Carbohydrate Research xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Carbohydrate Research

journal homepage: www.elsevier.com/locate/carres

Transglycosylations employing recombinant α- and β-galactosidases and novel donor substrates

Sven Schröder [a], Lars Kröger [a], Ralf Mattes [b], Joachim Thiem [a,*]

[a] University of Hamburg, Faculty of Science, Department of Chemistry, Martin-Luther-King-Platz 6, D-20146 Hamburg, Germany
[b] University of Stuttgart, Institute for Industrial Genetics, Allmandring 31, D-70569 Stuttgart, Germany

ARTICLE INFO

Article history:
Received 21 March 2014
Received in revised form 7 May 2014
Accepted 10 May 2014
Available online xxxx

Keywords:
Chemoenzymatic glycosylation
Recombinant galactosidases
Galactooligosaccharide synthesis

ABSTRACT

Recombinant α- and β-galactosidases could be prepared in larger amounts for chemoenzymatic syntheses of glycosylated oligosaccharides relevant in nutrition approaches. α-Galactosidase RafA from *Escherichia coli*, another thermophilic α-galactosidase AgaB from *Geobacillus stearothermophilus* KVE39, and also a thermophilic β-galactosidase BglT from *Thermus thermophilus* TH 125 could be employed in α- and in β-glycosylations, respectively. With model structures as well as sucrose, isomaltitol, and isomaltulose the stereo- and regiospecificities were studied. Further, a number of modified donor structures with structural variation and different leaving groups were synthesized, employed, and compared to classical donors for these transglycosylations.

1. Introduction

In human nutrition galactooligosaccharides represent an interesting group of bioactive compounds in the search for prebiotic nutrition additives. Some galactooligosaccharides support the proliferation of bifidobacteria and are thus of vital importance to the intestinal flora.[1,2] As a consequence of increasing malnutrition there is a strong demand for human health promoting additives to nourishment. Increased significance is given to galactooligosaccharides that are structural analogs of known mono-, di-, and trisaccharides. These structural analogs are supposed to be potentially prebiotic nutrition additives.

It was of interest to devise simple and non-toxic synthetic approaches to such galactooligosaccharides. The often high regioselectivity of enzymes allows the synthesis of a particular saccharide structure without the disadvantages of classical organic synthesis, such as long synthetic pathways with extended application of protecting groups. Further, in the course of classical organic reactions often toxic substances such as reagents, solvents, and catalysts are used, however, even small traces of toxic contaminants may rule out a product for nutrition purposes. In contrast, enzymatic syntheses are beneficial in that respect and to date, substantial advances have been made in synthesis employing β-galactosidases.[3,4]

2. Results and discussion

2.1. Preparation of enzymes

Previously, we have cloned the genes of the thermophilic α-galactosidase AgaB from *Geobacillus stearothermophilus* KVE39,[5] the β-galactosidase BglT from *Thermus thermophilus* TH125[6] and the mesophilic α-galactosidase RafA from *Escherichia coli*.[7] The enzymes were overexpressed in *E. coli* harboring the pBTac plasmid derivatives pAMG22 for expression of the *agaB* gene, pHWG543 for the *bglT* gene, and pREM1128 for *rafA* gene expression. The recombinant enzymes were produced in high amounts in *E. coli* RM448. After disruption of the cells by French press, the thermophilic enzymes AgaB[5] and BglT[6] were enriched to 80% homogeneity by heat precipitation of the host protein. Extracts of *E. coli*/pREM1128 were purified for RafA preparation as described by Spangenberg et al.[7]

2.2. Glycosylations employing RafA

Attractive donor substrates for α-galactosidases are glycopyranosyl fluorides. In contrast to β-glycopyranosyl fluorides, the α-anomers are stable even in aqueous medium and easy to handle. Additionally, these fluorides are non-toxic derivatives, and thus they meet the requirements for food-additive syntheses. For initial studies concerning regioselectivity and preparative capability of modified enzymes glycosyl fluorides **1**–**3** were incubated as donor substrates for glycosylation of methyl α-D-glucopyranoside (**4**) at pH 6.5 and 37 °C for 24 h in phosphate buffer to neutralize the

* Corresponding author. Tel.: +49 40 42838 4241; fax: +49 40 42838 4325.
E-mail address: thiem@chemie.uni-hamburg.de (J. Thiem).

http://dx.doi.org/10.1016/j.carres.2014.05.005
0008-6215/

released HF. Separation and purification of the reaction products were done on Biogel P2 to give the corresponding disaccharides. For structure assignments these were generally peracetylated in anhydrous pyridine with acetic anhydride.

Treatment of **4** with α-D-galactopyranosyl fluoride (**1**)[8] led to 19% of a mixture of the (α1–6)- (**5**) and the (α1–4)-linked disaccharide (**8**) in a ratio of 71:29. Assignment by MS and ^{1}H as well as ^{13}C NMR of the peracetylated regioisomeric derivatives **5Ac** and **8Ac** was unequivocal. It was further of interest to check, whether the enzyme could tolerate modified donor structures. Thus, under corresponding condition α-D-fucopyranosyl fluoride (**2**)[9] was employed, and in reaction with **4** lead to 16% of a disaccharide mixture having the (α1–6)- (**6**) and the (α1–4)-linked disaccharide (**9**) in a ratio of 69:31. As before, the assignment was done spectroscopically with the peracetylated regioisomers **6Ac** and **9Ac**. Finally, L-arabinose as the pentose analog of D-galactose was of interest to be linked since there are reports for this sugar in animal feed[10,11] as well as dietary food.[12] Thus, the β-L-arabinopyranosyl fluoride (**3**) was prepared[13] and reacted under similar conditions with **1** to give in 12% yield a mixture of the three disaccharides with (α1–6)- (**7**) (α1–4)- (**10**) and (α1–3)-linkage (**11**) in a ratio of 65:23:12. Their assignment was clearly achieved employing the peracetylated disaccharide derivatives **7Ac**, **10Ac**, and **11Ac** (Scheme 1).

