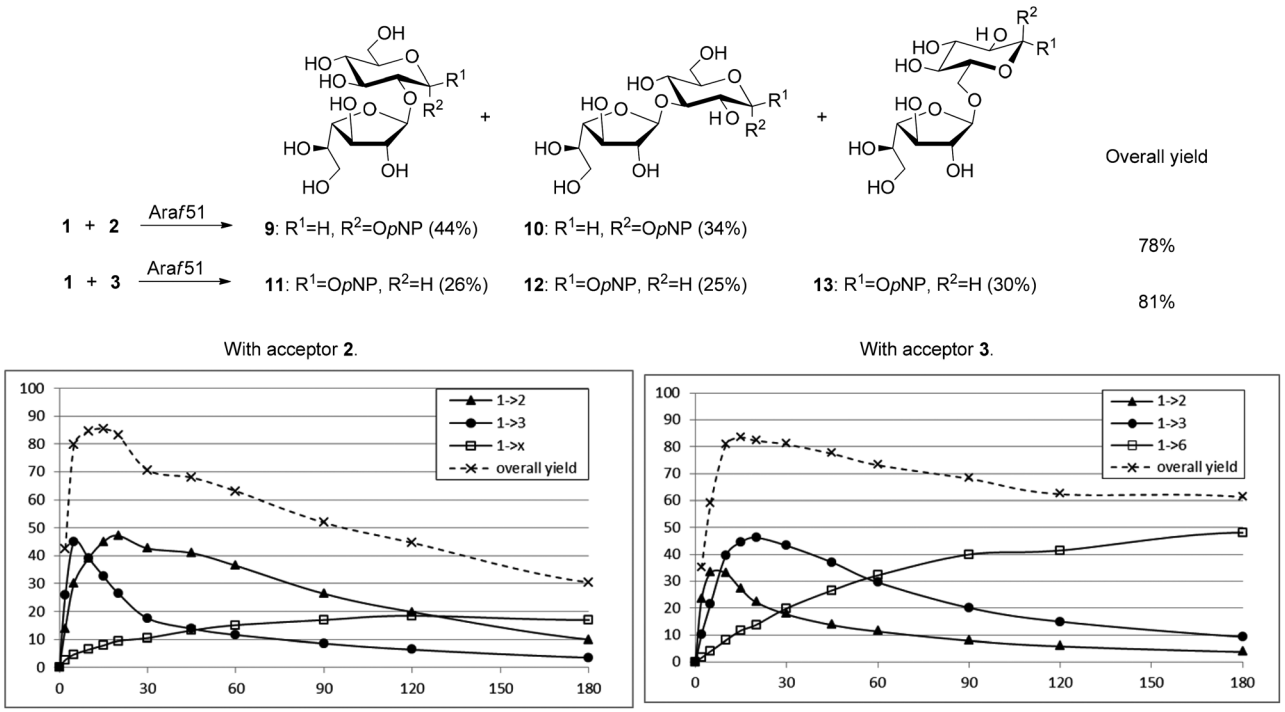


Fig. 2 Progress of the reaction of **1** with **2** or **3** catalyzed by Araf51.

ides were observed from the early stage of the reaction (5–10 minutes) and confirmed by mass spectrum analysis, but precise structures could not be elucidated.

Under similar conditions, using the β-glucopyranoside **3** as an acceptor (Fig. 2), and after 20 minutes of reaction, three disaccharides were chromatographically separated: *p*NP β-D-Gal*f*-(1→2)-β-D-Glc*p* **11**, *p*NP β-D-Gal*f*-(1→3)-β-D-Glc*p* **12**, and *p*NP β-D-Gal*f*-(1→6)-β-D-Glc*p* **13** were isolated in 26%, 25% and 30% yields, respectively. The time-course analysis revealed that the (1→2)-linked regioisomer **11** was the major kinetic product and is present together with the (1→3)-linked regioisomer **12** from the first minutes of the reaction. From about 15 minutes, the (1→6)-linked regioisomer **13** was formed and became the prevalent one after 1 hour. It was still detected in 15% yield after 24 hours. It is interesting to note that, when **12** was incubated with the biocatalyst and an excess of **3**, it was transformed into **13**. This emphasizes the striking stability of this (1→6)-bond in the presence of the furanosyl hydrolase Araf51.

To go further with this methodology, we applied the Araf-51-assisted synthesis of furanosyl-containing disaccharides to three other families of acceptors. The use of galactosidic acceptors **4** and **5** did not fundamentally change the observations previously exposed with the *gluco* series. Three disaccharides with (1→2)- (**14**), (1→3)- (**15**), and (1→4)-linkage (**16**) were obtained from the α-anomer **4** and isolated in 73% overall yield (Table 1, entry 1). Substrate **5**, characterized by a β-configuration, afforded only the (1→2)- (**17**) and (1→6)-disaccharides (**18**) in a near equimolecular ratio and in a 52% overall yield (entry 2).

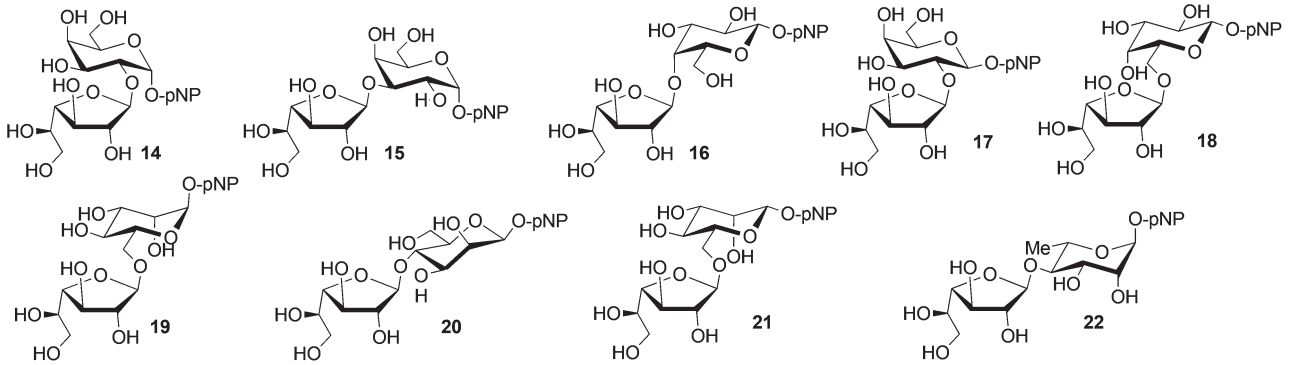
On the other hand, the reaction with the α-mannopyranoside **6** (entry 3) was relatively rapid compared to other pyranosidic acceptors and the degree of hydrolysis of formed disaccharides was slightly higher. The reaction in a preparative scale was thus carried out for only 10 minutes. *p*NP β-D-Gal*f*-(1→6)-α-D-Man*p* **19** was obtained in 15% yield. 2-D NMR analyses confirmed that three other regioisomers were obtained as a mixture in an overall yield of 43%. The time-course analysis monitored by TLC revealed that these regioisomers are readily formed from the first minutes of reaction with the maximum yields reached between 5 and 10 minutes. The (1→6)-linked regioisomer **19** was formed from about 5 minutes of reaction.

The transglycosylation with the β-anomer **7** was apparently slower than that of the α-anomer and the conversion of *p*NP β-D-Gal*f* still did not reach a maximum after two hours of reaction. However, the degree of hydrolysis was quite low, similarly to the other *p*NP pyranosides. After 25 minutes of the preparative-scale reaction, two regioisomers were isolated (entry 4). *p*NP β-D-Gal*f*-(1→4)-β-D-Man*p* **20** was obtained in a very high yield of 49%. The second regioisomer **21**, identified as the (1→6)-disaccharide, was isolated in 16% yield. The time-course analysis confirmed kinetic preferences for the (1→4)-linked disaccharide **20**. In about 40 minutes of reaction, both regioisomers were present equivalently. At the end of 2 hours incubation, the favoured (1→6)-linked regioisomer **21** prevailed. Structures were unambiguously established according to NMR data.

