A Chemoenzymatic Approach for the Synthesis of Unnatural Disaccharides Containing D-Galacto- or D-Fucofuranosides

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Unusual diglycosides composed of D-hexofuranosyl entities were prepared by a chemoenzymatic route using the α-L-arabinofuranosidase, AbfD3. The required, unprotected monosaccharidic donors were first prepared according to multistep syntheses. Since one goal of this study was the investigation of donor –1 subsite in the active site of the enzyme, we focused on D-fucofuranosyl and 6-deoxy-6-fluoro-D-galactofuranosyl derivatives which present stereochemical similarities with L-arabinose series, but also structural variations on the side arm. These substrates were then used in AbfD3-catalysed hydrolyses to determine the parameters K_m and k_{cat} and in AbfD3-catalysed transglycosylation to evaluate their ability to serve as donor/acceptor. Four disaccharides were thus isolated and characterised, two resulting from β-(1,2) connection along with two β-(1,3)-regioisomers.

Introduction

Retaining glycoside hydrolases exhibiting transglycosylation activity are increasingly involved in the synthesis of oligosaccharides and glycoconjugates.[1] It is well-established that these enzymes perform hydrolysis using a double-displacement mechanism: (i) The formation of a covalently-linked enzyme-substrate intermediate, (ii) the reaction of the enzyme-substrate intermediate with nucleophilic molecules. In the case of transglycosylation, these glycosyl acceptors, generally *O*-nucleophiles, compete with water to generate oligosaccharides or hydrolysed sugars.[2] Consequently, glycosides may be synthesised with glycoside hydrolases as catalysts under either thermodynamically or kinetically controlled conditions. However, as the former approach generally requires sophisticated procedures and often provides coupling products in poor yields, kinetic control of synthesis is largely preferred. In order to generate a reactive intermediate, suitable activated glycosyl donors including di- and oligosaccharides,[3]aryl glycosides[4] and glycosyl fluorides,[1a,5] are required. To date, very few studies have explored the use of glycoside hydrolases for the synthesis of oligosaccharides characterised by the presence of furanosyl residues, and more particularly hexofuranosyl entities. As part of our ongoing interest in oligosaccharides and glycoconjugates containing alternate furanoid and pyranoid residues,[6,7] we are also involved in the development of new chemoenzymatic approaches for the preparation of such rare bioactive carbohydrates. Indeed, galactofuranosyl-containing oligosaccharides are crucial constituents of polysaccharides in the cell walls in bacteria, fungi and parasitic microorganisms including some clinically significant pathogens.[8] Since these glycosides are not present in mammals, structurally well-defined, synthetic analogues are potentially antigenic and, as such, are of great interest for the development of new pharmacophores and new therapies.[9] However, those oligofuranosides are generally difficult to prepare according to classical methods of glycosidic synthesis. It is therefore highly desirable to explore new approaches and notably methodologies that combine the versatility of chemical methods and the simplicity and precision of enzymes. In particular, enzymes are likely to facilitate both the regio- and diastereoselective control of glycosidic coupling without having recourse to the use of protecting groups. Up to now, few galactofuranosidases have been identified[10] and, to the best of our knowledge, none of these biocatalysts have been used as synthetic tools for the preparation of galactofuranosyl-containing oligosaccharides. Nevertheless, on the basis of structural similarities, we expected that D-galactofuranosyl residues and stereochemically related analogues could be recognized as either donors or acceptors by more commonly available furanosyl

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hydrolases such as α-L-arabinofuranosidases (E.C. 3.2.1.55). These enzymes are glycoside hydrolases which are naturally involved in the degradation of plant biomass through the catalysis of hydrolysis of α-(1→2)-, α-(1→3)-, and/or α-(1→5)-L-arabinofuranosidic linkages in arabinoxylans, arabinans and other L-arabinose-containing compounds.[11] In an original report, Rémond et al.[12] reported the use of the thermostable arabinofuranosidase produced by *Thermobacillus xylanilyticus* (AbfD3) as an efficient tool to catalyze transglycosylation in the presence of various alcohols as well as *p*-nitrophenyl glycosides. More recently, an *exo*-arabinanase isolated from *Penicillium chysogenum* has been found to possess *trans*-arabinobiosylation activity on various acceptors such as aliphatic alcohols and common monosaccharides.[13] Based on the characterisation of the isolated α-L-arabinofuranosidase encoding gene, AbfD3, the arabinofuranosidase from *T. xylanilyticus* belongs to the vast family 51 within the glycoside hydrolase classification, which groups enzymes into families on the basis of amino acid sequence similarity.[14] This family is exclusively composed of retaining arabinofuranosidases which catalyze hydrolysis leading to net conservation of the anomeric configuration. Previous studies based on site-directed mutagenesis, have led to the localisation of both acid-base and nucleophilic catalytic sites, Glu^{176} and Glu^{298}, and a third glutamate residue (Glu^{28}) that is not directly involved in the catalytic mechanism, but which is probably a key determinant of substrate recognition in the –1 subsite.[15] In two recent studies,[16] the glycosynthetic ability of AbfD3 was further investigated using a variety of candidates as acceptors and/or donors. Likewise, it was shown that AbfD3 can catalyze the self-condensation of aromatic glycosides derived from α-L-arabinofuranose (α-L-Ara*f*), β-D-xylopyranose (β-D-Xyl*p*) and β-D-galactofuranose (β-D-Gal*f*), a hexose that is configurationally related to α-L-Ara*f*. These self-condensation reactions were characterised by variable degrees of regioselectivity. In the case of α-L-Ara*f*, the major product of transglycosylation was an α-L-(1,2)-arabinobioside, although other non-characterised species were generated. In the case of β-D-Gal*f*, regioselectivity was very tight and the dominant species was a β-D-(1,2)-galactofuranobioside. Therefore, in order to pursue our investigation into the selectivity of AbfD3, it appears useful to extend the rationale of the previous study. Likewise, in this study we have synthesised other novel donors that constitute structural variations of the galactofuranosyl skeleton. This strategy has allowed us to examine the effects of substitution of the primary hydroxy group on the 4-C-branched exocyclic arm of galactofuranosides on (i) substrate binding in the –1 subsite of AbfD3 and on (ii) the reactivity of secondary hydroxy functions owing to variation of inductive effects. In this paper, we report the original synthesis of required hexofuranosyl donors **1** and **2** that are galactofuranosyl derivatives modified at the 6 position, i.e. *p*-nitrophenyl β-D-fucofuranoside (*p*-NP-β-D-Fuc*f*, 6-deoxy-galactofuranosyl derivative) **1** and *p*-nitrophenyl 6-deoxy-6-fluoro-β-D-galactofuranoside (*p*-NP-6F-β-D-Gal*f*) **2** (Figure 1). In a second part, we have examined the AbfD3-mediated synthesis of novel furanose-containing disaccharides that have never been obtained through classical multi-step synthesis.

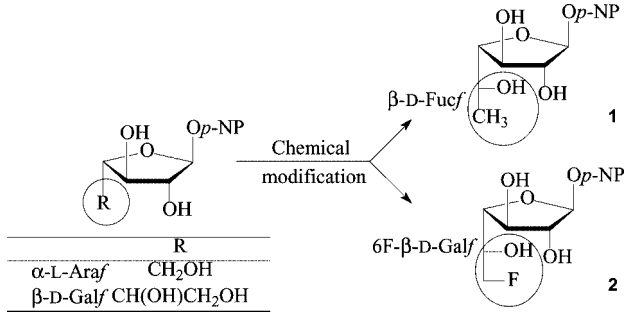


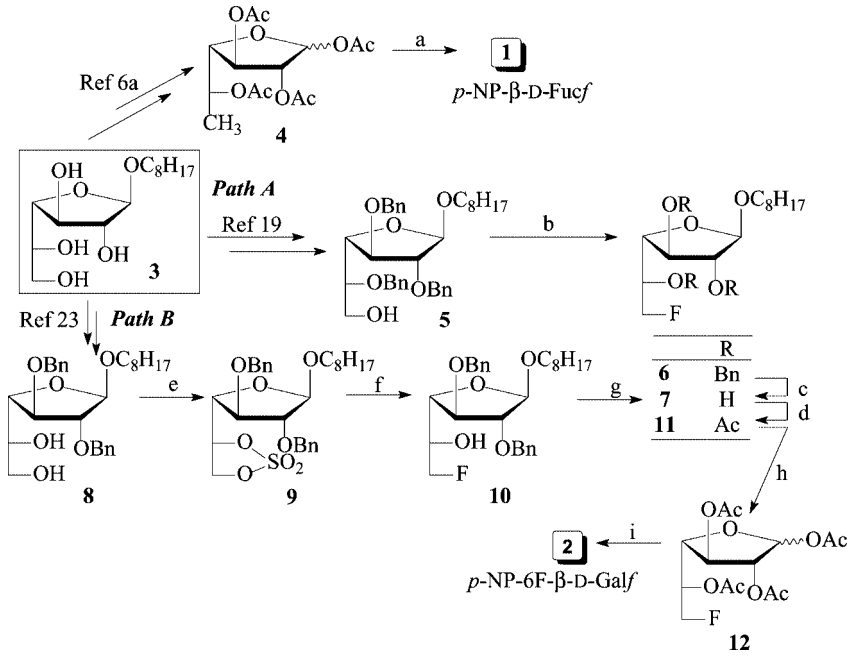
Figure 1. Chemical structures of targeted 6-modified galactofuranosides **1** and **2**.