Following these promising results the studies were extended to synthesize glycosylated sucrose (**14**). Indeed, by incubation of this acceptor with RafA and α-D-galactopyranosyl fluoride (**1**) the (α1–6)-galactosylated trisaccharide 17[14] resulted as the single regioisomer in 15.4% yield. With a slightly modified buffer system the corresponding reaction of **14** and the donor *p*-nitrophenyl α-D-galactopyranoside (**12**)[15] could be performed to give **17** in 17% yield. Finally, it was checked whether melibiose (**13**) could function as donor substrate, and indeed this was possible to give the trisaccharide **17** in 9% yield. Improved structural assignments again could be made by use of the undecaacetate **17Ac**. In case of the donor α-D-fucopyranosyl fluoride (**2**) a yield of only 2% of trisaccharide **18** resulted, and with the β-L-arabinopyranosyl fluoride (**3**) no transfer could be observed. Apparently, the required cooperative binding of substrates in the enzyme pockets is notably influenced by the donor structure.

Another interesting acceptor substrate was palatinitol [**15**, Glcp(α1–6)Glcol/Manol], which under the above conditions reacted with **1** catalyzed by RafA to give selectively the (α1–6)-linked trisaccharide **19** in 17% yield. By reaction with melibiose (**13**) again the same structure **19** resulted in 9% yield. Finally the enzymatic transfer was performed with isomaltulose (**16**) and fluoride **1** to give the (α1–6)-linked trisaccharide **20** in 12% yield. With melibiose (**13**) compound **20** was obtained in 8% yield. As before the peracetate **20Ac** could be nicely structurally assigned (Scheme 2).

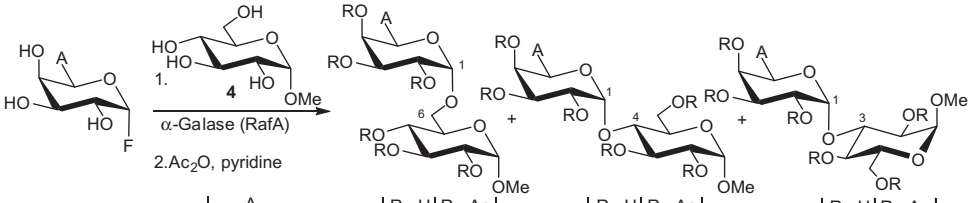
Except for the monosaccharide model the regioselectivity observed for RafA catalyzed reactions was remarkably high to give predominantly the (α1–6)-galactosylated products. This transfer was largely also possible with various donor substrates such as the glycopyranosyl fluorides, the *p*-nitrophenyl glycopyranosides and the disaccharide melibiose. Further, unusual compounds such as the D-fucopyranosyl and the L-arabinopyranosyl fluorides could be employed as donor substrates. Thus, RafA represents a synthetically interesting hydrolase for formation of various derivatives of interest in the context of novel food components.

2.3. Syntheses of 4-pyridinyl hexopyranosides

Even though *para*-nitrophenyl (*p*NP) groups show good leaving properties and are well suited for monitoring the hydrolytic reaction of glycosidases, their toxicity makes use critical for nutrition products. In search of alternative donor substrates excellent transfer properties as well as good water solubility are required. Again nitrophenyl glycopyranosides drop behind in this regard and following previous studies[16] pyridinyl glycosides became of interest. Thus, along classical glycosylation α-acetobromogalactose (**21**)[17] was glycosylated with silver 4-pyridoxide[18] in acetonitrile/dimethyl formamide 10:1 to give the β-D-galactopyranoside **24**, which after Zemplén deacetylation led to the desired donor substrate 4-pyridinyl β-D-galactopyranoside (**27**). In a corresponding approach α-acetobromofucose (**22**)[19] gave the glycoside **25** and after deacetylation the β-D-fucopyranoside donor **28**. To round up the series β-acetobromoarabinose (**23**)[20] led to glycoside **26**, and after transesterification according to Zemplén the structurally related donor substrate α-L-arabinopyranoside **29** was obtained (Scheme 3).