Finally, we also studied the L-rhamnopyranoside **8** since it presents significantly different reactivity and because it is widely found in Nature. While three disaccharides were

Table 1 Araf51-mediated synthesis of disaccharides **9–22** using pNP β-D-Galf **1** as donor and the linkages identified in individual regioisomers together with the isolated yields after two hours of reaction

			Product (yield, %)				
Entry	Acceptor	Time (min)	(1→2)	(1→3)	(1→4)	(1→6)	Overall yield (%)
1	**4** (α-D-Gal*p*)	20	**14** (41)	**15** (32)	**16**		73
2	**5** (β-D-Gal*p*)	20	**17** (27)			**18** (25)	52
3	**6** (α-D-Man*p*)	15	Mixture (1→2/3/4) (43)			**19** (15)	58
4	**7** (β-D-Man*p*)	25			**20** (49)	**21** (16)	65
5	**8** (α-L-Rha*p*)	20			**22** (38)		38



observed after 20 minutes of the biocatalytic process, exclusively one regioisomer **22**, characterized by a (1→4)-connection, was indeed isolated and in a fairly good 38% yield (entry 5). Evidence of the precise structure was established on the basis of NMR data. More specifically, the C-4 signal in the starting substrate **8** ($\delta_{C\text{-}4}$ = 71.4 ppm) was shifted to 77.8 ppm within the disaccharide **22**, thus demonstrating the (1→4)-coupling, and this result was corroborated by 2D NMR experiments.

Discussion

All results obtained for the transfer of a galactofuranosyl residue to pyranosidic acceptors mediated by the arabinofuranosidase Araf51 underline the ability of this enzyme to accept within its +1 subsite all the seven tested pyranosidic acceptors. Importantly, the anomeric configuration of the latter modulated the behavior of the furanosyl transfer. Usually, the (1→2)-disaccharides were the most common in the *gluco* and *galacto* series, and readily formed and stable. The (1→3) linkage was formed subsequently and was also frequently presented in these series. Interestingly, none of these α-anomers displayed the (1→6)-connection although for the β-anomers, it represents the major thermodynamically formed linkage. In Nature, *exo*-acting α-L-arabinofuranosidases release the arabinosyl decorations at C-2 and C-3 position of arabinogalactans and arabinoxylans from a range of plant structural polysaccharides, where both the galactose and xylose moieties are presented in a β-D-pyranose form. Thus, considering the structural similarity of xylose and glucose, the transglycosylation preferences correspond to the hydrolytic activity on natural substrates.

Starting from β-D-mannopyranoside and α-L-rhamnopyranoside, the kinetically preferred linkage was the (1→4). It results from this observation that the adopted conformations within the active site of Man*p* and Rha*p* derivatives vary to some extent compared to the glucosidic and galactosidic monosaccharides. Thus the axial C-2 hydroxyl group in mannose, in contrast to glucose, seems to impact the conformation within the active site more markedly than the orientation of C-4 OH group distinguishing glucose from galactose. Overall, in all the three series where both anomers were tested (D-Glc*p*, D-Gal*p*, D-Man*p*), the reactions involving the α-anomer proceeded markedly faster than those of their β-anomeric counterparts. The time-course analyses also revealed the crucial effect of the reaction time on the ratio of regioisomers formed in individual reactions. Thus, in most of cases, it is possible to isolate either kinetic or thermodynamic disaccharide with a very low quantity of the other regioisomer with respect to the time-course of the reaction.

In order to elucidate binding modes of acceptors **2–7** in transglycosylation reactions, complexes of Araf51 with all putative transglycosylation products were subjected to molecular dynamics simulations (24 × 10 ns). Each putative product was docked into the active site by rigid fit of its Gal*f* moiety onto the Ara*f* moiety in the experimental structure. The pyranosyl and *p*NP-moieties were adjusted manually before the simulation. The results of simulations for disaccharides **11**, **14**, **17** and **18** are shown in Fig. 3. These selected products showed stable binding in the active site illustrated by low root-mean-square deviation (RMSD). The results also demonstrated that binding modes of (1→2) products are very similar. The *p*NP-moiety is oriented perpendicularly to the access of the active site. On the other hand, the *p*NP-moiety in **18** [β-D-Gal*f*-(1→6)-

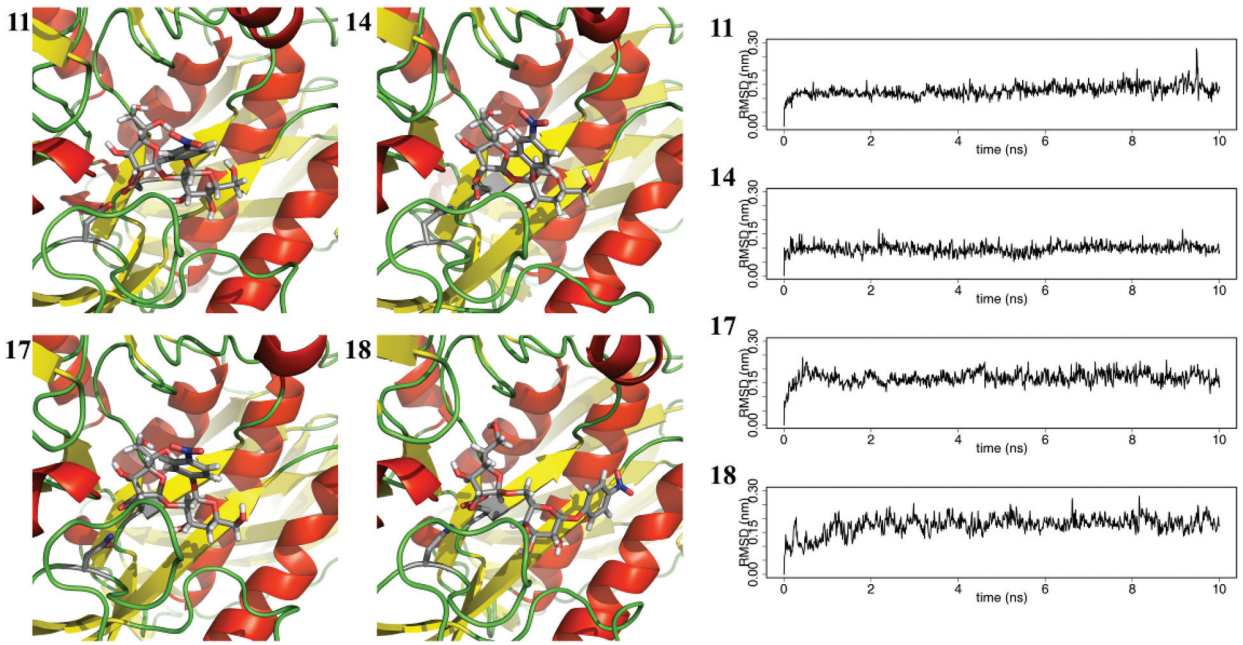


Fig. 3 Predicted binding modes of **11**, **14**, **17** and **18** by 10 ns molecular dynamics simulations. The figure shows predicted binding modes at the end of each simulation (left) and corresponding RMSD profiles (right). These were obtained by fitting protein Cα atoms and calculating RMSD for the product.

β-D-Gal*p*], as an example of (1→6) product, is placed along the axis of the active site. The fact that the *p*NP-moiety may orient in different angles explains relatively broad acceptor specificity. Most other putative products have shown high RMSD which indicate either inability of the product to bind into the active site or incorrect docking. Unfortunately, attempts to quantitatively predict acceptor preferences from molecular simulations or free-energy methods were not successful (not shown).

Among the disaccharides prepared throughout this part, several display a configuration reported from uncommon cell wall glycoconjugates of some pathogenic species. Galactofuranosyl residue connected to D-Glc*p* moiety through the (1→3)-linkage has been identified *e.g.* in *Streptococcus* species,[34] the one with the (1→6)-linkage is reported from *Escherichia coli* K-12 strain.[35] The (1→3)-linkage to D-Gal*p* was identified in *Fibrobacter succinogenes*[36] or in the O-antigen repeating unit of *Klebsiella pneumoniae*.[36] In *Mycobacterium* species[37–40] and *Plesiomonas shigelloides*,[41] β-D-Gal*f* is connected to L-Rha*p* through the (1→4)-linkage. The sequence β-D-Gal*f*-(1→2)-D-Man*p* is present in many microorganisms, among the pathogens notably *Cryphonectria parasitica*[42] or *Trichoderma*.[43] β-D-Gal*f*-(1→3)-D-Man*p* is frequent in *Aspergillus*,[44] *Trypanosoma cruzi*[12,45] and *Leishmania*,[11] β-D-Gal*f*-(1→6)-D-Man*p* *e.g.* in *Aspergillus*,[44] *Leishmania*[11] or *Paracoccidioides brasiliensis*.[46]

Conclusions

A chemo-enzymatic synthesis of galactofuranosyl-containing disaccharides was proposed mediated by the thermophilic arabinofuranosidase Ara*f*51. We have first demonstrated that a large excess of acceptor allowed overcoming the self-condensation side reaction. Secondly, transglycosylation products were obtained in the early stage of the biocatalyzed process and increased reaction times did not affect the target furanosyl-pyranoside disaccharides since the degree of hydrolysis of the latter remained very low. These factors together were fruitful from a synthetic point of view. Consequently, and with regard to the very good yields obtained with the assistance of the hydrolytic furanosidase Ara*f*51, this chemo-enzymatic approach constitutes a very interesting alternative to multi-step chemical synthesis of various mimetics of biologically significant structures. The +1 subsite of Ara*f*51 was able to recognize simple glycopyranosides as acceptors, even a Rha*p* derivative and its $^{1}C_{4}$ conformation. Among targets were prepared disaccharides biosynthesized by *Mycobacteria*, *Leishmania*, *Trypanosoma* or *Paracoccidioides* microorganisms.