Results and Discussion

Synthesis of Activated Donors

The *p*-nitrophenyl hexofuranosides **1** and **2** can be regarded as variants of D-galactofuranose because all three possess a common molecular backbone structure. Since AbfD3 performs both hydrolysis and transglycosylation with *p*-nitrophenyl β-D-galactofuranoside, we decided to prepare the desired activated donors. In order to limit the number of synthetic steps, *n*-octyl β-D-galactofuranoside (**3**), that was specifically obtained by galactosylation of *n*-octanol promoted by ferric chloride,[17] was used as a common building block. Accordingly, D-fucofuranose derivative **4**[6a] was obtained as described previously and used as a donor for the glycosylation of *p*-nitrophenol (Scheme 1). Initially, tin(IV) chloride[18] was chosen as the promoter for this reaction and subsequent deacylation afforded the target fucofuranoside **1** in 46% yield. This modest result was attributed to the strength of the Lewis acid which probably gave rise to degradation of both donor and product. Therefore, we decided to susbtitute tin(IV) chloride by a milder promoter. After experimentation, best results were obtained with the boron trifluoride–etherate complex in the presence of triethylamine.[19] Under such conditions and after Zemplen deacylation, the required fucofuranoside **1** was isolated in a significantly higher (66%) yield for two steps.

The preparation of the fluorinated target **2** required a free primary hydroxy which was differentiated from the secondary ones according to a three-step procedure (tritylation, benzylation, selective hydrolysis) in 62% overall yield.[20] Substitution of 6-hydroxy in **5** was further performed using the well-known fluorinating agent diethylaminosulfur trifluoride (DAST) (Scheme 1, path A). Unfortunately, initial attempts under standard conditions[21] resulted in the degradation of **5** and/or the target compound **6**. The lability of these molecules was undoubtedly provoked by the high acidity of the medium. Indeed, the addition of triethylamine to the reaction medium attenuated this effect and allowed the synthesis of the fluoro derivative



Scheme 1. (a) i. *p*-NPOH, $BF_3{\cdot}OEt_2$, Et_3N, CH_2Cl_2; ii. MeONa, MeOH (66%); (b) DAST, Et_3N, CH_2Cl_2 (47%); (c) $NaBrO_3$, Na_2HSO_3, H_2O, AcOEt (80%); (d) Ac_2O, Pyr (97%); (e) i. $SOCl_2$, Pyr, CH_2Cl_2; ii. $RuCl_3$, $NaIO_4$, CH_3CN, H_2O, CH_2Cl_2 (89%); (f) i. TBAF, THF, Me_2CO; ii. H_2SO_4, H_2O (86%); (g) H_2, $Pd(OAc)_2$, AcOH, AcOEt (92%) (h) Ac_2O, H_2SO_4, CH_2Cl_2 (98%); (i) i. *p*-NPOH, $BF_3{\cdot}OEt_2$, Et_3N, CH_2Cl_2; ii. MeONa, MeOH (61%).

6, which was obtained in moderate but classical 47% yield. Subsequently, debenzylation was performed under standard hydrogenolysis conditions. However, pallado-catalysed deprotection could not be completed, even in the presence of excess catalyst. This phenomenon was attributed to the presence of a fluorine atom at the primary position since the fucofuranosyl counterpart was readily deprotected under similar conditions. To surmount this difficulty, we turned our attention towards alternative methods and, after experimentation, best results were obtained using an oxidative procedure involving sodium bromate.[22] In this way, 6-fluorofuranoside **7** was obtained in 80% yield and in 20% overall yield over seven steps starting from **3**. In order to improve this result, we also investigated a second fluorination approach based on sulfate ring opening by fluoride anion[23] (Scheme 1, path B). For this purpose, dihydroxylated compound **8** was prepared in 79% yield from **3** as described by Reynolds (acetonation, benzylation, selective hydrolysis).[24] Further treatment by thionyl chloride gave a mixture of two inseparable cyclic diastereoisomeric sulfites with a 1.3:1 ratio. Oxidation by sodium periodate was catalysed by ruthenium(III) chloride[25] and led to the desired 5,6-sulfate **9**. Subsequent nucleophilic substitution with tetrabutylammonium fluoride (TBAF) followed by in situ acidic desulfation gave compound **10** in 86% yield for the last two steps. The 5-hydroxy now being unprotected, removal of other benzyl groups in 2 and 3 positions was catalytically performed under hydrogen to efficiently afford the desired 6-fluorofuranoside **7**. In conclusion, both pathways required seven steps, but the overall yield calculated from **3** was higher for path B (55%) than for path A (20%).

Having obtained **7**, the precursor **12** was prepared. First **7** was acetylated (→ **11**) and then smooth acetolysis[26] yielded **12**, which was subsequently used to glycosylate *p*-NPOH. Once again, it is interesting to note that the presence of a fluorine atom, albeit distant from the anomeric centre, was detrimental for the coupling reaction promoted by tin(IV) chloride. This is possibly due to the strong interactions between the fluoroglycosyl donor **12** and the Lewis acid. Fortunately, the softer promoter boron trifluoride–triethylamine prepared in situ was an interesting alternative to tin(IV) chloride. Finally, transesterification afforded the target activated donor **2** in 61% yield for the last two steps.

Hydrolytic Activity of Abfd3 on *p*-Nitrophenyl Furanosides 1 and 2

The specific activities of AbfD3 on the galactofuranoside analogues **1** and **2** were evaluated and compared to those obtained in the presence of *p*-NP-α-L-Ara*f* or *p*-NP-β-D-Gal*f* (Table 1). The specific activities of AbfD3 on **1** and **2** were lower than the activity observed on *p*-NP-α-L-Ara*f*, but were significantly higher than that estimated for *p*-NP-β-D-Gal*f*.[16] Investigation of the kinetic parameters revealed that the use of **1** and **2** as substrates caused only minor increases in K_m value when compared to the K_m value of the hydrolysis of *p*-NP-α-L-Ara*f*. Importantly, these increases were insignificant when compared to the increase in K_m value that was observed when *p*-NP-β-D-Gal*f* was used as the substrate.[16b] These results suggest that **1** and **2** are well recognized by AbfD3 and highlight the drastic ef-

Table 1. Hydrolytic parameters[a] of AbfD3 towards furanoside substrates.

	K_m [mm]	k_{cat} [s^{-1}]	k_{cat}/K_m [$mM^{-1}\cdot s^{-1}$]	Specific activity [$IU\cdot mg^{-1}$]
p-NP-α-L-Ara*f*	3.47 ± 0.45	425.79 ± 29.02	122.58 ± 24.35	465
p-NP-β-D-Gal*f* [16b]	> 50[b]	15.02 ± 0.46	0.13 ± 0.02	0.64
p-NP-β-D-Fuc*f* **1**	5.72 ± 0.44	193.13 ± 19.97	33.70 ± 6.05	119
p-NP-6F-β-D-Gal*f* **2**	7.59 ± 1.81	39.26 ± 5.21	5.28 ± 1.96	28

[a] Each value represents the mean of 3 measurements. [b] The K_m values were estimated to be extremely high and thus incompatible with the solubility and availability of the substrate.[16b]

fect that is caused by the presence of an extra hydroxymethyl group at the 5-C of the arabinofuranosyl skeleton. Moreover, AbfD3-mediated hydrolysis of **1** and **2** was characterised by a 2.2 to 10.8-fold lower activity (k_{cat}) and 3.6 to 23.2-fold lower catalytic efficiency (k_{cat}/K_m) compared with *p*-NP-α-L-Ara*f*. These results can be considered in the light of the findings of a recently published study in which the three-dimensional structure of another family 51 α-L-arabinofuranosidase was reported.[27] This work emphasises that the arabinofuranosyl moiety at the –1 subsite of the active site is tightly bound by a large number of hydrogen bonds, including some with the 5-OH. The data presented here using the synthetic derivatives **1** and **2** indicate that the addition of different hydrophobic groups, CH_3 and CH_2F, respectively, to the 5-C of the arabinofuranosyl skeleton does not necessarily have a major effect on substrate binding at the donor subsite. Indeed the addition of a methyl group has a relatively minor effect on both substrate binding and catalysis, whereas the presence of fluorine is mainly detrimental for catalysis. In contrast, it is interesting to note that the most detrimental effect on hydrolysis and substrate recognition is induced by the presence of a primary hydroxy on 6-C (β-D-Gal*f*). Presumably, this addition constitutes a steric hindrance for binding and, by possibly participating in new hydrogen bonds, might also cause the collapse of the normal hydrogen bonding network. Nevertheless, in previous work it was shown that, despite being a poor substrate for hydrolysis, *p*-NP-β-D-Gal*f* can act as a donor for transglycosylation. Therefore, in this work we assumed that **1** and **2** might also fulfil this role.