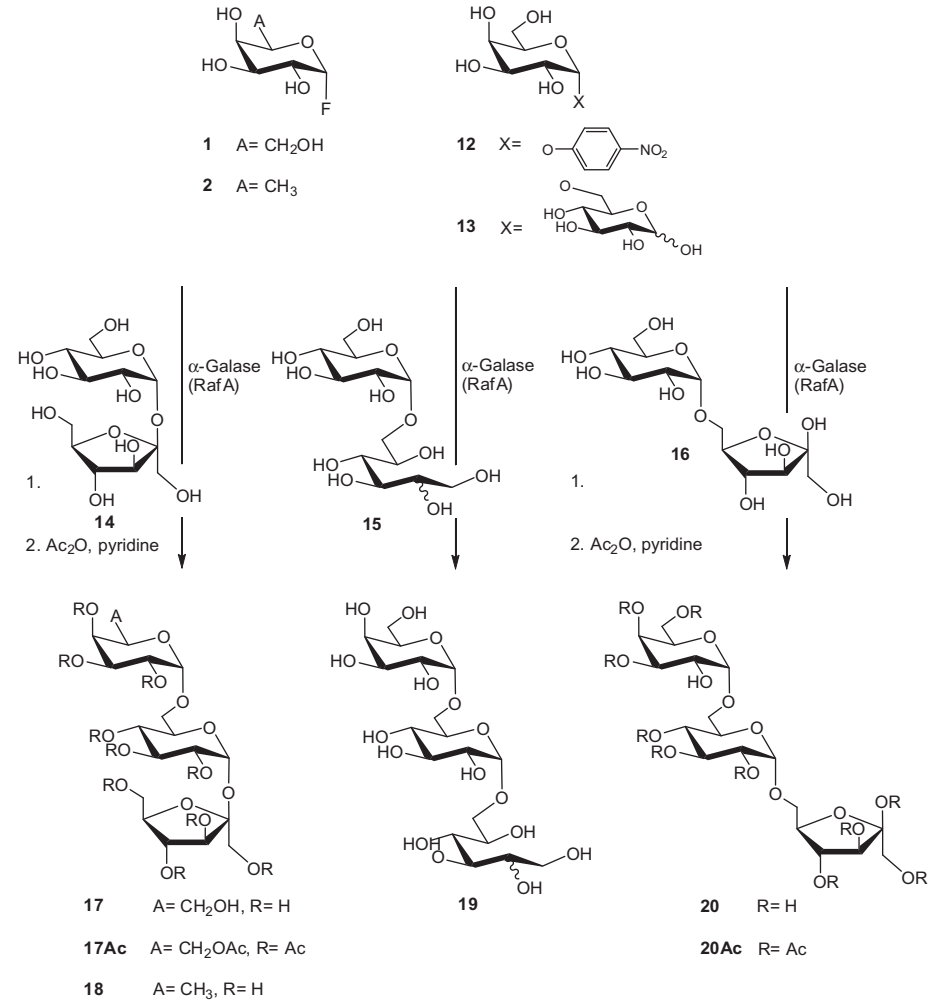
2.4. Glycosylations employing AgaB

It was of interest to study the thermophilic α-galactosidase AgaB for transfer reactions to mono and disaccharides at 65 °C. Thus, treatment of methyl α-D-glucopyranoside (**4**) with the α-D-galactopyranosyl fluoride (**1**) in phosphate buffer and the enzyme AgaB gave selectively the (α1–6)-linked derivative **5** (methyl α-melibioside) in 9% yield. Employing the donor substrate *p*-nitrophenyl α-D-galactopyranoside (**12**) enhanced the yield of **5** to 22%, and with melibiose (**13**) as donor the yield of **5** amounted to 15%. In attempts for fucosylation or arabinosylation with donors **2** and **3**, respectively, no transfer could be observed and apparently the enzyme exhibits enhanced donor specificity. With sucrose (**14**) as acceptor and **1** as donor compound **17** resulted in 3.4%, and with melibiose (**13**) the yield of trisaccharide **17** was 9%. Then palatinitol (**15**) was treated with **1** to give trisaccharide **19** in 6%, and with melibiose (**13**) in 12% yield. Finally, isomaltulose (**16**) was treated

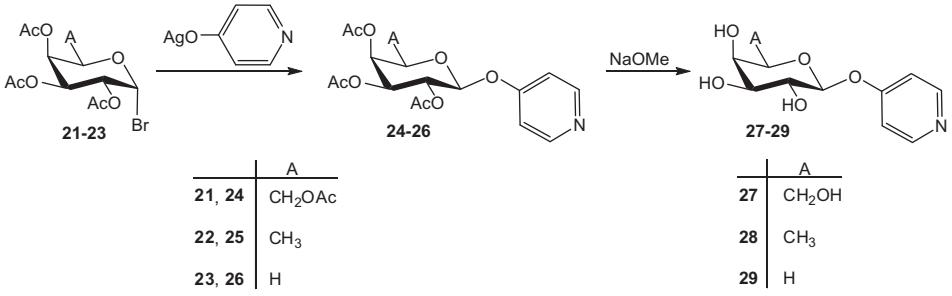


	A	R= H	R= Ac	R= H	R= Ac	R= H	R= Ac
1	CH_2OH	**5**	**5Ac**	**8**	**8Ac**	-	-
2	CH_3	**6**	**6Ac**	**9**	**9Ac**	-	-
3	H	**7**	**7Ac**	**10**	**10Ac**	**11**	**11Ac**

Scheme 1. Glycosylations of methyl α-D-glucopyranoside (**4**) employing α-galactosidase (RafA) [free compounds (R = H): **5**–**11**; peracetylated compounds (R = Ac): **5Ac**–**11Ac**].



Scheme 2. Glycosylations of sucrose (**14**), palatinitol (**15**), and isomaltulose (**16**) employing α-galactosidase (RafA).



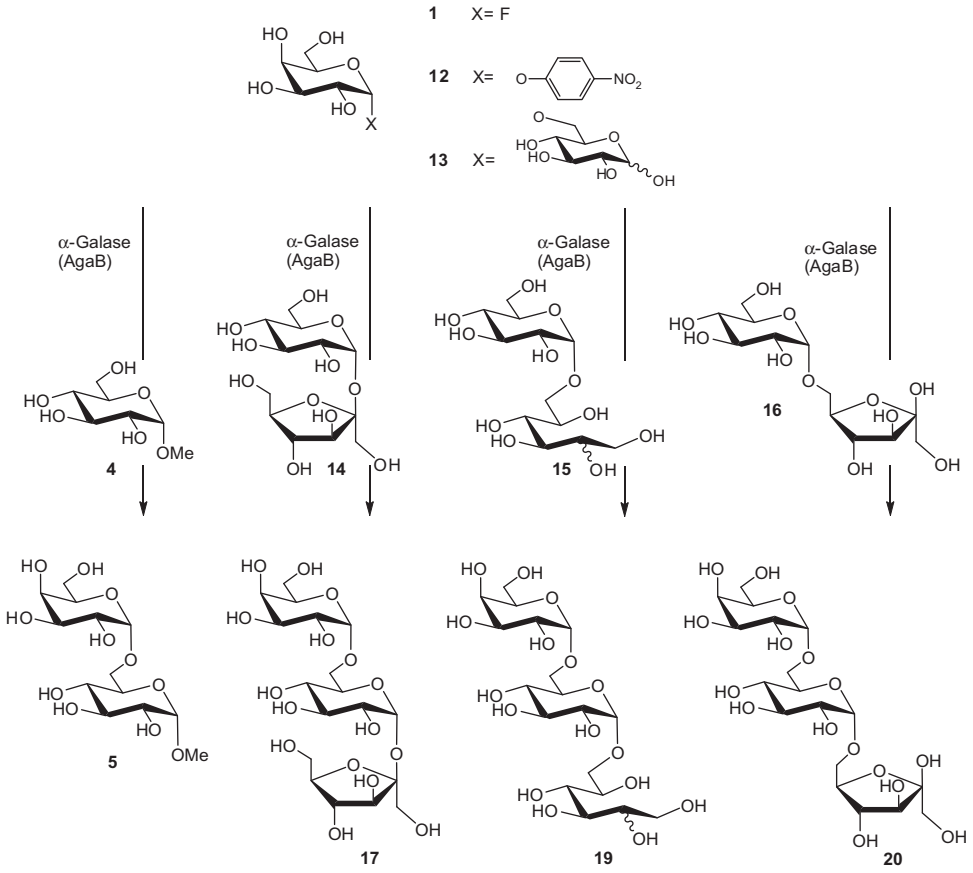
Scheme 3. Syntheses of 4-pyridinyl hexopyranosides.

with **1** to give the (α1–6)-galactosylated trisaccharide structure **20** in 7% and with melibiose (**13**) in 13% yield (Scheme 4).

These data show that the product spectrum of AgaB corresponds to that of RafA. Yields employing melibiose as donor substrate are higher in this application. Also, AgaB showed enhanced donor specificity, and is altogether more selective, thus less universally applicable.