Experimental section

General remarks

Prior to NMR analysis, fractions were exchanged in D_2O (99.9% purity) at room temperature with intermediate freeze-drying, and then dissolved in 400 μL of D_2O. ^{1}H, ^{13}C, COSY, HSQC, HMBC, TOCSY and NOESY NMR spectra were recorded at the Laboratory of NMR spectroscopy (ICT Prague, Czech Republic) on a Bruker 600 Avance spectrometer equipped with a cryoprobe at 600 MHz for ^{1}H and 125 MHz for ^{13}C, and on a Bruker ARX 400 at 400 MHz for ^{1}H, 100 MHz for ^{13}C at ENSCR (France). Chemical shifts are given in δ-units (ppm). Coupling constants *J* are given in Hz.

The HRMS were measured at the Centre Régional de Mesures Physiques de l'Ouest (CRMPO, Université de Rennes 1, France) with a MS/MS ZabSpec TOF Macromass using *m*-nitrobenzylic alcohol as the matrix and accelerated caesium ions for ionization and at the Laboratory of Mass Spectrometry (ICT Prague, Czech Republic) using a Q-TOF Micro (Waters, USA), where electrospray-ionisation mass spectra (ESI-MS) were recorded on samples dissolved in MeOH injected in a volume of 2–5 μL into a flow (100 μL min^{-1}) of MeOH. Sample cone voltage was 42 V and the source temperature was 150 °C. Measurements were performed in positive ($[M + Na]^+$ ion detection) mode in the range of 100–1000 Da. Finally, at the Mass Spectrometry Group (Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic) on LTQ-Orbitrap XL (THERMO), where ESI+ spectra were recorded on samples dissolved in MeOH–H_2O (1 : 1), sample cone voltage was 40 V.

Thin layer chromatography (TLC) analyses were conducted on Kieselgel 60 F254 (Merck) plates with 0.2 mm layer thickness. Spots were visualized by UV (254 nm) and by exposure to 0.2% w/v orcinol in H_2SO_4 (20% v/v) in ethanol. For column chromatography, either Si 60 (40–63 μm) Silica gel or prepacked Chromabond®Flash RS 15 SiOH columns (Macherey-Nagel) were used.

Gel permeation chromatography was performed on P-2 Bio-Gel (Bio-Rad) using FPLC system consisting of a solvent delivery system Biologic F40 DuoFlow, Biologic QuadTec UV-Vis Detector and Biologic BioFrac Fraction Collector (all Bio-Rad). Deionized filtrated (0.22 μm PVDF membrane, Millipore) water was used as a mobile phase with a flow rate of 0.15 mL min^{-1}. Separation was monitored by UV absorbance at 280 and 405 nm by operating software Biologic DuoFlow. Collected fractions were lyophilized (FreeZone Freeze Dry System, Labconco).

Recombinant arabinofuranosidase Araf51 was produced in *E. coli* and purified as already described.[47] The hydrolytic activity toward *p*-nitrophenyl pyranosides was tested by incubation of the solution of enzyme (obtained after affinity Ni-NTA chromatography and exchanged to 50 mM PBS pH 7.4 by PD10 gel chromatography) with individual glycopyranosides (5 mM) in 50 mM potassium phosphate buffer pH 7.4 at 60 °C. The release of *p*-nitrophenolate was continuously measured at 405 nm (Microplate Spectrophotomerer PowerWave XS/XS2, BioTek) and data evaluated with Gen5 Data Analysis Software (BioTek). *p*NP α-D-Gal*p*, *p*NP β-D-Gal*p*, *p*NP α-D-Glc*p*, *p*NP β-D-Glc*p*, *p*NP α-D-Man*p*, *p*NP β-D-Man*p* and *p*NP α-L-Rha*p* are commercially available.

General procedure for the transglycosylation reactions

Prior to preparative-scale reactions, the time-course analyses were performed in the analytical scale (5 μmol of individual *p*NP pyranosides, 0.5 μmol of *p*NP β-D-galactofuranoside and 36 U of Araf51 in 100 μL of 50 mM PBS pH 7.4). The reaction was monitored by TLC (7 : 2 : 2 EtOAc–AcOH–H_2O). Preparative-scale reactions were performed at 60 °C by incubation of the *p*NP β-D-galactofuranoside (20 mg, 66 μmol; or 10 mg, 33 μmol) with individual *p*NP pyranosides (200 mg, 660 μmol for *p*NP D-Glc*p* and *p*NP D-Gal*p*; 100 mg, 330 μmol for *p*NP D-Man*p* and *p*NP L-Rha*p*) and 4800 U of Araf51 (for reactions with *p*NP D-Glc*p* and *p*NP D-Gal*p*) or 2400 U of Araf51 (for reactions with *p*NP D-Man*p* and *p*NP L-Rha*p*) in 50 mM PBS pH 7.4 in a total volume with regard to the solubility of substrates: 5 mL (reactions with α- and β-D-Gal*p* and β-D-Glc*p*) or 10 mL (α-D-Glc*p* and α- and β-D-Man*p*) or 15 mL (α-L-Rha*p*).

Based on the results from analytical-scale reactions, individual reactions proceeded for 20 min with the exception of α-D-Man*p* (15 min) and β-D-Man*p* (25 min) to obtain the maximum ratio of transglycosylation/hydrolysis. The reactions were stopped by enzyme denaturation at 100 °C for 10 min. The reaction products were repeatedly separated by silica gel flash chromatography using EtOAc–AcOH–H_2O in ratios from 15 : 1 : 1 to 40 : 1 : 1. Separations were monitored by TLC, fractions corresponding to individual regioisomers were collected, evaporated and lyophilized several times with intermediate dissolving in H_2O, finally in D_2O and subjected to structural analyses.

***p*-Nitrophenyl β-D-galactofuranosyl-(1→2)-α-D-glucopyranoside (9).** This compound was obtained according to the described general procedure by incubation of 20 mg (66 μmol) of **1** with 200 mg (660 μmol) of *p*NP α-D-glucopyranoside **2** in the presence of 4800 U of Araf51 and was isolated in 44% yield (13.3 mg) after purification. TLC: R_f = 0.45 (AcOEt–AcOH–H_2O, 7/2/2). ^{1}H NMR (400 MHz, D_2O): δ = 8.19 (d, 2H, J = 9.2 Hz, H*m*, C_6H_4), 7.23 (d, 2H, J = 9.2 Hz, H*o*, C_6H_4), 5.87 (d, 1H, J = 3.6 Hz, 1a-H), 5.08 (d, 1H, J = 2.0 Hz, 1b-H), 4.07 (dd, 1H, J = 2.0, 4.2 Hz, 2b-H), 3.93 (dd, 1H, J = 4.2, 6.8 Hz, 3b-H), 3.92 (dd, 1H, J = 9.0, 9.8 Hz, 3a-H), 3.70 (dd, 1H, J = 3.6, 9.8 Hz, 2a-H), 3.60–3.50 (m, 3H, 6a-H, 5a-H), 3.56 (dd, 1H, J = 3.1, 6.8 Hz, 4b-H), 3.53–3.47 (m, 1H, 5b-H), 3.50 (dd, 1H, J = 9.0, 9.8 Hz, 4a-H), 3.10 (dd, 1H, J = 8.1, 11.5 Hz, 6b-H), 2.86 (dd, 1H, J = 4.0, 11.5 Hz, 6′b-H) ppm. ^{13}C NMR (100 MHz, D_2O): δ = 161.6 (C*ipso* C_6H_4), 142.7 (C*p* C_6H_4), 126.1 (C*m* C_6H_4), 116.7 (C*o* C_6H_4), 109.5 (1b-C), 96.3 (1a-C), 82.6 (4b-C), 81.1 (2b-C), 79.3 (2a-C), 76.1 (3b-C), 72.4 (5a-C), 71.5 (3a-C), 69.7 (5b-C), 69.0 (4a-C), 62.7 (6b-C), 60.1 (6a-C) ppm. HRMS (ESI): *m*/*z* calcd for $C_{18}H_{25}O_{13}NNa$ $[M + Na]^+$ 486.12181; found 486.12173.