Abfd3-Catalysed Synthesis of Homo-Disaccharides

When compounds **1** or **2** (5 mM) were incubated with 12 IU AbfD3 at 60 °C for short periods (not exceeding 30 min), transglycosylation products were detected by high performance thin layer chromatography (HPTLC) analysis. In both cases, in addition to the starting furanosides and the products of hydrolysis (reducing fucose or 6-deoxy-6-fluorogalactose and *p*-nitrophenol), two novel species could be detected at 300 nm (Figure 2).

These products were isolated after one pass by preparative TLC using a normal stationary phase and identified by careful examination of the corresponding NMR spectra. Since the ^{13}C NMR spectra revealed the presence of only two peaks between δ = 104 and 110 ppm, it was concluded that all four products correspond to disaccharides **13**–**16** (Figure 3). A more precise analysis of the ^{13}C NMR spectroscopic data for **13**, the major disaccharide, obtained from *p*-NP-β-D-Fuc*f* **1**, showed a downfield shift of the 2a-C of the reducing D-fucofuranosyl moiety from 81.5 (for, **1**) to 87.6 ppm, as well as weak upfield shifts ($\Delta\delta$ = –1.3 ppm and –2.3 ppm) for the signals of the neighbouring carbons, 1a-C and 3a-C, respectively. Moreover, an intense three-bond coupling between 2a-C (δ = 87.6 ppm) and 1b-H (δ = 5.11 ppm) on ^1H-^{13}C (HMBC) spectrum allowed the unambiguous identification of **13** as a (1,2)-disaccharide. Finally, the small coupling constant observed between 1b-H and 2b-H ($J_{1b,2b}$ = 1.4 Hz) clearly indicated that the glycosidic bond in **13** exhibited β-stereochemistry. The minor disaccharide **14** produced in the same reaction as **13** was also

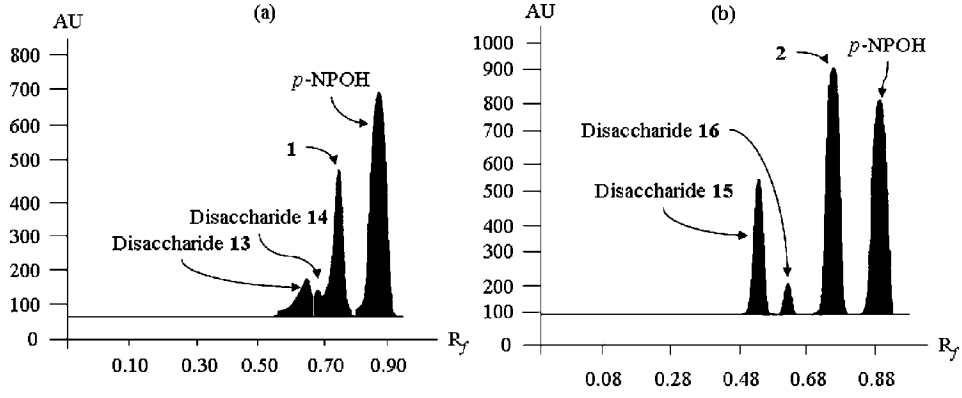


Figure 2. HPTLC chromatograms at 300 nm for the AbfD3-catalysed transformations of **1** (a) and **2** (b).

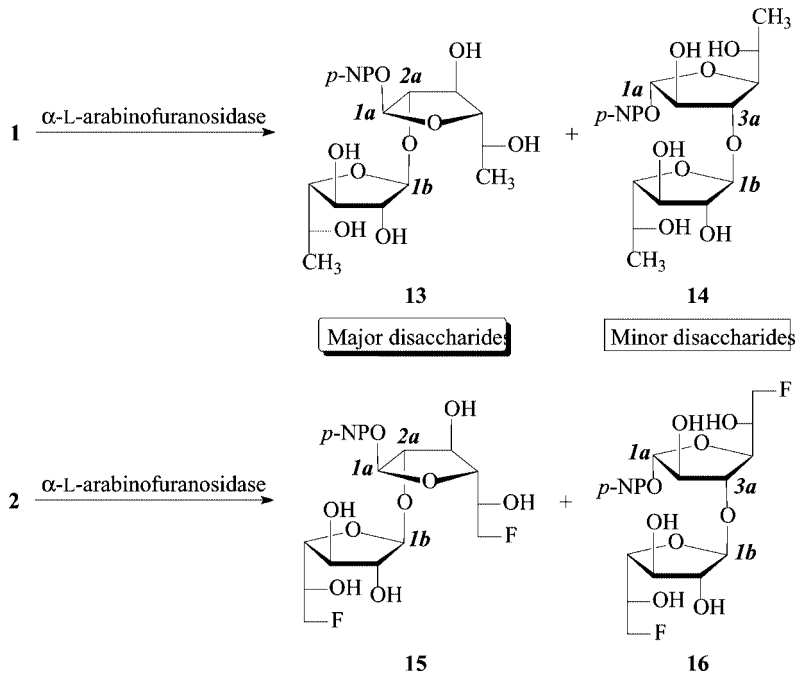


Figure 3. Structures of disaccharides **13**, **14** and **15**, **16** obtained from the AbfD3-catalysed condensation of **1** and **2**, respectively.

isolated and analysed. For this compound, the ^{13}C chemical signal for 3a-C was significantly downfield shifted ($\Delta\delta$ = +4.8 ppm), whereas 2a-C and 4a-C exhibited upfield-shifted signals ($\Delta\delta$ = –2.3 ppm and –1.5 ppm, respectively). These data corroborated the results obtained from the ^{1}H-^{13}C (HMBC) spectrum which showed a three-bond coupling between 3a-C (δ = 82.2 ppm) and 1b-H (δ = 5.14 ppm), the latter being characterised by a low coupling constant with 2b-H ($J_{1b,2b}$ = 1.1 Hz). This result lead us to conclude that the two monosaccharide moieties in **14** are linked through a β-(1,3) glycosidic bond.

As expected, the structural analysis of the products **15** and **16** arising from the action of AbfD3 on *p*-NP-6F-β-D-Gal*f* (**2**) revealed similar results. The major compound **15** was identified as a β-(1,2) disaccharide (Figure 3) on the basis of 1D and 2D NMR experiments. Indeed, *O*-2 glycosylation involved a rather large, downfield shift of 2a-C ($\Delta\delta$ = +4.7 ppm) and the HMBC spectrum showed a three-bond coupling between this carbon atom (δ = 87.6 ppm) and a signal at δ = 5.12 ppm assigned to 1b-H. According to HPTLC analysis of AbfD3-catalysed condensation of **2** (Figure 2, b), the regioselectivity of the reaction appeared to be higher than the one involving *p*-NP-β-D-Fuc*f* **1** and largely orientated towards the formation of the β-(1,2) disaccharide **15** (**15/16** = 6.6:1). However, the minor disaccharide **16** produced in this reaction was characterised by 1D and 2D NMR (COSY) experiments. As expected, the data showed a downfield chemical shift of +4.7 ppm for 3a-C and upfield chemical shifts of –1.0 and –2.3 ppm for 2a-C and 1a-C, respectively. Moreover, low coupling constants between 1b-H and 2b-H appeared to be relevant for two β-glycosidic linkages for **15** and **16** ($J_{1b,2b}$ = 1.6 Hz and 1.4 Hz, respectively).