2.5. Glycosylations employing BglT

Excellent transgalactosylations for formation of (β1–3)-linked oligosaccharides[21] can be achieved employing raw β-galactosidase from bovine testis.[22,23] However, isolation of the protein from life stock is laborious and the activity generally varies affected by various factors. Thus, the genetically modified β-galactosidase BglT from *Thermus thermophilus* TH 125, a thermophilic enzyme with a maximum efficiency at 65 °C, was of interest for synthetic applications.[6] This enzyme with a preference for establishing (β1–3)-linkages was to be tested regarding the stereo- and regiospecificity in transgalactosylations employing a number of novel as well as established donor and acceptor substrates. As novel donor substrates the three 4-pyridinyl hexopyranosides **27**–**29** were used and compared to the known *p*-nitrophenyl β-D-galactopyranoside (**30**)[24] as well as lactose (**31**).



Scheme 4. Glycosylations of methyl α-D-glucopyranoside (**4**), sucrose (**14**), palatinitol (**15**), and isomaltulose (**16**) employing α-galactosidase (AgaB).

In galactosylation of the model structure **4** the new donor **27** showed to be advantageous and led to the (β1–3)-disaccharide glycoside (isolactoside, **32**) in 27% yield. With the *p*-nitrophenyl donor **30** a yield of 13%, and with lactose as donor 23% yield was obtained. Structure assignments were facilitated after transfer to the heptaacetate **32Ac**.

Corresponding experiments were done in the case of sucrose (**14**) as acceptor structure. With the 4-pyridinyl β-D-galactopyranoside (**27**) the trisaccharide **33** resulted as the single stereo- and regioisomer in 31% yield, whereas lactose gave only 12% yield. Again the NMR assignment was favorably done for the peracetate **33Ac**.The enzyme BglT showed also to recognize the D-fucose donor **28**, which led to the modified trisaccharide **34** in 2.6% yield. On top of this the L-arabinose donor **29** could be used to give the corresponding trisaccharide product **35** in 3% yield.

As in the case of sucrose (**14**) palatinitol (**15**) could be galactosylated with the enzyme BglT employing the 4-pyridinyl derivative **27** and lactose (**31**) to yield the (β1–3)-galactosylated trisaccharide **36** in 11.2% and 8% yield, respectively. Finally, isomaltulose (**16**) was treated under similar conditions, which led to the terminally galactosylated trisaccharide **37** in 13% and 8%, respectively. As above, the structure assignment was performed on the peracetate **37Ac** (Scheme 5).

In all cases stereospecific glycosylations were observed, and the regiospecificity was (1–3) exclusively to the terminal glucopyranosyl unit. With these features the novel β-galactosidase BglT proved to be superior to the one isolated from bovine testis, which opens up synthetic pathways for a large number of different, terminally (β1–3)-galactosylated oligosaccharides.