***p*-Nitrophenyl β-D-galactofuranosyl-(1→3)-α-D-glucopyranoside (10).** This compound was obtained according to the described general procedure by incubation of **1** (20 mg, 66 μmol) with *p*NP α-D-glucopyranoside **2** (200 mg, 660 μmol) in the presence of 4800 U of Araf51. It was isolated in 34% yield (10.5 mg). TLC: R_f = 0.5 (AcOEt–AcOH–H_2O, 7/2/2). ^{1}H NMR (400 MHz, D_2O): δ = 8.12 (d, 2H, J = 9.2 Hz, H*m*, C_6H_4), 7.18 (d, 2H, J = 9.2 Hz, H*o*, C_6H_4), 5.70 (d, 1H, J = 3.5 Hz, 1a-H), 5.24 (d, 1H, J = 1.5 Hz, 1b-H), 4.10 (dd, 1H, J = 1.5, 3.5 Hz, 2b-H), 4.03–3.99 (m, 2H, 3b-H, 4b-H), 3.96 (dd, 1H, J = 9.2, 9.6 Hz, 3a-H), 3.80 (dd, 1H, J = 3.5, 9.6 Hz, 2a-H), 3.76 (ddd, 1H, J = 3.2, 4.4, 7.6 Hz, 5b-H), 3.67–3.57 (m, 5H, 6a-H, 6′a-H, 6b-H, 6′b-H, 5a-H), 3.48 (dd, 1H, J = 9.2, 9.6 Hz, 4a-H) ppm. ^{13}C NMR (100 MHz, D_2O): δ = 161.1 (C*ipso* C_6H_4), 141.9 (C*p* C_6H_4), 125.7 (C*m* C_6H_4), 116.4 (C*o* C_6H_4), 108.3 (1b-C), 96.6

(1a-C), 82.9 (4b-C), 81.2 (2b-C), 79.4 (3a-C), 76.7 (3b-C), 72.7 (5a-C), 70.8 (2a-C), 70.6 (5b-C), 67.5 (4a-C), 62.8 (6b-C), 60.1 (6a-C) ppm. HRMS (ESI): *m*/*z* calcd for $C_{18}H_{25}O_{13}NNa$ $[M + Na]^+$ 486.12181; found 486.12160.

***p*-Nitrophenyl β-D-galactofuranosyl-(1→2)-β-D-glucopyranoside (11).** This compound was obtained according to the described general procedure by incubating **1** (20 mg, 66 μmol) with *p*NP β-D-glucopyranoside **3** (200 mg, 660 μmol) in the presence of 4800 U of Araf51, and was isolated in 26% yield (7.9 mg). TLC: R_f = 0.4 (AcOEt–AcOH–H_2O, 7/2/2). ^{1}H NMR (400 MHz, D_2O): δ = 8.18 (d, 2H, J = 9.2 Hz, H*m*, C_6H_4), 7.17 (d, 2H, J = 9.2 Hz, H*o*, C_6H_4), 5.28 (d, 1H, J = 7.2 Hz, 1a-H), 5.26 (d, 1H, J = 1.4 Hz, 1b-H), 4.03 (dd, 1H, J = 1.4, 2.5 Hz, 2b-H), 3.94–3.90 (m, 2H, 3b-H, 4b-H), 3.81 (dd, 1H, J = 5.7, 12.4 Hz, 6a-H), 3.70–3.65 (m, 2H, 5b-H, 2a-H), 3.65 (dd, 1H, J = 9.5, 9.5 Hz, 3a-H), 3.64 (dd, 1H, J = 2.2, 12.4 Hz, 6′a-H), 3.59–3.55 (m, 1H, 5a-H), 3.44 (dd, 1H, J = 4.3, 11.6 Hz, 6b-H), 3.43 (t, 1H, J = 9.5 Hz, 4a-H), 3.39 (dd, 1H, J = 7.3, 11.6 Hz, 6′b-H) ppm. ^{13}C NMR (100 MHz, D_2O): δ = 161.6 (C*ipso* C_6H_4), 142.7 (C*p* C_6H4), 126.1 (C*m* C_6H_4), 116.5 (C*o* C_6H_4), 108.3 (1b-C), 98.1 (1a-C), 83.7 (4b-C), 81.0 (2b-C), 78.2 (2a-C), 76.7 (3b-C), 76.1 (5a-C), 75.6 (3a-C), 70.8 (5b-C), 69.0 (4a-C), 62.7 (6b-C), 60.3 (6a-C) ppm. HRMS (ESI): *m*/*z* calcd for $C_{18}H_{25}O_{13}NNa$ $[M + Na]^+$ 486.12181; found 486.12173.

***p*-Nitrophenyl β-D-galactofuranosyl-(1→3)-β-D-glucopyranoside (12).** This disaccharide was prepared as described in the general procedure starting from donor **1** (20 mg, 66 μmol) and *p*NP β-D-glucopyranoside **3** (200 mg, 660 μmol) in the presence of 4800 U of Araf51. The target compound **12** was isolated in 25% yield (7.6 mg) after purification. TLC: R_f = 0.48 (AcOEt–AcOH–H_2O, 7/2/2). ^{1}H NMR (400 MHz, D_2O): δ = 8.18 (d, 2H, J = 9.2 Hz, H*m*, C_6H_4), 7.17 (d, 2H, J = 9.2 Hz, H*o*, C_6H_4), 5.26 (d, 1H, J = 1.7 Hz, 1b-H), 5.21 (d, 1H, J = 7.6 Hz, 1a-H), 4.10 (dd, 1H, J = 1.7, 3.3 Hz, 2b-H), 4.02 (dd, 1H, J = 3.8, 6.4 Hz, 4b-H), 4.01 (ddd, 1H, J = 0.4, 3.3, 6.4 Hz, 3b-H), 3.87–3.84 (m, 1H, 6a-H), 3.77–3.72 (m, 1H, 5b-H), 3.72–3.68 (m, 1H, 3a-H), 3.70–3.66 (m, 2H, 2a-H, 6′a-H), 3.66–3.57 (m, 2H, 5a-H, 6b-H), 3.61–3.55 (m, 1H, 6′b-H), 3.49 (dd, 1H, 4a-H) ppm. ^{13}C NMR (100 MHz, D_2O): δ = 161.6 (C*ipso* C_6H_4), 142.7 (C*p* C_6H_4), 126.1 (C*m* C_6H_4), 116.5 (C*o* C_6H_4), 108.1 (1b-C), 99.2 (1a-C), 83.0 (4b-C), 81.5 (2a-C), 81.1 (2b-C), 76.6 (3b-C), 76.0 (5a-C), 72.8 (3a-C), 70.6 (5b-C), 67.7 (4a-C), 62.8 (6b-C), 60.5 (6a-C) ppm. HRMS (ESI): *m*/*z* calcd for $C_{18}H_{25}O_{13}NNa$ $[M + Na]^+$ 486.12181; found 486.12171.