Overall, the analysis of the products **13**–**16** revealed that AbfD3-mediated transglycoylation of **1** and **2** was characterised by moderately relaxed regioselectivity. This is in sharp contrast to the AbfD3-mediated transglycosylation reactions involving *p*-NP-β-D-Gal*f*, but is similar to the situation observed in reactions involving *p*-NP-α-L-Ara*f*. This suggests that although *p*-NP-β-D-Gal*f* is an extremely poor substrate for hydrolysis, mainly because it is poorly recognized by AbfD3, the radically altered binding mode for this substrate in the –1 and/or +1 subsites of AbfD3 might actually increase discrimination of reactivity towards the 2-OH vs. 3-OH of the furanosyl acceptor molecule.

Conclusions

Here we provide a further demonstration of the catalytic versatility of the α-L-arabinofuranosidase AbfD3 and its usefulness for the synthesis of unnatural disaccharides containing hexofuranosyl entities. This study was based on the use of two hexofuranosides whose structures are related to α-L-Ara*f* and whose electronic and steric properties were chosen to be intermediate between those of α-L-Ara*f* and β-D-Gal*f*, i.e. D-fucofuranosyl and 6-deoxy-6-fluoro-D-galactofuranosyl. In particular, the kinetic parameters determined for the AbfD3-catalysed hydrolysis of **1** reveal that *p*-NP-β-D-Fuc*f* is a very good substrate. Moreover, the analysis of transglycosylation products has confirmed that using hexofuranosides **1** and **2**, AbfD3 can synthesise both β-(1,2) and β-(1,3) regioisomeric disaccharides. Such β-(1,2) and β-(1,3)-linked galactofuranosides were previously identified in polysaccharides of *Talaromyces* species and were used as chemotaxonomic markers.[28] Consequently, these results open new opportunities for the use of AbfD3 as an

efficient biocatalyst for the synthesis of a variety of hexofuranosyl-containing oligosaccharides. Taken together with the three-dimensional structure of AbfD3, which will be published in the near future, these data provide insight into the way in which different donor compounds are bound in the –1 subsite. Likewise, it is expected that such knowledge will help us to optimise the use of AbfD3 for synthetic purposes.

Experimental Section

General Remarks: Melting points were determined with a Reichert microscope and are uncorrected. Thin layer chromatography (TLC) analyses were conducted with E. Merck 60 F_{254} Silica Gel nonactivated plates, and compounds were revealed using a 5% solution of H_2SO_4 in EtOH followed by heating. High performance TLC (HPTLC) experiments were performed with a CAMAG TLC SCANNER 3, designed for densitometric measurement of thin-layer chromatograms of 100 × 100 mm in size and controlled by the newly designed software WinCATS. For column chromatography, Geduran Si 60 (40–63 μM) Silica Gel was used. Preparative TLC was performed by using 0.25 mm silica gel Si 60 plates (200 × 200 mm, E. Merck). Optical rotations were measured with a Perkin–Elmer 341 polarimeter. ^{1}H, ^{13}C, HMBC and COSY NMR spectra were recorded with a Bruker ARX 400 spectrometer at 400 MHz for ^{1}H, 100 MHz for ^{13}C and 376 MHz for ^{19}F analyses. One-dimensional and two-dimensional spectra of the disaccharides **13**–**16** were all recorded with a Bruker Avance 500 spectrometer at 500 MHz for ^{1}H and 125 MHz for ^{13}C. Chemical shifts are given in δ units (ppm) measured downfield from Me_4Si. Assignments of ^{1}H and ^{13}C for carbohydrate residues of compounds **1**, **2**, **6**, **7**, **9**–**12** are given in Table 2 and Table 3. Disaccharides carbon atoms and protons are designated with the prefix "a" for the reducing residue and "b" for the other one. Microanalyses were performed by the Service de Microanalyse de l'ICSN (Gif sur Yvette, France) and by the Service de Microanalyse du Centre Régional de Mesures Physiques de l'Ouest (CRMPO, Rennes, France). The HRMS were measured at the CRMPO with a MS/MS ZabSpec TOF Micromass.

***p*-Nitrophenyl β-D-Fucofuranoside (1):** To a solution of **4**[6a] (196 mg, 0.59 mmol) in CH_2Cl_2 (4 mL), were successively added *p*-nitrophenol (123 mg, 0.88 mmol), Et_3N (41 μL, 0.29 mmol) and $BF_3{\cdot}OEt_2$

Table 2. ^{1}H NMR (400 MHz) chemical shifts and coupling constants (H,H and H,F) for compounds **6**–**7**, **9**–**12** and **2**.

Compound	δ (ppm), J (Hz)						
	1-H ($J_{1,2}$)	2-H ($J_{2,3}$)	3-H ($J_{3,4}$)	4-H ($J_{4,5}$)	5-H ($J_{5,6a}/J_{5,F}$)	6a-H ($J_{6a,6b}/J_{6a,F}$)	6b-H ($J_{6b,5}/J_{6b,F}$)
6[b]	5.04	3.98	4.01	4.08	3.83	4.72–4.31	4.72–4.31
	(< 1.0)	(3.3)	(6.9)	(3.3)	(6.3/17.3)	(nd/nd)[c]	(4.3/nd)
7[a]	4.85	3.93	4.02	3.88	3.97–3.91	4.48	4.42
	(1.8)	(4.0)	(6.6)	(3.8)	(4.8/–)	(9.4/46.8)	(6.8/47.8)
9[b]	5.06	4.03	3.96	4.21	4.96	4.65–4.46	4.65–4.46
	(1.3)	(3.3)	(6.6)	(4.8)	(7.4/–)	(nd/nd)	(6.8/–)
10[b]	5.04	4.01	4.06	4.12	3.97–3.89	4.43	4.43
	(< 1.0)	(2.6)	(6.2)	(2.8)	(5.8/–)	(nd/47.1)	(5.8/47.1)
11[b]	5.01	5.04	5.00	4.26	5.39	4.58	4.55
	(< 1.0)	(1.8)	(5.6)	(4.1)	(5.6/16.3)	(9.7/46.8)	(5.1/46.5)
12α[b]	6.33	5.32	5.58	4.25	5.25	4.66–4.44	4.66–4.44
	(4.6)	(7.1)	(6.4)	(7.1)	(4.1/20.9)	(nd/nd)	(4.1/ nd)
12β[b]	6.19	5.18	5.11	4.40	5.36	4.66–4.44	4.66–4.44
	(< 1.0)	(1.8)	(5.1)	(4.3)	(5.1/16.5)	(nd/nd)	(5.1/nd)
2[a]	5.66	4.28	4.20	4.05	3.98	4.46	4.42
	(2.0)	(4.3)	(6.4)	(3.3)	(5.1/14.5)	(9.6/46.8)	(6.8/47.8)

[a] Recorded in CD_3OD. [b] Recorded in $CDCl_3$. [c] nd: not determined.

Table 3. ^{13}C NMR (400 MHz) chemical shifts and coupling constants (C,F) for compounds **6**–**7**, **9**–**12** and **2**.

Compound	1-C	2-C	3-C	4-C ($J_{4-C,F}$)	5-C ($J_{5-C,F}$)	6-C ($J_{6-C,F}$)
6[b]	106.1	88.3	82.6	80.0	76.0	84.3
				(7.0)	(19.0)	(171.0)
7[a]	109.5	83.5	78.4	83.2	70.2	85.3
				(6.0)	(20.0)	(169.0)
9[b]	106.1	87.4	82.5	78.4	80.0	68.9
10[b]	106.4	87.3	83.2	80.5	69.7	84.1
				(6.0)	(21.0)	(171.0)
11[b]	105.5	81.4	76.6	79.3	69.9	81.1
				(5.0)	(22.0)	(173.0)
12α[b]	93.2	75.5	73.3	78.6	71.3	81.0
				(6.0)	(21.0)	(173.0)
12β[b]	99.1	80.7	76.3	81.6	69.8	80.8
				(6.0)	(21.0)	(172.0)
2[a]	107.7	83.3	77.9	85.0	70.0	85.1
				(6.0)	(20.0)	(169.0)

[a] Recorded in CD3OD. [b] Recorded in $CDCl_3$.