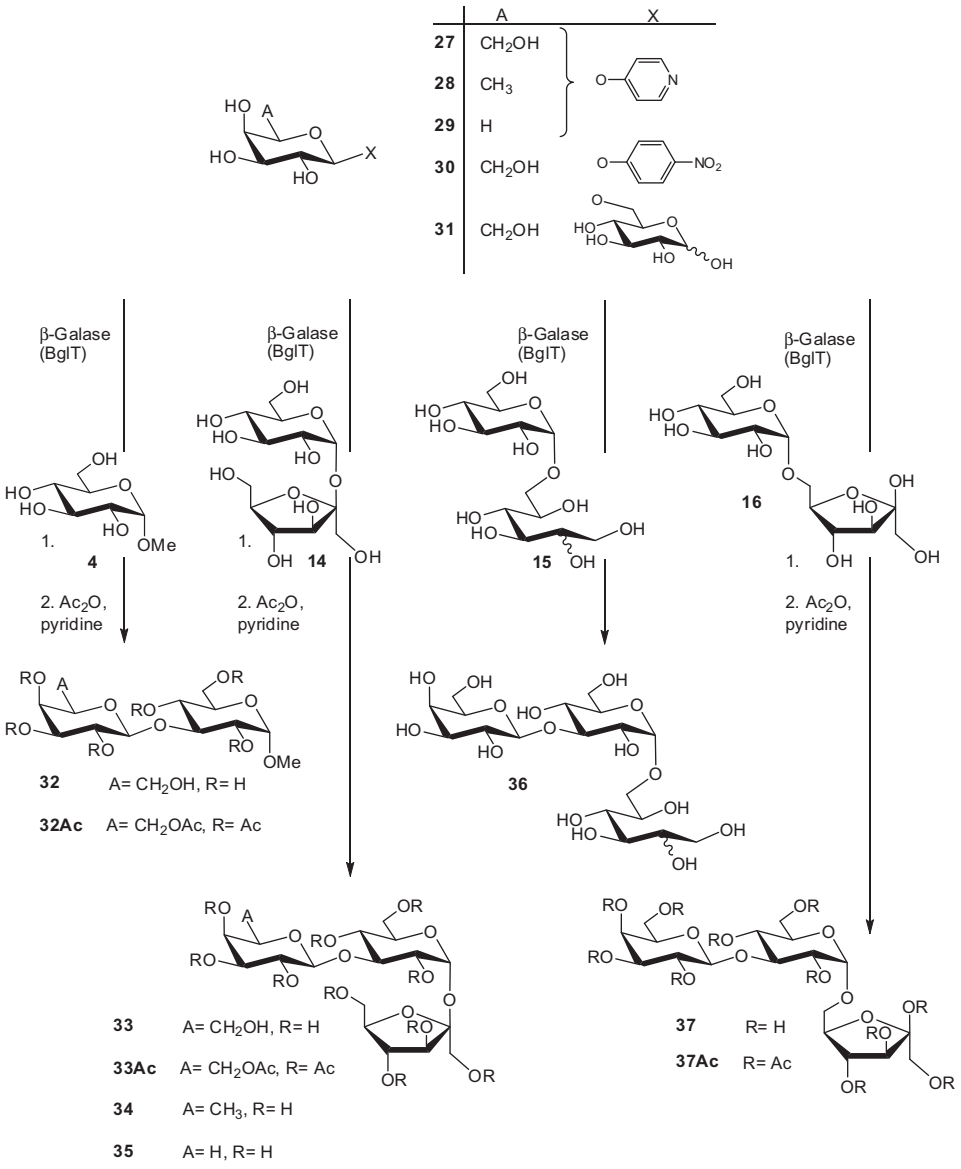
3. Conclusion

In this contribution recombinant α-(RafA and AgaB) and β-galactosidases (BglT) could be prepared in large amounts and employed in chemoenzymatic syntheses of glycosylated oligosaccharides, several of which are relevant in nutrition approaches. As donor substrates hexopyranosyl fluorides of D-galactose, D-fucose and L-arabinose, *p*NP α-D-galactopyranoside and melibiose proved to be efficient with acceptor structures methyl α-D-glucopyranoside, sucrose, palatinitol, and isomaltulose to give stereo- and regiospecifically the terminally glycosylated oligosaccharides. In addition to *p*NP β-D-galactopyranoside and lactose a number of novel 4-pyridinyl hexopyranosides turned out to be efficient donor substrates in transglycosylations of the mentioned acceptor substrates to give further terminally glycosylated oligosaccharides.

4. Experimental

4.1. General methods

Commercially available starting materials were used without further purification. Solvents were dried according to standard methods. TLC was performed on precoated aluminium plates (Silica Gel 60 F_{254}, Merck 5554), and charring was with 10% H_2SO_4 in ethanol for visualization. For column chromatography Silica Gel 60, 230–400 mesh, 40–63 μm (Merck) was used. ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker AMX-400 (400 MHz for ^{1}H, 100.6 MHz for ^{13}C) or on Bruker DRX-500 (500 MHz for ^{1}H, 125.8 MHz for ^{13}C) at 300 K. Chemical shifts



Scheme 5. Glycosylations of methyl α-D-glucopyranoside (**4**), sucrose (**14**), palatinitol (**15**), and isomaltulose (**16**) employing β-galactosidase (BglT).

were calibrated to solvent residual peaks. Signals were assigned by H,H-COSY, HSQC, and HMBC experiments. Optical rotations were measured using Perkin–Elmer polarimeter 341 (589 nm) at 20 °C. Melting points were measured on a Reichert heating microscope or on ST-apotec and are uncorrected. MALDI-TOF-MS was performed on a Bruker Biflex III with dihydroxybenzoic acid as matrix in positive reflector mode. HRFAB-MS was performed on a VG 70S mass spectrometer in positive ion mode with a xenon FAB-gun and *m*-nitrobenzyl alcohol as matrix at 5000 resolution.

4.2. General procedures

4.2.1. Transgalactosylation with α-galactosidase RafA (GP 1)

The donor component (1 equiv), the acceptor substrate (2 equiv), and α-galactosidase RafA (20 U/mmol acceptor) were dissolved in potassium phosphate buffer (100 μL, version A or version B) and incubated in a thermomixer for 24 h at 37 °C. For termination the temperature was enhanced to 95 °C for 10 min. Separation and purification of the products were done either on Biogel P2 with water as eluent or on Sephadex LH-20 with water/ethanol 1:5 as eluent. Version A for glycopyranosyl fluoride donors: 0.3 M KH_2PO_4/K_2HPO_4, pH 6.5. Version B for other donors: 0.1 M K_2HPO_4/K_2HPO_4, pH 6.5.

4.2.2. Transgalactosylation with α-galactosidase AgaB (GP 2)

The donor component (1 equiv), the acceptor substrate (2.5 equiv), and α-galactosidase AgaB (10 U/mmol acceptor) were dissolved in potassium phosphate buffer (100 μL, version A or version B) and incubated in a thermomixer for 24 h at 65 °C. For termination the temperature was enhanced to 95 °C for 15 min. Separation and purification of the products were done either on Biogel P2 with water as eluent or on Sephadex LH-20 with water/ethanol 1:5 as eluent. Version A for glycopyranosyl fluoride donors: 0.3 M KH_2PO_4/K_2HPO_4, pH 6.5. Version B for other donors: 0.1 M K_2HPO_4/K_2HPO_4, pH 6.5.

4.2.3. Transgalactosylation with β-galactosidase BglT (GP 3)

The donor component (1 equiv), the acceptor substrate (2 equiv), and β-galactosidase BglT (10 U/mmol acceptor) were dissolved in potassium phosphate buffer (0.1 M K_2HPO_4/K_2HPO_4, pH 6.5) and

incubated in a thermomixer for 24 h at 65 °C. For termination the temperature was enhanced to 95 °C for 15 min. Separation and purification of the products were done either on Biogel P2 with water as eluent or on Sephadex LH-20 with water/ethanol 1:5 as eluent.

4.3. Syntheses

4.3.1. Methyl α-D-galactopyranosyl-(1→6)-α-D-glucopyranoside (5) and methyl α-D-galactopyranosyl-(1→4)-α-D-glucopyranoside (8)

(a) Methyl α-D-glucopyranoside (**4**, 194 mg, 1.0 mmol) and α-D-galactopyranosyl fluoride (**1**,[8] 91 mg, 0.5 mmol) dissolved in potassium phosphate buffer (1 mL, 0.3 M, KH_2PO_4/K_2HPO_4, pH = 6.5) were incubated according to GP 1, version A. Workup and chromatography on Biogel P2 gave 33.8 mg (19%) of a colorless, amorphous solid. ^{1}H NMR showed the mixture to be **5**:**8** = 71: 19.

(b) Methyl α-D-glucopyranoside (**4**, 242 mg, 1.25 mmol) and α-D-galactopyranosyl fluoride (**1**, 91 mg, 0.5 mmol) dissolved in potassium phosphate buffer (1 mL, 0.3 M, KH_2PO_4/K_2HPO_4, pH = 6.5) were incubated according to GP 2, version A. Workup and chromatography on Biogel P2 gave 16.0 mg (9%) of a colorless, amorphous solid.

(c) Methyl α-D-glucopyranoside (**4**, 242 mg, 1.25 mmol) and *p*-nitrophenyl α-D-galactopyranoside (**12**,[15] 150 mg, 0.5 mmol) dissolved in potassium phosphate buffer (1 mL, 0.1 M, KH_2PO_4/K_2HPO_4, pH = 6.5) were incubated according to GP 2, version B. Workup and chromatography on Biogel P2 gave 32.9 mg (22%) of a colorless, amorphous solid.