***p*-Nitrophenyl β-D-galactofuranosyl-(1→6)-β-D-glucopyranoside (13).** This compound was synthesized as described in the general procedure using **1** (20 mg, 66 μmol), *p*NP β-D-glucopyranoside **3** (200 mg, 660 μmol), and 4800 U of Araf51. The desired disaccharide **13** was isolated in 30% yield (9.2 mg). TLC: R_f = 0.4 (AcOEt–AcOH–H_2O, 7/2/2). ^{1}H NMR (600 MHz, D_2O): δ = 8.22 (d, 2H, J = 9.1 Hz, H*m*, C_6H_4), 7.21 (d, 2H, J = 9.1 Hz, H*o*, C_6H_4), 5.22 (d, 1H, J = 2.7 Hz, 1a-H), 4.94 (d, 1H, J = 1.5 Hz, 1b-H), 4.04–3.97 (m, 2H, 6a-H, 2b-H), 4.98 (dd, 1H, J = 3.4, 6.2 Hz, 3b-H), 3.90–3.86 (m, 1H, 4b-H), 3.80–3.71 (m, 3H, 5a-H, 5b-H, 6′a-H), 3.57–3.48 (m, 5H, 3a-H, 2a-H, 6b-H, 6′b-H, 4a-H) ppm; ^{13}C NMR (125 MHz, D_2O): δ = 161.6 (C*ipso* C_6H_4), 142.7 (C*p* C_6H_4), 126.1 (C*m* C_6H_4), 116.5 (C*o* C6H4), 107.9 (1b-C), 99.3 (1a-C), 83.0 (4b-C), 81.0 (2b-C), 76.8 (3b-C), 75.3 (3a-C), 75.2 (5a-C), 72.7 (2a-C), 70.8 (5b-C), 69.4 (4a-C), 66.3 (6a-C), 62.7 (6b-C) ppm. HRMS (ESI): *m*/*z* calcd for $C_{18}H_{25}O_{13}NNa$ $[M + Na]^+$ 486.12181; found 486.12172.

***p*-Nitrophenyl β-D-galactofuranosyl-(1→2)-α-D-galactopyranoside (14).** This compound was obtained according to the general procedure starting from donor **1** (20 mg, 66 μmol), acceptor **4** (200 mg, 660 μmol) and 4800 U of Araf51. Compound **14** was isolated in 41% yield (12.6 mg). TLC: R_f = 0.4 (AcOEt–AcOH–H_2O, 7/2/2). ^{1}H NMR (400 MHz, D_2O): δ = 8.19 (d, 2H, J = 9.2 Hz, H*m*, C_6H_4), 7.23 (d, 2H, J = 9.2 Hz, H*o*, C_6H_4), 5.89 (d, 1H, J = 3.2 Hz, 1a-H), 5.08 (s, 1H, 1b-H), 4.10 (dd, 1H, J = 3.3, 10.4 Hz, 3a-H), 4.07 (dd, 1H, J = 2.1, 4.3 Hz, 2b-H), 3.99 (dd, 1H, J = 2.0, 3.3 Hz, 4a-H), 3.93 (ddd, 1H, J = 0.7, 4.3, 6.9 Hz, 3b-H), 3.91 (dd, 1H, J = 3.7, 10.3 Hz, 2a-H), 3.89 (ddd, 1H, J = 1.9, 4.7, 7.7 Hz, 5a-H), 3.67–3.57 (m, 2H, 6a-H, 6′a-H), 3.56 (dd, 1H, J = 2.9, 6.9 Hz, 4b-H), 3.52 (ddd, 1H, J = 2.9, 4.2, 8.2 Hz, 5b-H), 3.42 (dd, 1H, J = 4.0, 6.4 Hz, 6b-H), 3.11 (dd, 1H, J = 8.0, 11.2 Hz, 6′b-H) ppm. ^{13}C NMR (100 MHz, D_2O): δ = 161.6 (C*ipso* C_6H_4), 142.7 (C*p* C_6H_4), 126.2 (C*m* C_6H_4), 116.8 (C*o* C_6H_4), 109.7 (1b-C), 96.6 (1a-C), 82.5 (4b-C), 81.2 (2b-C), 76.2 (2a-C and 3b-C), 71.8 (5a-C), 69.6 (5b-C), 69.0 (4a-C), 68.0 (3a-C), 62.6 (6b-C), 60.8 (6a-C) ppm. HRMS (ESI): *m*/*z* calcd for $C_{18}H_{25}O_{13}NNa$ $[M + Na]^+$ 486.12181; found 486.12163.

***p*-Nitrophenyl β-D-galactofuranosyl-(1→3)-α-D-galactopyranoside (15).** The disaccharide **15** was obtained according to the described general procedure by incubation of donor **1** (20 mg, 66 μmol) with acceptor **4** (200 mg, 660 μmol) in the presence of 4800 U of Araf51, and was isolated in regioisomeric mixture with **16** in 32% yield (9.9 mg). According to the ^{1}H NMR signal integration, it was obtained in 18% yield. TLC: R_f = 0.48 (AcOEt–AcOH–H_2O, 7/2/2). ^{1}H NMR (400 MHz, D_2O): δ = 8.19 (d, 2H, J = 9.2 Hz, H*m*, C_6H_4), 7.23 (d, 2H, J = 9.2 Hz, H*o*, C_6H_4), 5.74 (d, 1H, J = 3.8 Hz, 1a-H), 5.12 (d, 1H, J = 2.1 Hz, 1b-H), 4.10 (dd, 1H, J = 3.7, 10.2 Hz, 3a-H), 4.07 (dd, 1H, J = 2.2, 4.7 Hz, 2b-H), 4.02 (dd, 1H, J = 1.3, 3.0 Hz, 4a-H), 3.97 (dd, 1H, J = 3.7, 10.4 Hz, 2a-H), 3.95 (dd, 1H, J = 4.4, 7.1 Hz, 3b-H), 3.92–3.87 (m, 1H, 5a-H), 3.85 (dd, 1H, J = 3.7, 7.1 Hz, 4b-H), 3.72–3.67 (m, 1H, 5b-H), 3.57–3.50 (m, 4H, 6a-H, 6′a-H, 6b-H, 6′b-H) ppm; ^{13}C NMR (100 MHz, D_2O): δ = 161.6 (C*ipso* C_6H_4), 142.7 (C*p* C_6H_4), 126.2 (C*m* C_6H_4), 116.8 (C*o* C_6H_4), 108.9 (1b-C), 96.6 (1a-C), 82.2 (4b-C), 81.4 (2b-C), 76.1 (3b-C), 75.6 (4a-C), 71.9 (5a-C), 70.1 (5b-C), 69.6 (3a-C), 67.8 (2a-C), 62.7 (6b-C), 61.3 (6a-C) ppm. HRMS (ESI): *m*/*z* calcd for $C_{18}H_{25}O_{13}NNa$ $[M + Na]^+$ 486.12181; found 486.12161.

***p*-Nitrophenyl β-D-galactofuranosyl-(1→4)-α-D-galactopyranoside (16).** This compound was synthesized according to the described general procedure starting from **1** (20 mg, 66 μmol), *p*NP α-D-galactopyranoside **4** (200 mg, 660 μmol), 4800 U of Araf51, and was isolated in regioisomeric mixture with **15** in 32% yield (9.9 mg). According to the ^{1}H NMR signal integration, this compound was obtained in 14% yield. TLC: R_f = 0.5 (AcOEt–AcOH–H_2O, 7/2/2). ^{1}H NMR (400 MHz, D_2O): δ = 8.19 (d, 2H, J = 9.2 Hz, H*m*, C_6H_4), 7.23 (d, 2H, J = 9.2 Hz, H*o*,

C_6H_4), 5.75 (d, 1H, J = 3.2 Hz, 1a-H), 5.15 (d, 1H, J = 1.8 Hz, 1b-H), 4.10 (dd, 1H, J = 1.8, 3.6 Hz, 2b-H), 4.09–4.02 (m, 2H, 4a-H, 3a-H), 4.03 (dd, 1H, J = 3.3, 10.4 Hz, 2a-H), 3.96 (ddd, 1H, J = 0.9, 3.4, 6.7 Hz, 3b-H), 3.94 (dd, 1H, J = 4.0, 6.6, 4a-H), 3.90–3.86 (m, 1H, 5a-H), 3.74–3.68 (m, 1H, 5b-H), 3.58–3.52 (m, 4H, 6a-H, 6′a-H, 6b-H, 6′b-H) ppm; ^{13}C NMR (100 MHz, D_2O): δ = 161.6 (C*ipso* C_6H_4), 142.7 (C*p* C_6H_4), 126.2 (C*m* C_6H_4), 116.8 (C*o* C_6H_4), 109.2 (1b-C), 96.6 (1a-C), 82.7 (4b-C), 81.4 (2b-C), 76.7 (3b-C), 72.0 (3a-C), 71.9 (5a-C), 71.9 (5a-C), 70.6 (5b-C), 66.9 (2a-C), 62.6 (6b-C), 60.8 (6a-C) ppm. HRMS (ESI): m/z calcd for $C_{18}H_{25}O_{13}NNa$ $[M + Na]^+$ 486.12181; found 486.12162.