(187 μL, 1.48 mmol). After stirring for 1 h at room temperature, the mixture was diluted with CH_2Cl_2 (20 mL) and washed with a saturated solution of aqueous $NaHCO_3$ until complete discolouration. The aqueous layers thus obtained were extracted with CH_2Cl_2 (2×10 mL), and the combined organic layers were dried ($MgSO_4$), filtered and finally concentrated. The residue was purified by flash chromatography on silica gel eluting with light petroleum/AcOEt, 3:2. The fractions containing *p*-nitrophenyl 2,3,5-tri-*O*-acetyl-β-D-fucofuranoside R_f = 0.39 (light petroleum/AcOEt, 3:2) were collected and concentrated under reduced pressure. To the isolated mixture, a 0.1 M solution of sodium methylate (590 μL, 0.06 mmol) in MeOH (10 mL) was added. After stirring at room temperature overnight, the mixture was neutralised by glacial AcOH and concentrated under diminished pressure. A flash chromatography on silica gel eluting with CH_2Cl_2/MeOH (9:1) afforded **1** as a pale yellow foam with a two-step yield of 66% (111 mg, 0.39 mmol). $[\alpha]_D^{20}$ = –144.2 (*c* = 0.3, MeOH), $[\alpha]_D^{20}$[18] = –145.0 (*c* = 1.4, MeOH). TLC (CH_2Cl_2/MeOH, 9:1): R_f = 0.26. NMR spectroscopic data were compatible with those previously published.[18a]

***n*-Octyl 2,3,5-Tri-*O*-benzyl-6-deoxy-6-fluoro-β-D-galactofuranoside (6):** To a solution of **5** (2.04 g, 3.63 mmol) in CH_2Cl_2 (41 mL) were successively added Et_3N (1 mL, 7.12 mmol) and DAST (567 μL, 4.33 mmol). After stirring at room temperature for 15 min, the reaction was quenched by a 2:1 solution of Et_3N and MeOH (6 mL) and concentrated. The resulting residue was then purified by flash chromatography eluting with light petroleum/AcOEt (19:1 → 9:1) to give the fluoro-compound **6** (962 mg) in 47% yield. $[\alpha]_D^{20}$ = –53.8 (*c* = 1.0, CH_2Cl_2). TLC (light petroleum/AcOEt, 9:1): R_f = 0.44. ^{19}F NMR ($CDCl_3$): δ = –227 ppm. ^{1}H NMR ($CDCl_3$): δ (carbohydrate ring protons, see Table 2) = 7.38–7.22 (m, 15 H, C_6H_5), 4.73–4.31 (m, 8 H, H-6a, H-6b, OCH_2Ph), 3.65 (dt, ^2J = 9.9, ^3J = 6.6 Hz, 1 H, OCH_2CH$_2$), 3.38 (dt, ^3J = 6.9 Hz, 1 H, OCH_2CH$_2$), 1.59–1.53 (m, 2 H, OCH$_2$CH_2), 1.34–1.27 [m, 10 H, $(CH_2)_5$], 0.88 (t, ^3J = 7.1 Hz, 3 H, CH_3) ppm. ^{13}C NMR ($CDCl_3$): δ (carbohydrate ring carbon atoms, see Table 3) = 138.0, 137.8, 137.7 (C_{ipso} C_6H_5), 128.5, 128.4, 128.4, 128.3, 128.1, 128.0, 127.9, 127.9 (C_6H_5), 73.6 (d, $^4J_{C,F}$ = 2 Hz, O*C*H$_2$Ph), 72.1, 72.0 (O*C*H$_2$Ph), 67.8 (O*C*H$_2$CH$_2$), 31.9, 29.6, 29.4, 29.4, 26.2, 22.8 [$(CH_2)_6$], 14.2 (CH_3) ppm. HRMS (ESI$^+$): $[C_{35}H_{45}FO_5 + Na]^+$: calcd. 587.3149; found 587.3154. $C_{35}H_{45}FO_5$ (564.7): calcd. C 74.44, H 8.03; found C 74.41, H 7.97.

***n*-Octyl 6-Deoxy-6-fluoro-β-D-galactofuranoside (7). Procedure A:** An aqueous solution of $NaBrO_3$ (33 mL, 4.71 g, 31.21 mmol) was added to a solution of **6** (1.96 g, 3.47 mmol) in AcOEt (40 mL). This mixture was incubated at room temperature for 10 min, and then an aqueous solution of $NaHSO_3$ (83 mL, 6.40 g, 31.24 mmol) was added. In order to complete the debenzylation, the mixture was vigorously stirred for 30 min before more $NaBrO_3$ (2.00 g, 13.25 mmol) was added. After 30 min, the reaction mixture was diluted with AcOEt (60 mL) and a 20% aqueous solution of $Na_2S_2O_3$ (40 mL) and stirred for more 20 min. The resulting suspension was filtered and the organic layer successively washed with a 20% aqueous solution of $Na_2S_2O_3$ (4×30 mL) and a saturated solution of $NaHCO_3$ (2×30 mL). The aqueous layer was then extracted with AcOEt (4×30 mL), and the combined organic layers were dried ($MgSO_4$), concentrated under diminished pressure and chromatographed (light petroleum/AcOEt, 1:1) to afford **7** (820 mg, 80%) as a white solid. **Procedure B:** A solution of dibenzylated intermediate **10** (1.77 g, 3.73 mmol) in AcOH/AcOEt (1:1, 18 mL) added of palladium(II) acetate (177 mg) was stirred for 72 h under 1 atm of hydrogen. After completion of the deprotection, the solvents were removed by co-evaporation with MeOH (5×50 mL) and a chromatographic purification (CH_2Cl_2/MeOH, 49:1) gave **7** (1.00 g, 92%) as a white solid. M.p. (light petroleum) 48 °C. $[\alpha]_D^{20}$ = –83.5 (*c* = 0.3, MeOH). TLC (1:1 light petroleum/AcOEt): R_f = 0.17. ^{19}F NMR (CD_3OD): δ = –230 ppm. ^{1}H NMR (CD_3OD): δ (carbohydrate ring protons, see Table 2) = 3.68 (dt, ^2J = 9.7, ^3J = 6.6 Hz, 1 H, OCH_2CH$_2$), 3.42 (dt, ^3J = 6,6 Hz, 1 H, OCH_2CH$_2$), 1,61–1,55 (m, 2 H, OCH$_2$CH_2), 1.38–1.30 [m, 10 H, $(CH_2)_5$], 0.90 (t, ^3J = 6.9 Hz, 3 H, CH_3) ppm. ^{13}C NMR (CD_3OD): δ (carbohydrate ring carbon atoms, see Table 3) = 69.0 (O*C*H$_2$CH$_2$), 33.0, 30.7, 30.5, 30.4, 27.2, 23.7 [$(CH_2)_6$], 14.4 (CH_3) ppm. HRMS (ESI$^+$): $[C_{14}H_{27}FO_5 + Na]^+$: calcd. 317.1740; found 317.1748. $C_{14}H_{27}FO_5$ (294.4): calcd. C 57.12, H 9.25; found C 56.85, H 9.11.

***n*-Octyl 2,3-Di-*O*-benzyl-5,6-*O*-sulfonyl-β-D-galactofuranoside (9):** Pyridine (3.5 mL, 43.3 mmol) and thionyl chloride (1.5 mL, 20.6 mmol) diluted in CH_2Cl_2 (5 mL) were added dropewise to a cooled (0 °C) solution of **8** (4.92 g, 10.4 mmol) in CH_2Cl_2 (25 mL). After 10 min at room temperature, the reaction mixture was partitioned between CH_2Cl_2 (200 mL) and 5% aqueous HCl (25 mL) and decanted. The organic layer was further washed with 5% aqueous HCl (2×25 mL), saturated aqueous Na_2CO_3 (2×25 mL) and brine (2×25 mL). Yield could be improved by treating the resulting aqueous layer with CH_2Cl_2 (3×20 mL). Finally, the combined organic layers were dried ($MgSO_4$), and the residue could be used without further purification. Nevertheless, main physical data were determined directly from the mixture of both diastereoisomeric thionyl intermediates. TLC (light petroleum/AcOEt, 5:1): R_f = 0.34. HRMS (ESI$^+$): $[C_{28}H_{38}O_7S + Na]^+$: calcd. 541.2236; found 541.2247. $C_{28}H_{38}O_7S$ (518.7): calcd. C 64.84, H 7.38, S 6.18; found C 64.81, H 7.43, S 6.17.