(d) Methyl α-D-glucopyranoside (**4**, 242 mg, 1.25 mmol) and melibiose (**13**, 171 mg, 0.5 mmol) dissolved in potassium phosphate buffer (1 mL, 0.1 M, KH_2PO_4/K_2HPO_4, pH = 6.5) were incubated according to GP 2, version B. Workup and chromatography on Biogel P2 gave 26.7 mg (15%) of a colorless, amorphous solid.

$[\alpha]_D^{20}$ +106 (*c* 0.7, H_2O); selected signals of **5**: ^{1}H NMR (D_2O, 500 MHz): δ 4.93 (d, 1H, $J_{1',2'}$ = 3.8 Hz, H-1′), 4.76 (d, 1H, $J_{1',2'}$ = 3.8 Hz, H-1), 3.97–3.91 (m, 3H, H-4′, H-6a, H-6b), 3.82 (dd, 1H, $J_{2',3'}$ = 9.9, $J_{3',4'}$ = 3.5 Hz, H-3′), 3.77 (m_c, 1H, H-2′), 3.72–3.67 (m, 3H, H-5, H-6′a, H-6′b), 3.64–3.59 (m, 1H, H-3), 3.52 (dd, 1H, $J_{1,2}$ = 3.8, $J_{2,3}$ = 9.8 Hz, H-2), 3.46 (t, 1H, H-4, $J_{3,4}$ = $J_{4,5}$ = 9.8 Hz, H-4). ^{13}C NMR (D_2O, 100 MHz): δ 99.76 (C-1), 98.47 (C-1′), 73.68 (C-3), 71.56 (C-2), 71.35 (C-4), 68.83 (C-2′), 66.01 (C-6), 61.48 (C-6′), 55.57 (OCH_3). Selected signals of **6**: ^{1}H NMR (D_2O, 500 MHz): δ 5.10 (d, 1H, $J_{1',2'}$ = 3.8 Hz, H-1′), 4.92 (d, 1H, $J_{1,2}$ = 3.8 Hz, H-1), 4.18 (dd, 1H, $J_{1,2}$ = 3.8, $J_{2,3}$ = 10.2 Hz, H-2), 4.10 (t, 1H, $J_{2,3}$ = $J_{3,4}$ = 10.2, H-3). ^{13}C NMR (D_2O, 100 MHz): δ 96.96 (C-1), 96.53 (C-1′), 71.45 (C-3), 68.97 (C-2).

Peracetate **5Ac**: ^{1}H NMR (C_6D_6, 500 MHz): δ 5.50 (t, 1H, $J_{2,3}$ = $J_{3,4}$ = 9.8 Hz, H-3), 5.46 (s, 1H, H-4′, J < 1), 5.36 (dd, 1H, $J_{2',3'}$ = 11.0, $J_{3',4'}$ = 3.5 Hz, H-3′), 5.18 (d, 1H, $J_{1',2'}$ = 3.5 Hz, H-1′), 5.11 (dd, 1H, $J_{1',2'}$ = 3.5, $J_{2',3'}$ = 11.0 Hz, H-2′), 5.02 (dd, 1H, $J_{2,3}$ = $J_{3,4}$ = 9.8 Hz, H-4), 4.91 (d, 1H, $J_{1,2}$ = 3.5 Hz, H-1), 4.85 (dd, 1H, $J_{1,2}$ = 3.5, $J_{2,3}$ = 9.8 Hz, H-2), 4.28 (m, 1H, H-5′), 4.12 (dd, 1H, $J_{5',6'a}$ = 6.5, $J_{6'a,6'b}$ = 11.4 Hz, H-6′a), 4.06 (dd, 1H, $J_{5',6'b}$ = 6.5 Hz, $J_{6'a,6'b}$ = 11.4 Hz, H-6′b), 3.95 (ddd, 1H, $J_{4,5}$ = 2.2, $J_{5,6a}$ = 5.8, $J_{5,6b}$ = 11.1 Hz, H-5), 3.73 (dd, 1H, $J_{5,6a}$ = 5.8, $J_{6a,6b}$ = 11.1 Hz, H-6a), 3.53 (dd, 1H, $J_{5,6b}$ = 2.2, $J_{6a,6b}$ = 11.1 Hz, H6b), 3.35 (s, 3H, OCH_3), 2.14, 2.12, 2.08, 2.06, 2.04, 2.01, 1.99 (7s, each 3H, CO—CH_3). ^{13}C NMR (C_6D_6, 100 MHz): δ 170.80, 170.59, 170.45, 170.29, 169.93 (*C*=O), 96.90 (C-1′), 96.52 (C-1), 71.22 (C-3), 70.54 (C-2), 69.48 (C-5), 68.53 (C-4′), 68.47 (C-2′, C-4), 67.85 (C-5′), 66.82 (C-6), 66.50 (C-3′), 62.20 (C-6′), 55.75 (OCH_3), 20.41–19.95 (CO-CH_3).

Peracetate **8Ac**: ^{1}H NMR (C_6D_6, 500 MHz): δ 5.42 (t, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.8 Hz, H-4), 5.32 (d, 1H, $J_{1',2'}$ = 3.5 Hz, H-1′), 5.25 (dd, 1H, $J_{1',2'}$ = 3.5, $J_{2',3'}$ = 10.8 Hz, H-2′), 4.97 (m, 1H, H-3′), 4.82 (d, 1H, $J_{1,2}$ = 3.5 Hz, H-1), 4.45 (t, 1H, $J_{2,3}$ = $J_{3,4}$ = 11.2 Hz, H-3), 4.23–4.16 (m, 3H, H-4′, H-6a, H-6b), 4.09 (m_c, 1H, H-6′a), 4.04 (m_c, 1H, H-6′b), 4.01 (m, 1H, H-5′), 3.85 (ddd, 1H, $J_{4,5}$ = 2.5, $J_{5,6a}$ = 6.5, $J_{5,6b}$ = 12.1 Hz, H-5), 3.80 (dd, 1H, $J_{1,2}$ = 3.5, $J_{2,3}$ = 11.2 Hz, H-2), 3.42 (s, 3H, OCH_3), 2.14, 2.11, 2.09, 2.06, 2.03, 2.01, 1.99 (7s, each 3H, CO-CH_3). ^{13}C NMR (C_6D_6, 100 MHz): δ 96.87 (C-1), 96.48 (C-1′), 75.17 (C-2), 71.83 (C-4), 70.84 (C-3′), 69.84 (C-5), 69.53 (C-2′), 68.92 (C-3), 67.89 (C-5′), 66.54 (C-4′), 62.43 (C-6), 62.29 (C-6′), 54.98 (OCH_3), 20.41–19.95 CO-CH_3.