***p*-Nitrophenyl β-D-galactofuranosyl-(1→2)-β-D-galactopyranoside (17).** The disaccharide was synthesized as described in the general procedure incubating furanosyl donor **1** (20 mg, 66 μmol) with *p*NP β-D-galactopyranoside **5** (200 mg, 660 μmol) in the presence of 4800 U of Ara*f*51, and was isolated in 27% yield (8.1 mg). TLC: R_f = 0.4 (AcOEt–AcOH–H_2O, 7/2/2). ^{1}H NMR (400 MHz, D_2O): δ = 8.18 (d, 2H, J = 9.2 Hz, H*m*, C_6H_4), 7.17 (d, 2H, J = 9.2 Hz, H*o*, C_6H_4), 5.25 (d, 1H, J = 1.5 Hz, 1b-H), 5.24 (d, 1H, J = 7.5 Hz, 1a-H), 4.05 (dd, 1H, J = 2.9, 1.5 Hz, 2b-H), 3.95 (dd, 1H, J = 2.9, 5.8 Hz, 3b-H), 3.96–3.91 (m, 1H, 5a-H), 3.91 (dd, 1H, J = 4.1, 5.8 Hz, 4b-H), 3.88 (dd, 1H, J = 7.5, 9.7 Hz, 2a-H), 3.84–3.78 (m, 2H, 3a-H, 4a-H), 3.73–3.67 (m, 3H, 5b-H, 6a-H, 6′a-H), 3.45 (dd, 1H, J = 4.4, 11.7 Hz, 6b-H), 3.39 (dd, 1H, J = 7.3, 11.7 Hz, 6′b-H) ppm. ^{13}C NMR (100 MHz, D_2O): δ = 161.9 (C*ipso* C_6H_4), 142.6 (C*p* C_6H_4), 125.9 (C*m* C_6H_4), 116.4 (C*o* C_6H_4), 108.4 (1a-C), 98.5 (1b-C), 83.5 (4a-C), 81.0 (2a-C), 76.7 (3a-C), 76.4 (2b-C), 75.5 (4b-C), 72.6 3b-C), 70.8 (5a-C), 68.4 (5b-C), 62.6 (6a-C), 60.6 (6b-C) ppm. HRMS (ESI): m/z calcd for $C_{18}H_{25}O_{13}NNa$ $[M + Na]^+$ 486.12181; found 486.12190.

***p*-Nitrophenyl β-D-galactofuranosyl-(1→6)-β-D-galactopyranoside (18).** This compound was prepared according to the described general procedure by incubation of **1** (20 mg, 66 μmol) with of *p*NP β-D-galactopyranoside **5** (200 mg, 660 μmol) in the presence of 4800 U of Ara*f*51, and was isolated in 25% yield (7.6 mg). TLC: R_f = 0.4 (AcOEt–AcOH–H_2O, 7/2/2). ^{1}H NMR (400 MHz, D_2O): δ = 8.18 (d, 2H, J = 9.2 Hz, H*m*, C_6H_4), 7.17 (d, 2H, J = 9.2 Hz, H*o*, C_6H_4), 5.12 (d, 1H, J = 7.6 Hz, 1a-H), 4.90 (dd, 1H, J = 1.2, 0.6 Hz, 1b-H), 4.00 (ddd, 1H, J = 0.9, 3.8, 8.4 Hz, 4a-H), 3.97 (d, 1H, J = 1.2 Hz, 2b-H), 3.96 (ddd, 1H, J = 3.3, 0.6 Hz, 3b-H), 3.93 (dd, 1H, J = 0.9, 3.4 Hz, 4a-H), 3.91 (dd, 1H, J = 1.5, 4.4 Hz, 4b-H), 3.83 (dd, 1H, J = 3.8, 11.6 Hz, 6a-H), 3.76 (dd, 1H, J = 7.6, 9.9 Hz, 2a-H), 3.73–3.72 (m, 1H, 5b-H), 3.71 (dd, 1H, J = 8.4, 11.6 Hz, 6′a-H), 3.70 (dd, 1H, J = 3.4, 9.9 Hz, 3a-H), 3.63–3.57 (m, 1H, 6b-H), 3.56–3.51 (m, 1H, 6′b-H) ppm. ^{13}C NMR (100 MHz, D_2O): δ = 161.6 (C*ipso* C_6H_4), 142.7 (C*p* C_6H_4), 126.1 (C*m* C_6H_4), 116.5 (C*o* C_6H_4), 107.8 (1b-C), 99.8 (1a-C), 83.1 (4b-C), 82.0 (2b-C), 76.7 (3b-C), 74.5 (5a-C), 72.3 (3a-C), 70.7 (5b-C), 70.2 (2a-C), 68.5 (4a-C), 66.9 (6a-C), 62.5 (6b-C) ppm. HRMS (ESI): m/z calcd for $C_{18}H_{25}O_{13}NNa$ $[M + Na]^+$ 486.12181; found 486.12172.

***p*-Nitrophenyl β-D-galactofuranosyl-(1→6)-α-D-mannopyranoside (19).** This compound was prepared according to the described general procedure by incubation of **1** (20 mg, 66 μmol) with of *p*NP α-D-mannopyranoside **6** (200 mg, 660 μmol) in the presence of 4800 U of Ara*f*51, and was isolated in 15% yield (4.6 mg). ^{1}H NMR (600 MHz, D_2O): δ = 8.22 (d, 2H, J = 9.3 Hz, H*m*, C_6H_4), 7.23 (d, 2H, J = 9.3 Hz, H*o*, C_6H_4), 5.71 (d, 1H, J = 1.7 Hz, 1a-H), 4.91 (d, 1H, J = 1.7 Hz, 1b-H), 4.14 (dd, 1H, J = 1.7, 3.4 Hz, 2a-H), 3.99 (dd, 1H, J = 3.4, 9.1 Hz, 3a-H), 3.91–3.82 (m, 3H, 5a-H, 2b-H, 3b-H), 3.72–3.62 (m, 3H, 4a-H, 6a-H, 6a′-H), 3.62–3.56 (m, 1H, 5b-H), 3.52–3.44 (m, 3H, 4b-H, 6b-H, 6a′-H) ppm. ^{13}C NMR (125 MHz, D_2O): δ = 160.7 (C*ipso* C_6H_4), 142.0 (C*p* C_6H_4), 126.0 (C*m* C_6H_4), 116.8 (C*o* C_6H_4), 108.1 (1b-C), 97.6 (1a-C), 82.5 (4b-C), 81.1 (2b-C), 76.5 (3b-C), 73.2 (4a-C), 70.5 (5b-C), 70.2 (3a-C), 69.6 (2a-C), 67.3(6a-CH_2), 66.7 (5a-C), 62.8 (6b-CH_2) ppm.

***p*-Nitrophenyl β-D-galactofuranosyl-(1→4)-β-D-mannopyranoside (20).** This compound was obtained according to the general procedure by incubation of **1** (10 mg, 33 μmol) with mannopyranosidic acceptor **7** (100 mg, 330 μmol) in the presence of 2400 U of Ara*f*51, and was isolated in 49% yield (7.5 mg). TLC: R_f = 0.49 (AcOEt–AcOH–H_2O, 7/2/2). ^{1}H NMR (400 MHz, D_2O): δ = 8.16 (d, 2H, J = 9.2 Hz, H*m*, C_6H_4), 7.13 (d, 2H, J = 9.2 Hz, H*o*, C_6H_4), 5.43 (s, 1H, 1a-H), 5.02 (d, 1H, J = 1.80 Hz, 1b-H), 4.18 (d, 1H, J = 2.6 Hz, 2a-H), 4.06–4.03 (m, 2H, 4b-H, 3b-H), 4.02 (d, 1H, 2b-H), 3.87 (dd, 1H, J = 1.9, 12.2 Hz, 6a-H), 3.79 (dd, 1H, J = 2.6, 9.2 Hz, 3a-H), 3.80–3.76 (m, 1H, 4a-H), 3.75–3.73 (m, 1H, 5b-H), 3.72 (dd, 1H, J = 4.5, 12.2 Hz, 6′a-H), 3.63 (ddd, 1H, J = 1.9, 5.4, 9.5 Hz, 5a-H), 3.60 (d, 1H, J = 6.7, 11.9 Hz, 6b-H), 3.58 (dd, 1H, J = 4.1, 11.9 Hz, 6′b-H) ppm. ^{13}C NMR (100 MHz, D_2O): δ = 161.5 (C*ipso* C_6H_4), 142.4 (C*p* C_6H_4), 126.1 (C*m* C_6H_4), 116.2 (C*o* C_6H_4), 108.0 (1b-C), 97.0 (1a-C), 82.8 (3b-C), 81.0 (4b-C), 76.0 (2b-C), 74.6 (4a-C), 71.2 (3a-C), 70.5 (5b-C), 70.1 (2a-C), 62.6 (6b-C), 61.1 (5a-C), 60.3 (6a-C) ppm. HRMS (ESI): m/z calcd for $C_{18}H_{25}O_{13}NNa$ $[M + Na]^+$ 486.12181; found 486.12204.