Diastereoisomer a: ^{1}H NMR ($CDCl_3$): δ = 7.31–7.17 (m, 10 H, C_6H_5), 4.99 (d, $J_{1,2}$ = 1.5 Hz, 1 H, 1-H), 4.52–4.37 (m, 6 H, 5-H, 6a-H, OCH_2Ph), 4.25–4.19 (m, 2 H, 4-H, 6b-H), 3.95 (dd, $J_{2,3}$ = 3.0 Hz, 1 H, 2-H), 3.87 (dd, $J_{3,4}$ = 6.9 Hz, 1 H, 3-H), 3.63 (dt, ^2J = 9.6, ^3J = 6.8 Hz, 1 H, OCH_2CH$_2$), 3.33 (dt, 1 H, ^3J = 6.9 Hz, OCH_2CH$_2$), 1.53–1.46 (m, 2 H, OCH$_2$CH_2), 1.27–1.12 [m, 10 H, $(CH_2)_5$], 0.80 (t, ^3J = 6.8 Hz, 3 H, CH_3) ppm. ^{13}C NMR ($CDCl_3$): δ = 137.3 (C_{ipso} C_6H_5), 128.6, 128.2, 128.1 (C_6H_5), 106.6 (1-C), 87.8 (2-C), 83.8 (3-C), 82.8 (5-C), 79.9 (4-C), 72.3, 72.2 (O*C*H$_2$Ph), 68.2 (O*C*H$_2$CH$_2$), 67.3 (6-C), 31.9, 29.5, 29.4, 26.2, 24.1, 22.7 [$(CH_2)_6$], 14.2 (CH_3) ppm. **Diastereoisomer b:** ^{1}H NMR ($CDCl_3$): δ = 7.31–7.17 (m, 10 H, C_6H_5), 4.99–4.94 (m, 1 H, 5-H), 4.93 (d, $J_{1,2}$ = 1.3 Hz, 1 H,1-H), 4.53–4.37 (m, 5 H, 6a-H, OCH_2Ph), 4.27 (dd, $J_{6a,6b}$ = 8.4, $J_{5,6b}$ = 5.3 Hz, 1 H, 6b-H), 4.00 (dd, $J_{3,4}$ = 6.9, $J_{4,5}$ = 4.3 Hz, 1 H, 4-H), 3.93 (dd, $J_{2,3}$ = 3.0 Hz, 1 H, 2-H), 3.83 (dd, 1 H, 3-H), 3.59 (dt, ^2J = 9.7, ^3J = 6.6 Hz, 1 H, OCH_2CH$_2$), 3.32 (dt, ^3J = 6.6 Hz, 1 H, OCH_2CH$_2$), 1.53–1.46 (m, 2 H, OCH$_2$CH_2), 1.27–1.13 [m, 10 H, $(CH_2)_5$], 0.80 (t, ^3J = 7.1 Hz, 3 H, CH_3) ppm. ^{13}C NMR ($CDCl_3$): δ = 137.2 (C_{ipso} C_6H_5), 128.6, 128.2, 128.1 (C_6H_5), 106.4 (1-C), 87.7 (2-C), 82.9 (3-C), 79.7 (4-C), 78.9 (5-C), 72.4, 72.2 (O*C*H$_2$Ph), 68.3 (6-C), 68.1 (O*C*H$_2$CH$_2$), 31.9, 29.5, 29.4, 29.3, 26.2, 22.7 [$(CH_2)_6$], 14.2 (CH_3) ppm. The crude oil previously obtained was diluted in a CH_2Cl_2/CH_3CN/H_2O mixture (2:2:3, 77 mL) before adding $NaIO_4$ (4.46 g, 20.9 mmol) and ruthenium(III) chloride (10 mg). This solution was vigorously stirred at room temperature for 30 min and then diluted with CH_2Cl_2 (200 mL). After separation, the organic layer was washed twice with water (50 mL) and the combined aqueous layers extracted with CH_2Cl_2 (3×25 mL). Finally, all organic phases were dried with $MgSO_4$, concentrated under diminished pressure before chromatographic purification (light petroleum/AcOEt, 9:1) to give **9** (4.95 g) in 89% yield for two steps. $[\alpha]_D^{20}$ = –58.2 (*c* = 1.1, CH_2Cl_2). TLC (light petroleum/AcOEt, 5:1): R_f = 0.30. ^{1}H NMR ($CDCl_3$): δ (carbohydrate ring protons, see Table 2) = 7.40–7.26 (m, 10 H,

C_6H_5), 4.65–4.46 (m, 6 H, 6-aH, 6-bH, OCH_2Ph), 3.69 (dt, ^2J = 9.6, ^3J = 6.6 Hz, 1 H, OCH_2CH$_2$), 3.41 (dt, ^3J = 6.9 Hz, 1 H, OCH_2CH$_2$), 1.62–1.55 (m, 2 H, OCH$_2$CH_2), 1.35–1.21 [m, 10 H, (CH$_2$)$_5$], 0.89 (t, ^3J = 7.1 Hz, 3 H, CH$_3$) ppm. ^{13}C NMR ($CDCl_3$): δ (carbohydrate ring carbon atoms, see Table 3) = 137.1, 137.0 (C_{ipso} C_6H_5), 128.7, 128.3, 129.2, 128.2 (C_6H_5), 72.5, 72.3 (OCH$_2$Ph), 68.4 (OCH$_2$CH$_2$), 31.9, 29.5, 29.4, 29.3, 26.2, 22.7 (OCH$_2$$CH_2$), 14.2 (CH$_3$) ppm. HRMS (ESI$^+$): [$C_{28}H_{38}O_8S$ + K]$^+$: calcd. 573.1925; found 573.1923. $C_{28}H_{38}O_8S$ (534.7): calcd. C 62.90, H 7.16, S 6.00; found C 62.81, H 7.11, S 5.98.

***n*-Octyl 2,3-Di-*O*-benzyl-6-deoxy-6-fluoro-β-D-galactofuranoside (10):** To a solution of **9** (4.30 g, 8.04 mmol) in acetone (43 mL) was added a 1 M solution of TBAF in THF (12.1 mL, 12.1 mmol) and stirred at room temperature for 1 h. After concentration, the crude oil was diluted with THF (43 mL) before adding a solution of concentrated H_2SO_4 (1.3 mL, 24.4 mmol) and H_2O (434 μL, 24.1 mmol). The reaction media was stirred at room temperature for 15 min and then neutralised with Et_3N, concentrated under reduced pressure and partitioned between AcOEt (200 mL) and brine (50 mL). The organic phase was washed with aqueous saturated Na_2CO_3 and the aqueous layers extracted with AcOEt (3 × 25 mL). Finally, the combined organic layers were dried ($MgSO_4$), the solvent removed and the residue chromatographically purified (light petroleum/AcOEt, 9:1 → 5:1). This procedure afforded the target compound **10** as colourless oil (3.28 g, 86%). $[\alpha]_D^{20}$ = –69.9 (*c* = 1.1, CH_2Cl_2). TLC (5:1 light petroleum/AcOEt): R_f = 0.20. ^{19}F NMR ($CDCl_3$): δ = –229 ppm. ^{1}H NMR ($CDCl_3$): δ (carbohydrate ring protons, see Table 2) = 7.38–7.28 (m, 10 H, C_6H_5), 4.62–4.49 (m, 4 H, OCH_2Ph), 3.67 (dt, ^2J = 9.7, ^3J = 6.9 Hz, 1 H, OCH_2CH$_2$), 3.40 (dt, ^3J = 6.6 Hz, 1 H, OCH_2CH$_2$), 2.37 (d, ^3J = 7.6 Hz, 1 H, OH), 1.61–1.54 (m, 2 H, OCH$_2$CH_2), 1.35–1.21 [m, 10 H, (CH$_2$)$_5$], 0.88 (t, ^3J = 7.1 Hz, 3 H, CH$_3$) ppm. ^{13}C NMR ($CDCl_3$): δ (carbohydrate ring carbon atoms, see Table 3) = 137.7, 137.3 (C_{ipso} C_6H_5), 128.6, 128.5, 128.1, 128.1, 128.0, 127.9 (C_6H_5), 72.4, 72.1 (OCH$_2$Ph), 67.9 (OCH$_2$CH$_2$), 31.9, 29.6, 29.4, 29.4, 26.2, 22.8 [(CH$_2$)$_6$], 14.2 (CH$_3$) ppm. HRMS (ESI$^+$): [$C_{28}H_{39}FO_5$ + K]$^+$: calcd. 513.2419; found 513.2421. $C_{28}H_{39}FO_5$ (474.6): calcd. C 70.86, H 8.28; found C 70.85, H 8.31.