Calcd for **5Ac**/**8Ac**: $C_{27}H_{38}O_{18}$ (650.20). MALDI-TOF: *m*/*z* 673.05 $[M+Na]^+$, 689.19 $[M+K]^+$.

4.3.2. Methyl α-D-fucopyranosyl-(1→6)-α-D-glucopyranoside (6) and methyl α-D-fucopyranosyl-(1→4)-α-D-glucopyranoside (9)

Methyl α-D-glucopyranoside (**4**, 194 mg, 1 mmol) and α-D-fucopyranosyl fluoride (**2**,[9] 83 mg, 0.5 mmol) dissolved in potassium phosphate buffer (1 mL, 0.3 M, KH_2PO_4/K_2HPO_4, pH = 6.5) were incubated according to GP 1, version A. Workup and chromatography on Sephadex LH-20 gave 27.2 mg (16%) of a colorless, amorphous solid. The mixture of **6** and **9** was peracetylated with acetic anhydride/anhydrous pyridine at room temperature and usual workup to give **6Ac**:**9Ac** 69:31 according to ^{1}H NMR.

Peracetate **6Ac**: ^{1}H NMR (C_6D_6, 500 MHz): δ 5.91 (t, 1H, $J_{2,3}$ = $J_{3,4}$ = 9.8 Hz, H-3), 5.80 (dd, 1H,, $J_{2',3'}$ = 11.3, $J_{3',4'}$ = 3.2 Hz, H-3′), 5.60 (dd, 1H, $J_{1',2'}$ = 3.8, $J_{2',3'}$ = 11.3 Hz, H-2′), 5.55 (d, 1H, $J_{3',4'}$ = 3.2 Hz, H-4′), 5.40 (d, 1H, $J_{1',2'}$ = 3.8 Hz, H-1′), 5.33 (t, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.8 Hz, H-4), 5.09 (d, 1H, $J_{1,2}$ = 3.8, $J_{2,3}$ = 10.3 Hz, H-2), 4.93 (d, 1H, $J_{1,2}$ = 3.8 Hz, H-1), 4.11 (q, 1H, $J_{5',6'}$ = 5.8 Hz, H-5′), 3.96 (ddd, 1H, $J_{4,5}$ = 9.8, $J_{5,6a}$ = 5.4, $J_{5,6b}$ = 2.2 Hz, H-5), 3.70 (dd, 1H, $J_{5,6a}$ = 5.4, $J_{6a,6b}$ = 11.4 Hz, H-6a), 3.49 (dd, 1H, $J_{5,6b}$ = 2.2, $J_{6a,6b}$ = 11.4 Hz, H-6b), 1.05 (d, 3H, $J_{5',6'}$ = 5.8 Hz, 6′-CH_3), 2.14, 2.11, 2.09, 2.06, 2.03, 2.01, (6s, each 3H, CO-CH_3). ^{13}C NMR (C_6D_6, 100 MHz): δ 169.76, 169.62, 169.56, 169.49, (C=O), 96.58 (C-1), 96.29 (C-1′), 71.35 (C-4′), 70.90 (C-3), 70.40 (C-4), 69.30 (C-2′), 68.32 (C-5), 68.27 (C-5′), 68.01 (C-2′), 67.98 (C-3′), 65.74 (C-6), 55.32 (OCH_3), 15.47 (C-6′).

Peracetate **9Ac**: ^{1}H NMR (C_6D_6, 500 MHz): δ 5.76 (t, 1H, $J_{2,3}$ = $J_{3,4}$ = 9.2 Hz, H-3), 5.67 (dd, 1H, $J_{2',3'}$ = 10.7, $J_{3',4'}$ = 3.2 Hz, H-3′), 5.45 (s, 1H, H-4′), 5.42 (dd, 1H, $J_{1',2'}$ = 3.8, $J_{2',3'}$ = 10.7 Hz, H-2′), 5.30 (t, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.2 Hz, H-4), 5.27 (d, 1H, $J_{1',2'}$ = 3.8 Hz, H-1′), 4.69 (d, 1H, H-1, $J_{1,2}$ = 3.8 Hz, H-1), 4.38–4.33 (m, 2H, H-6a, H-6b), 4.14 (d, 1H, H-5′, $J_{5',6'}$ = 5.8 Hz, H-5′), 3.87 (ddd, 1H, $J_{4,5}$ = 2.2, $J_{5,6a}$ = 5.8, $J_{5,6b}$ = 11.1 Hz, H-5), 3.63 (dd, 1H, $J_{1,2}$ = 3.8, $J_{2,3}$ = 9.2 Hz, H-2), 3.07 (s, 3H, OCH_3), 1.00 (d, 3H, $J_{5',6'}$ = 5.8 Hz, 6′-CH_3), 2.13, 2.10, 2.09, 2.05, 2.03, 2.01, (6s, each 3H, CO-CH_3). ^{13}C NMR (C_6D_6, 100 MHz): δ 169.29, 169.19, 169.12, 169.01, 168.77 (C=O), 96.58 (C-1′), 95.87 (C-1), 75.99 (C-4), 73.56 (C-2), 72.35 (C-2′), 71.35 (C-4′), 70.97 (C-3), 68.54 (C-3′), 67.98 (C-5′), 67.74 (C-5), 61.61 (C-6), 55.34 (OCH_3), 15.27 (C-6′). Calcd for **6Ac**/**9Ac**: $C_{25}H_{36}O_{16}$ (592.20) MALDI-TOF *m*/*z* 615.07 $[M+Na]^+$, 631.25 $[M+K]^+$.

4.3.3. Methyl β-L-arabinopyranosyl-(1→6)-α-D-glucopyranoside (7), methyl β-L-arabinopyranosyl-(1→4)-α-D-glucopyranoside (10) and methyl β-L-arabinopyranosyl-(1→3)-α-D-glucopyranoside (11)

Methyl α-D-glucopyranoside (**4**, 194 mg, 1 mmol) and β-L-arabinopyranosyl fluoride (**3**,[13] 76 mg, 0.5 mmol) dissolved in potassium phosphate buffer (1 mL, 0.3 M, KH_2PO_4/K_2HPO_4, pH = 6.5) were incubated according to GP 1, version A. Workup and chromatography on Sephadex LH-20 gave 19.5 mg (12%) of a colorless, amorphous solid. The mixture of **7**, **10,** and **11** was peracetylated with acetic anhydride/anhydrous pyridine at room temperature and usual workup to give **7Ac**:**10Ac**:**11Ac** 65:23:12 according to ^{1}H NMR.