***p*-Nitrophenyl β-D-galactofuranosyl-(1→6)-β-D-mannopyranoside (21).** This compound was prepared according to the described general procedure by incubation of **1** (20 mg, 66 μmol) with of *p*NP α-D-mannopyranoside **6** (200 mg, 660 μmol) in the presence of 4800 U of Ara*f*51, and was isolated in 15% yield (4.9 mg). ^{1}H NMR (600 MHz, D_2O): δ = 8.20 (d, 2H, J = 9.3 Hz, H*m*, C_6H_4), 7.17 (d, 2H, J = 9.3 Hz, H*o*, C_6H_4), 5.46 (br s, 1H, 1a-H), 4.95 (d, J = 4.4 Hz, 1H, 1b-H), 4.18 (d, 1H, J = 3.0 Hz, 2a-H), 4.00–3.00 (m, 2H, 5a-H, 2b-H), 3.97 (dd, 1H, J = 3.5, 6.0 Hz, 3b-H), 3.91 (dd, 1H, J = 4.3, 6.0 Hz, 4b-H), 3.76–3.63 (m, 5H, 3a-H, 4a-H, 5b-H, 6a-H, 6a′-H), 3.60–3.53 (m, 2H, 6b-H, 6b′-H) ppm. ^{13}C NMR (125 MHz, D_2O): δ = 161.5 (C*ipso* C_6H_4), 142.5 (C*p* C_6H_4), 126.1 (C*m* C_6H_4), 116.3 (C*o* C_6H_4), 107.9(1b-C), 97.0 (1a-C), 83.1 (4b-C), 80.9 (2b-C), 76.8 (3b-C), 75.5 (4a-C), 72.5 (3a-C), 70.8 (5b-C), 70.2 (2a-C), 66.6 (5a-C), 66.6 (6a-CH_2), 62.7 (6b-CH_2) ppm.

***p*-Nitrophenyl β-D-galactofuranosyl-(1→4)-α-L-rhamnopyranoside (22).** This compound was obtained according to the described general procedure by incubation of *p*NP β-D-galactofuranoside **1** (10 mg, 33 μmol) with *p*NP α-L-rhamnopyranoside **8** (100 mg, 330 μmol) in the presence of 2400 U of Ara*f*51, and was isolated in 38% yield (5.5 mg). TLC: R_f = 0.5 (AcOEt–

AcOH–H_2O, 7/2/2). ^{1}H NMR (400 MHz, D_2O): δ = 8.15 (d, 2H, J = 9.2 Hz, H*m*, C_6H_4), 7.13 (d, 2H, J = 9.2 Hz, H*o*, C_6H_4), 5.55 (d, 1H, J = 1.2 Hz, 1a-H), 5.21 (d, 1H, J = 1.7 Hz, 1b-H), 4.06 (dd, 1H, J = 1.2, 3.4 Hz, 2a-H), 4.04 (dd, 1H, J = 1.8, 3.9 Hz, 2b-H), 4.04–4.00 (m, 1H, 3a-H), 3.97 (dd, 1H, J = 3.9, 6.3 Hz, 3b-H), 3.87 (dd, J = 3.8, 6.3 Hz, 1H, 4b-H), 3.72 (ddd, J = 4.4, 4.5, 7.2 Hz, 1H, 5b-H), 3.58 (dd, 1H, J = 4.4, 11.6 Hz, 6b-H), 3.59–3.55 (m, 1H, 5a-H), 3.57–3.53 (m, 1H, 4a-H), 3.54 (dd, 1H, J = 7.2, 11.6 Hz, 6′b-H), 1.11 (d, 1H, J = 5.7 Hz, CH_3) ppm. ^{13}C NMR (100 MHz, D_2O): δ = 161.3 (C*ipso* C_6H_4), 142.0 (C*p* C_6H_4), 125.8 (C*m* C_6H_4), 116.2 (C*o* C_6H_4), 108.4 (1b-C), 97.5 (1a-C), 82.7 (4b-C), 81.5 (2b-C), 77.8 (4a-C), 70.4 (5b-C), 70.3 (3a-C), 69.9 (2a-C), 68.2 (5a-C), 62.7 (6b-C), 17.1 (6a-C) ppm. HRMS (ESI): *m*/*z* calcd for $C_{18}H_{25}O_{12}NNa$ $[M + Na]^+$ 470.12690; found 470.12665.

Molecular modeling

All simulations were performed in Gromacs 4.5.5.[48] Each system included one molecule of Ara*f*51 (from the experimental structure, PDB I.D. 2C8N),[49] one molecule of putative transglycosylation product, ~18 300 water molecules and 15 sodium counter-ions. Protein was modelled using Amber99SB force field,[50] transglycosylation products were modelled using Glycam06[51] (carbohydrates) and General Amber Force Field[52] (*p*NP-moiety). Charges were calculated by RESP method on the basis of HF/6-31G*//HF/6-31G* wavefunction calculated for individual fragments in Gaussian 03.[53] Molecules of putative products were placed to the active site by 3D-alignment of the Gal*f* moiety with the Ara*f* moiety in the experimental structure. This was followed by manual adjustment of glycosidic bond torsions to avoid steric clashes. Finally, the system was minimized (3000 steps of L-BFGS and 500 steps of steepest descent) and simulated by 1 ns of equilibration and 10 ns of production simulation. Non-hydrogen atoms of the protein and Gal*f* were restrained during the equilibration run by a harmonic restraint potential.

Acknowledgements

I.C. thanks the Czech Ministry of Education for a grant (MSM: 6046137305). Financial support by the Ministère Français des Affaires Etrangères, the Programme Hubert Curien (Barrande), the Agence Nationale de la Recherche (JCJC06_140075), and the GDR-RDR2 "Aller vers une chimie éco-compatible" are gratefully acknowledged, as well as Prof. G. Davies for supplying the plasmid of Ara*f*51. Molecular modeling was supported by access to high performance computing facilities (MetaCentrum – LM2010005 and CERIT-SC – CZ. 1.05/3.2.00/08.0144), by COST (CM1102 – MultiGlycoNano) and the Ministry of Education, Youth and Sports of the Czech Republic (LD13024 and 7AMB13FR014). The authors are finally grateful to the GlycoOuest network, supported by the Région Bretagne and the Région Pays de la Loire.