***n*-Octyl 2,3,5-Tri-*O*-acetyl-6-deoxy-6-fluoro-β-D-galactofuranoside (11):** Acetylation of **7** (780 mg, 2.65 mmol) was performed at room temperature in dry pyridine (6.5 mL, 79.71 mmol) using Ac_2O (7.5 mL, 79.86 mmol) as acylating agent. After 48 h stirring, concentration under reduced pressure and co-distillation with MeOH (3 × 10 mL), the crude oil was diluted with AcOEt (100 mL) and successively washed with 5% aqueous HCl (2 × 20 mL), saturated aqueous Na_2CO_3 (2 × 20 mL) and brine (20 mL). The resulting aqueous layers were then extracted with AcOEt (3 × 10 mL) and the combined organic layers were dried ($MgSO_4$) and concentrated. Finally, purification of the crude oil by flash chromatography (light petroleum/AcOEt, 4:1) yielded the desired product **11** (1.08 g, 97%). $[\alpha]_D^{20}$ = –47.4 (*c* = 0.6, CH_2Cl_2). TLC (light petroleum/AcOEt, 4:1): R_f = 0.31. ^{19}F NMR ($CDCl_3$): δ = –231 ppm. ^{1}H NMR ($CDCl_3$): δ (carbohydrate ring protons, see Table 2) = 3.64 (dt, ^2J = 9.7, ^3J = 6.6 Hz, 1 H, OCH_2CH$_2$), 3.43 (dt, ^3J = 6.4 Hz, 1 H, OCH_2CH$_2$), 2.14, 2.09, 2.08 (3 s, 9 H, CH$_3$CO), 1.61–1.52 (m, 2 H, OCH$_2$CH_2), 1.37–1.21 [m, 10 H, (CH$_2$)$_5$], 0.87 (t, ^3J = 6.9 Hz, 3 H, CH$_3$) ppm. ^{13}C NMR ($CDCl_3$): δ (carbohydrate ring carbon atoms, see Table 3) = 170.1, 169.7 (CO), 67.9 (OCH$_2$CH$_2$), 31.9, 29.4, 29.3, 26.0, 22.7 [(CH$_2$)$_6$], 20.9, 20.7 (*C*H$_3$CO), 14.2 (CH$_3$) ppm. HRMS (ESI$^+$): [$C_{20}H_{33}FO_8$ + Na]$^+$: calcd. 443.2057; found 443.2060. $C_{20}H_{33}FO_8$ (420.5): calcd. C 57.13, H 7.91; found C 57.39, H, 7.91.

1,2,3,5-Tetra-*O*-acetyl-6-deoxy-6-fluoro-β-D-galactofuranose (12): Ac_2O (50 μL, 0.53 mmol) and H_2SO_4 (1.4 μL, 0.03 mmol) were successively added to a solution of **11** (56 mg, 0.13 mmol) in dry CH_2Cl_2 (560 μL). After stirring at room temperature for 24 h, the reaction was quenched by adding Et_3N, the solvent removed under diminished pressure and the crude residue purified by chromatography (light petroleum/AcOEt, 4:1 → 3:2). This acetolysis procedure yielded an inseparable anomeric mixture of **12** (α/β = 1:4.2, 46 mg, 98%) as a colourless oil. TLC (light petroleum/AcOEt, 3:2): R_f = 0.40. HRMS (ESI$^+$): [$C_{14}H_{19}FO_8$ + Na]$^+$: calcd. 373.0911; found 373.0916. $C_{14}H_{19}FO_9$ (350.3): calcd. C 48.00, H 5.47; found C 48.05, H 5.57. **α-Anomer:** ^{19}F NMR ($CDCl_3$): δ = –233 ppm. ^{1}H NMR ($CDCl_3$): δ (carbohydrate ring protons, see Table 2) = 2.13, 2.12, 2.11, 2.08 (4 s, 12 H, CH$_3$CO) ppm. ^{13}C NMR ($CDCl_3$): δ = carbohydrate ring carbon atoms (see Table 3), 170.1, 169.9, 169.5, 169.3 (CO), 21.1, 20.9, 20.7, 20.5 (*C*H$_3$CO). **β-Anomer:** ^{19}F NMR ($CDCl_3$): δ = –231 ppm. ^{1}H NMR ($CDCl_3$): δ (carbohydrate ring protons, see Table 2) = 2.14, 2.12, (4 s, 12 H, CH$_3$CO) ppm. ^{13}C NMR ($CDCl_3$): δ (carbohydrate ring carbon atoms, see Table 3) = 170.1, 169.9, 169.5, 169.2 (CO), 21.1, 20.9, 20.7 (*C*H$_3$CO) ppm.

***p*-Nitrophenyl 6-Deoxy-6-fluoro-β-D-galactofuranoside (2):** To a solution of **12** (218 mg, 0.62 mmol) in CH_2Cl_2 (4 mL), were successively added *p*-nitrophenol (130 mg, 0.93 mmol), Et_3N (44 μL, 0.31 mmol) and BF_3·OEt_2 (197 μL, 1.56 mmol). After stirring at room temperature for 1 h, the mixture was diluted with CH_2Cl_2 (20 mL), and washed with a saturated solution of aqueous $NaHCO_3$ until complete discolouration. The aqueous layers thus obtained were extracted with CH_2Cl_2 (2 × 10 mL) and the combined organic layers were dried ($MgSO_4$), filtered and finally concentrated. The residue was purified by flash chromatography on silica gel eluting with light petroleum/AcOEt (7:3). The fractions containing *p*-nitrophenyl 2,3,5-tri-*O*-acetyl-6-deoxy-6-fluoro-β-D-galactofuranoside [TLC (light petroleum/AcOEt, 3:2): R_f = 0.42] were collected and concentrated under reduced pressure. To the isolated mixture dissolved in anhydrous MeOH (12 mL), a decimolar solution of sodium methylate (620 μL, 0.06 mmol) in MeOH was added. After stirring overnight at room temperature, the mixture was neutralised by glacial AcOH and concentrated under reduced pressure. A flash chromatography on silica gel eluting with CH_2Cl_2/MeOH (9:1) afforded **2**, as a colourless solid with a two-step yield of 61% (115 mg, 0.38 mmol). Mp 144 °C. $[\alpha]_D^{20}$ = –197.5 (*c* = 0.4, MeOH). TLC (CH_2Cl_2/MeOH, 9:1): R_f = 0.53. ^{19}F NMR (CD_3OD): δ = –230 ppm. ^{1}H NMR (CD_3OD): δ (carbohydrate ring protons, see Table 2) = 8.22 (d, ^3J = 9.4 Hz, 2 H, H_m C_6H_4), 7.21 (d, 2 H, H_o C_6H_4) ppm. ^{13}C NMR (CD_3OD): δ (carbohydrate ring carbon atoms, see Table 3) = 163.4 (C_{ipso} C_6H_4), 143.6 (C_p C_6H_4), 126.6 (C_m C_6H_4), 117.6 (C_o C_6H_4) ppm. HRMS (ESI$^+$): [$C_{12}H_{14}FNO_7$ + Na]$^+$: calcd. 342.0391; found 342.0388.

AbfD3 Production and Activity Measurement: Recombinant arabinofuranosidase AbfD3 expressed from the plasmid-borne AbfD3 gene was produced and purified from *Escherichia coli* cells as described previously.[15,29] The hydrolytic activity of AbfD3 was quantified after incubation of the enzyme with *p*-NP furanosides (5 mM in 900 μL 50 mM sodium acetate, pH 5.8) at 60 °C. In each case 100 μL of enzyme solution was employed, but the amounts of enzyme were adapted for the different substrates: *p*-NP-α-L-Ara*f*, 0.10 IU, *p*-NP-β-D-Gal*f* 1.60 IU, *p*-NP-6F-β-D-Gal*f*, 0.32 IU and *p*-NP-β-D-Fuc*f*, 0.11 IU. Continuous release of *p*-nitrophenol was measured at 401 nm. One unit of activity corresponds to the amount of enzyme releasing 1 μmol of *p*-nitrophenol per minute. Initial rate conditions and a suitable substrate concentration (0.5 to 40 mM) were used in order to determine the kinetic parameters K_m and k_{cat} from Lineweaver–Burk plots.

Transglycosylation Reactions: A 5 mM aqueous solution of **1** or **2** (1.4 mL) was incubated with 12 IU AbfD3 under magnetic stirring for 30 min at 60 °C. Reactions were quenched by enzyme denaturation at 100 °C for 10 min. After lyophilizing, the residue was dissolved in water (100 μL) and the products of transglycosylation reactions were purified by preparative TLC using 4:1 CH_2Cl_2/MeOH as the mobile phase. The desired products were detected by UV absorption (254 nm), collected from the plates and extracted with 1:1 CH_2Cl_2/MeOH (4 mL). After filtration and freeze-drying, the isolated disaccharides **13**, **14** and **15**, **16**, respectively obtained form **1** and **2**, were characterised by NMR and high resolution mass spectrometry.