Notes and references

1 R. Mody, S. H. A. Joshi and W. Chaney, *J. Pharmacol. Toxicol. Methods*, 1995, **33**, 1–10.
2 E. Gorelik, U. Galili and A. Raz, *Cancer Metastasis Rev.*, 2001, **20**, 245–277.
3 P. Peltier, R. Euzen, R. Daniellou, C. Nugier-Chauvin and V. Ferrières, *Carbohydr. Res.*, 2008, **343**, 1897–1923.
4 M. R. Richards and T. L. Lowary, *ChemBioChem*, 2009, **10**, 1920–1938.
5 H. A. Taha, M. R. Richards and T. L. Lowary, *Chem. Rev.*, 2013, **113**, 1851–1876.
6 J. B. Houseknecht, T. L. Lowary and C. M. Hadad, *J. Phys. Chem. A*, 2002, **107**, 372–378.
7 P. Nassau, S. Martin, R. Brown, A. Weston, D. Monsey, M. McNeil and K. Duncan, *J. Bacteriol.*, 1996, **178**, 1047–1052.
8 R. Köplin, J. R. Brisson and C. Whitfield, *J. Biol. Chem.*, 1997, **272**, 4121–4128.
9 F. Pan, M. Jackson, Y. Ma and M. McNeil, *J. Bacteriol.*, 2001, **183**, 3991–3998.
10 S. M. Beverley, K. L. Owens, M. Showalter, C. L. Griffith, T. L. Doering, V. C. Jones and M. R. McNeil, *Eukaryot. Cell*, 2005, **4**, 1147–1154.
11 M. J. McConville, S. W. Homans, J. E. Thomas-Oates, A. Dell and A. Bacic, *J. Biol. Chem.*, 1990, **265**, 7385–7394.
12 R. M. de Lederkremer and W. Colli, *Glycobiology*, 1995, **5**, 547–552.
13 F. A. Shaikh and S. G. Withers, *Biochem. Cell Biol.*, 2008, **86**, 169–177.
14 M. L. Sinnott, *Chem. Rev.*, 1990, **90**, 1171–1202.
15 K. N. Jayaprakash, J. Lu and B. Fraser-Reid, *Angew. Chem., Int. Ed.*, 2005, **117**, 6044–6048.
16 B. Fraser-Reid, J. Lu, K. N. Jayaprakash and J. C. López, *Tetrahedron: Asymmetry*, 2006, **17**, 2449–2463.
17 A. Hölemann, B. L. Stocker and P. H. Seeberger, *J. Org. Chem.*, 2006, **71**, 8071–8088.
18 Y. Ito and S. Hanashima, in *Experimental Glycoscience*, ed. N. Taniguchi, A. Suzuki, Y. Ito, H. Narimatsu, T. Kawasaki and S. Hase, Springer, Japan, 2008, pp. 210–216.
19 P. Bojarova and V. Kren, *Trends Biotechnol.*, 2009, **27**, 199–209.
20 R. W. Gantt, P. Peltier-Pain and J. S. Thorson, *Nat. Prod. Rep.*, 2011, **28**, 1811–1853.
21 M. Joe, Y. Bai, R. C. Nacario and T. L. Lowary, *J. Am. Chem. Soc.*, 2007, **129**, 9885–9901.
22 M. McGeachy, Y. Chen, C. Tato, A. Laurence, B. Joyce-Shaikh, W. Blumenschein, T. McClanahan, J. O'Shea and D. Cua, *Nat. Immunol.*, 2009, **10**, 314–324.
23 C. A. G. M. Weijers, M. C. R. Franssen and G. M. Visser, *Biotechnol. Adv.*, 2008, **26**, 436–456.
24 I. Chlubnova, L. Legentil, R. Dureau, A. Pennec, M. Almendros, R. Daniellou, C. Nugier-Chauvin and V. Ferrières, *Carbohydr. Res.*, 2012, **356**, 44–61.
25 C. Rémond, R. Plantier-Royon, N. Aubry and M. J. O'Donohue, *Carbohydr. Res.*, 2005, **340**, 637–644.

26 M. G. Szczepina, R. B. Zheng, G. C. Completo, T. L. Lowary and B. M. Pinto, *ChemBioChem*, 2009, **10**, 2052–2059.
27 N. L. Rose, G. C. Completo, S.-J. Lin, M. R. McNeil, M. M. Palcic and T. L. Lowary, *J. Am. Chem. Soc.*, 2006, **128**, 6721–6729.
28 N. L. Rose, G. C. Completo, S.-J. Lin, M. McNeil, M. M. Palcic and T. L. Lowary, *J. Am. Chem. Soc.*, 2006, **128**, 6721–6729.
29 N. L. Rose, R. B. Zheng, J. Pearcey, R. Zhou, G. C. Completo and T. L. Lowary, *Carbohydr. Res.*, 2008, **343**, 2130–2139.
30 C. D. Brown, M. S. Rusek and L. L. Kiessling, *J. Am. Chem. Soc.*, 2012, **134**, 6552–6555.
31 C. Zhang, B. R. Griffith, Q. Fu, C. Albermann, X. Fu, I.-K. Lee, L. Li and J. S. Thorson, *Science*, 2006, **313**, 1291–1294.
32 R. W. Gantt, P. Peltier-Pain, W. J. Cournoyer and J. S. Thorson, *Nat. Chem. Biol.*, 2011, **7**, 685–691.
33 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.
34 J. E. Germond, M. Delley, N. D'Amico and S. J. F. Vincent, *Eur. J. Biochem.*, 2001, 5149–5156.
35 G. Stevenson, B. Neal, D. Liu, M. Hobbs, N. H. Packer, M. Batley, J. W. Redmond, L. Lindquist and P. Reeves, *J. Bacteriol.*, 1994, **176**, 4144–4156.
36 E. Vinogradov, E. E. Egbosimba, M. B. Perry, J. S. Lam and C. W. Forsberg, *Eur. J. Biochem.*, 2001, 3566–3576.
37 M. McNeil, M. Daffe and P. J. Brennan, *J. Biol. Chem.*, 1990, **265**, 18200–18206.
38 M. Daffe, P. J. Brennan and M. McNeil, *J. Biol. Chem.*, 1990, **265**, 6734–6743.
39 G. S. Besra, K. H. Khoo, M. R. McNeil, A. Dell, H. R. Morris and P. J. Brennan, *Biochemistry*, 1995, **34**, 4257–4266.
40 D. Kaur, T. L. Lowary, V. D. Vissa, D. C. Crick and P. J. Brennan, *Microbiology*, 2002, **148**, 3049–3057.
41 M. Linnerborg, A. Weintraub and G. Widmalm, *Eur. J. Biochem.*, 1999, 460–466.
42 A. Molinaro, V. Piscopo, R. Lanzetta and M. Parrilli, *Carbohydr. Res.*, 2002, **340**, 1707–1713.
43 A. Prieto, J. A. Leal, A. Poveda, J. Jimenéz-Barbero, B. Gómez-Miranda, J. Domenech, O. Ahrazem and M. Bernabé, *Carbohydr. Res.*, 1997, **304**, 281–291.
44 J. P. Latgé, H. Kobayashi, J. P. Debeaupuis, M. Diaquin, J. Sarfati, J. M. Wieruszeski, E. Parra, J. P. Bouchara and B. Fournet, *Infect. Immun.*, 1994, **62**, 5424–5433.
45 R. M. de Lederkremer, C. Lima, M. I. Ramirez, M. A. Ferguson, S. W. Homans and J. Thomas-Oates, *J. Biol. Chem.*, 1991, 23670–23675.
46 S. B. Levery, M. S. Toledo, E. Suzuki, M. E. K. Salyan, S.-i. Hakomori, A. H. Straus and H. K. Takahashi, *Biochem. Biophys. Res. Commun.*, 1996, **222**, 639–645.
47 E. Taylor, N. Smith, J. Turkenburg, S. D'Souza, H. Gilbert and G. Davies, *Biochem. J.*, 2006, **395**, 31–37.
48 B. Hess, C. Kutzner, D. v. d. Spoel and E. Lindahl, *J. Chem. Theory Comput.*, 2008, **4**, 435–447.
49 E. J. Taylor, N. L. Smith, J. P. Turkenburg, S. D'Souza, H. J. Gilbert and G. J. Davies, *Biochem. J.*, 2006, **395**, 31–37; PDB code: 32C38N.
50 V. Hornak, R. Abel, A. Okur, B. Strockbine, A. Roitberg and C. Simmerling, *Proteins*, 2006, **65**, 712–725.
51 K. N. Kirschner, A. B. Yongye, S. M. Tschampel, J. González-Outeiriño, C. R. Daniels, B. L. Foley and R. J. Woods, *J. Comput. Chem.*, 2008, **29**, 622–655.
52 J. Wang, R. M. Wolf, J. W. Caldwell, P. A. Kollman and D. A. Case, *J. Comput. Chem.*, 2004, **25**, 1157–1174.
53 M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, J. J. A. Montgomery, T. Vreven, K. N. Kudin, J. C. Burant, J. M. Millam, S. S. Iyengar, J. Tomasi, V. Barone, B. Mennucci, M. Cossi, G. Scalmani, N. Rega, G. A. Petersson, H. Nakatsuji, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, M. Klene, X. Li, J. E. Knox, H. P. Hratchian, J. B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. W. Ochterski, P. Y. Ayala, K. Morokuma, G. A. Voth, P. Salvador, J. J. Dannenberg, V. G. Zakrzewski, S. Dapprich, A. D. Daniels, M. C. Strain, O. Farkas, D. K. Malick, A. D. Rabuck, K. Raghavachari, J. B. Foresman, J. V. Ortiz, Q. Cui, A. G. Baboul, S. Clifford, J. Cioslowski, B. B. Stefanov, G. Liu, A. Liashenko, P. Piskorz, I. Komaromi, R. L. Martin, D. J. Fox, T. Keith, M. A. Al-Laham, C. Y. Peng, A. Nanayakkara, M. Challacombe, P. M. W. Gill, B. Johnson, W. Chen, M. W. Wong, C. Gonzalez and J. A. Pople, *Gaussian 03, Revision C.02*, Gaussian, Inc., Wallingford, CT, 2004.