***p*-Nitrophenyl β-D-Fucofuranosyl-(1→2)-β-D-fucofuranoside (13):** HPTLC (CH_2Cl_2/MeOH, 4:1): R_f = 0.64. ^{1}H NMR (D_2O): δ = 8.16 (d, ^3J = 9.3 Hz, 2 H, H_m C_6H_4), 7.16 (d, 2 H, H_o C_6H_4), 5.84 (s, 1 H, 1a-H), 5.11 (d, $J_{1b,2b}$ = 1.4 Hz, 1 H, 1b-H), 4.32 (dd, $J_{1a,2a}$ < 1, $J_{2a,3a}$ = 4.0 Hz, 1 H, 2a-H), 4.12 (dd, $J_{3a,4a}$ = 6.0 Hz, 1 H, 3a-H), 4.02 (dd, $J_{2b,3b}$ = 3.8 Hz, 1 H, 2b-H), 3.87–3.82 (m, 2 H, 4a-H, 3b-H), 3.77–3.72 (m, 1 H, 5b-H), 3.70–3.64 (m, 1 H, 4b-H), 3.55–3.48 (m, 1 H, 5a-H), 1.13 (d, $J_{5a,6a}$ = 6.11 Hz, 3 H, 6a-H), 0.99 (d, 1 H, 6b-H, $J_{5b,6b}$ = 6.5 Hz) ppm. ^{13}C NMR (D_2O): δ = 161.2 (C_{ipso} C_6H_4), 142.1 (C_p C_6H_4), 126.1 (C_m C_6H_4), 116.7 (C_o C_6H_4), 107.3 (1b-C), 104.3 (1a-C), 87.6 (2a-C), 87.5 (4b-C), 87.2 (4a-C), 81.6 (2b-C), 77.3 (3b-C), 75.3 (3a-C), 66.8 (5b-C), 62.5 (5a-C), 18.2 (6a-C, 6b-C) ppm. HRMS (ESI$^+$): $[C_{18}H_{25}NO_{11} + Na]^+$: calcd. 454.1325; found 454.1331.

***p*-Nitrophenyl β-D-Fucofuranosyl-(1→3)-β-D-fucofuranoside (14):** HPTLC (CH_2Cl_2/MeOH, 4:1): R_f = 0.68. ^{1}H NMR (D_2O): δ = 8.19 (d, ^3J = 9.3 Hz, 2 H, H_m C_6H_4), 7.17 (d, ^3J = 9.3 Hz, 2 H, H_o C_6H_4), 5.79 (d, $J_{1a,2a}$ = 1.1 Hz, 1 H, 1a-H), 5.14 (d, $J_{1b,2b}$ = 1.4 Hz, 1 H, 1b-H), 4.49 (dd, $J_{2a,3a}$ = 2.1 Hz, 1 H, 2a-H), 4.14 (dd, $J_{3a,4a}$ = 4.8 Hz, 1 H, 3a-H), 4.07 (dd, $J_{2b,3b}$ = 3.1 Hz, 1 H, 2b-H), 3.99–3.93 (m, 2 H, 4a-H, 5a-H), 3.87 (dd, $J_{3b,4b}$ = 6.1 Hz, 1 H, 3b-H), 3.83 (m, 1 H, 5b-H), 3.69 (t, $J_{4b,5b}$ = 6.1 Hz, 1 H, 4b-H), 1.22 (d, $J_{5a,6a}$ = 6.9 Hz, 3 H, 6a-H), 1.19–1.16 (m, 3 H, 6b-H) ppm. ^{13}C NMR (D_2O): δ = 161.2 (C_{ipso} C_6H_4), 141.9 (C_p C_6H_4), 126.1 (C_m C_6H_4), 116.8 (C_o C_6H_4), 106.6 (1b-C), 105.7 (1a-C), 87.6 (4b-C), 87.4 (4a-C), 82.2 (3a-C), 81.6 (2b-C), 79.2 (2a-C), 77.4 (3b-C), 67.5 (5b-C), 66.9 (5a-C), 20.0 (6a-C), 18.3 (6b-C) ppm. HRMS (ESI$^+$): $[C_{18}H_{25}NO_{11} + Na]^+$: calcd. 454.1325; found 454.1321.

***p*-Nitrophenyl 6-Deoxy-6-fluoro-β-D-galactofuranosyl-(1→2)-6-deoxy-6-fluoro-β-D-galactofuranoside (15):** HPTLC (CH_2Cl_2/MeOH, 4:1): R_f = 0.54. ^{1}H NMR (D_2O): δ = 8.17 (d, J = 9.3 Hz, 2 H, H_m C_6H_4), 7.14 (d, 2 H, H_o C_6H_4), 5.81 (d, $J_{1a,2a}$ = 1.7 Hz, 1 H, 1a-H), 5.10 (d, $J_{1b,2b}$ = 1.6 Hz, 1 H, 1b-H), 4.51–4.41 (m, $J_{6a,F}$ = 46.0 Hz, 2 H, 6a-H), 4.33 (dd, $J_{2a,3a}$ = 4.3 Hz, 1 H, 2a-H), 4.29–4.26 (m, 1 H, 4a-H), 4.27 (dd, $J_{3a,4a}$ = 6.9 Hz, 1 H, 3a-H), 4.20–4.08 (m, $J_{6b,F}$ = 47.0 Hz, 2 H, 6b-H), 4.06 (dd, $J_{2b,3b}$ = 3.6 Hz, 1 H, 2b-H), 4.05–3.98 (m, 2 H, 5a-H, 3b-H), 3.85 (m, 2 H, 4b-H, 5b-H) ppm. ^{13}C NMR (D_2O): δ = 161.3 (C_{ipso} C_6H_4), 142.0 (C_p C_6H_4), 126.3 (C_m C_6H_4), 116.9 (C_o C_6H_4), 108.1 (1b-C), 104.7 (1a-C), 88.0 (2a-C), 85.0 (6a-C, $J_{6a,F}$ = 161.0 Hz), 84.0 (4a-C), 83.5 (6b-C, $J_{6b,F}$ = 160.0 Hz), 83.0 (4b-C), 81.8 (2b-C), 76.9 (3b-C), 75.1 (3a-C), 69.0 (5a-C, 5b-C) ppm.

***p*-Nitrophenyl 6-Deoxy-6-fluoro-β-D-galactofuranosyl-(1→3)-6-deoxy-6-fluoro-β-D-galactofuranoside (16):** HPTLC (CH_2Cl_2/MeOH, 4:1): R_f = 0.62. ^{1}H NMR (D_2O): δ = 8.16 (d, J = 9.1 Hz, 2 H, H_m C_6H_4), 7.12 (d, 2 H, H_o C_6H_4), 5.76 (d, $J_{1a,2a}$ = 1.5 Hz, 1 H, 1a-H), 5.13 (d, $J_{1b,2b}$ = 1.4 Hz, 1 H, 1b-H), 4.56–4.37 (m, $J_{6a,F}$ = 46.5 Hz, 2 H, 6a-H), 4.49 (dd, $J_{2a,3a}$ = 3.56 Hz, 1 H, 2a-H), 4.23 (dd, $J_{3a,4a}$ = 5.4 Hz, 1 H, 3a-H), 4.15–4.13 (m,1 H, 4a-H), 4.13–3.87 (m, $J_{6b,F}$ = 47.1 Hz, 2 H, 6b-H), 4.06 (dd, $J_{2b,3b}$ = 3.1 Hz, 1 H, 2b-H), 4.01–3.98 (m, 1 H, 5a-H), 3.90–3.88 (m, 1 H, 4b-H), 3.86–3.83 (m, 1 H, 5b-H), 3.84 (dd, $J_{3b,4b}$ = 6.5 Hz, 1 H, 3b-H) ppm. ^{13}C NMR (D_2O): δ = 160.9 (C_{ipso} C_6H_4), 142.3 (C_p C_6H_4), 125.8 (C_m C_6H_4), 117.3 (C_o C_6H_4), 107.2 (1b-C), 105.4 (1a-C), 86.1 (6a-C, $J_{6a,F}$ = 160.3 Hz), 84.2 (6b-C, $J_{6b,F}$ = 161.2 Hz), 82.9 (4a-C), 82.6 (3a-C), 82.3 (2a-C), 81.6 (4b-C), 81.5 (2b-C), 76.4 (3b-C), 71.3 (5b-C), 69.9 (5a-C) ppm.

Supporting Information: (see also the footnote on the first page of this article) One-dimensional and two-dimensional correlation NMR spectra for new monosaccharides and disaccharides.

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