Peracetate **7Ac**: ^{1}H NMR (C_6D_6, 500 MHz): δ 5.90 (t, 1H, $J_{2,3} = J_{3,4} = 10.1$ Hz, H-3), 5.74 (dd, 1H,, $J_{2',3'} = 11.0$, $J_{3',4'} = 1.6$ Hz, H-3′), 5.63 (dd, 1H, $J_{1',2'} = 3.8$, $J_{2',3'} = 11.0$ Hz, H-2′), 5.55 (dd, 1H, $J_{3',4'} = J_{4',5'a} = 1.9$ Hz, H-4′), 5.40 (d, 1H, $J_{1',2'} = 3.8$ Hz, H-1′), 5.29 (t, 1H, $J_{3,4} = J_{4,5} = 10.1$ Hz, H-4), 5.08 (d, 1H, $J_{1,2} = 3.8$, $J_{2,3} = 10.1$ Hz, H-2), 4.90 (d, 1H, $J_{1,2} = 3.8$ Hz, H-1), 3.95 (m, 1H, H-5), 3.89 (d, 1H, $J_{5'a,5'b} = 12.9$ Hz, H-5′a), 3.67 (dd, 1H, $J_{5,6a} = 5.7$, $J_{6a,6b} = 11.8$ Hz, H-6a), 3.54 (dd, 1H, $J_{4',5'b} = 1.9$, $J_{5'a,5'b} = 12.9$ Hz, H-5′b), 3.45 (dd, 1H, $J_{5,6b} = 3.1$, $J_{6a,6b} = 11.8$ Hz, H-6b), 3.11 (s, 3H, OC*H*$_3$), 2.14, 2.11, 2.09, 2.06, 2.03, 2.01, (6s, each 3H, CO-C*H*$_3$). ^{13}C NMR (C_6D_6, 100 MHz): δ 170.51, 170.30, 170.15, 170.10, 170.00, 169.91 (*C*=O), 97.70 (C-1), 97.41 (C-1′), 71.71 (C-2′), 71.19 (C-2), 70.07 (C-3), 69.68 (C-4′), 69.46 (C-4), 69.32 (C-3′), 68.05 (C-5), 66.54 (C-6), 61.13 (C-5′), 55.40 (OCH$_3$).

Peracetate **10Ac**: ^{1}H NMR (C_6D_6, 500 MHz): δ 5.78 (t, 1H, $J_{2,3} = J_{3,4} = 10.1$ Hz, H-3), 5.74 (dd, 1H, $J_{2',3'} = 10.9$, $J_{3',4'} = 1.6$ Hz, H-3′), 5.54 (d, 1H, $J_{3',4'} = 1.6$ Hz, H-4′), 5.42 (m, 1H, H-2′), 5.45 (d, 1H, $J_{1,2} = 3.8$, $J_{2,3} = 10.1$ Hz, H-2), 5.25 (d, 1H, $J_{1',2'} = 3.8$ Hz, H-1′), 4.64 (d, 1H, H-1, $J_{1,2} = 3.8$ Hz, H-1), 4.34 (dd, 1H, $J_{4',5'a} = 4.4$, $J_{5'a,5'b} = 12.3$ Hz, H-5′a), 4.22 (m, 1H, H-4), 4.10 (dd, 1H, $J_{4',5'b} = 2.2$, $J_{5'a,5'b} = 12.3$ Hz, H-5′b), 3.93 (m, 1H, H-5), 3.60 (dd, 1H, $J_{5,6a} = 4.7$, $J_{6a,6b} = 11.3$ Hz, H-6a), 3.48 (dd, 1H, $J_{5,6b} = 2.8$, $J_{6a,6b} = 11.3$ Hz, H-6b), 3.06 (s, 3H, OC*H*$_3$), 2.12, 2.10, 2.07, 2.05, 2.02, 2.01, (6s, each 3H, CO-C*H*$_3$). ^{13}C NMR (C_6D_6, 100 MHz): δ 170.55, 170.35, 170.05, 170.00, 169.72, 169.53 (*C*=O), 97.70 (C-1), 96.42 (C-1′), 73.87 (C-4), 73.03 (C-3′), 71.88 (C-3), 70.92 (C-2), 69.98 (C-4′), 69.92 (C-2′), 67.72 (C-5), 62.46 (C-5′), 61.65 (C-6), 55.51 (OCH$_3$).

Peracetate **11Ac**: ^{1}H NMR (C_6D_6, 500 MHz): δ 5.58 (d, 1H, $J_{1',2'} = 3.8$ Hz, H-1′), 5.49 (m, 1H, H-2′), 5.32 (d, 1H, $J_{3',4'} = 1.8$ Hz, H-4′), 5.01 (d, 1H, H-1, $J_{1,2} = 3.5$ Hz, H-1), 4.85 (d, 1H, $J_{1,2} = 3.5$, $J_{2,3} = 9.9$ Hz, H-2), 4.46 (t, 1H, $J_{2,3} = J_{3,4} = 9.9$ Hz, H-3), 3.84 (ddd, 1H, $J_{4,5} = 2.2$, $J_{5,6a} = 4.7$, $J_{5,6b} = 12.3$ Hz, H-5), 2.97 (s, 3H, OC*H*$_3$), 2.10, 2.08, 2.05, 2.02, 1.99, 1.97, (6s, each 3H, CO-C*H*$_3$). ^{13}C NMR (C_6D_6, 100 MHz): δ 170.50, 170.32, 170.03, 169.80, 169.72, 169.43 (*C*=O), 99.51 (C-1), 97.91 (C-1′), 76.26 (C-3), 71.45 (C-4′), 71.02 (C-2), 69.91 (C-2′), 62.53 (C-5′), 55.19 (OCH$_3$). Calcd for **7Ac**/**10Ac**/**11Ac** $C_{24}H_{34}O_{18}$ (578.13) MALDI-TOF *m*/*z* 600.71 [M+Na]$^+$, 618.65 [M+K]$^+$.

4.3.4. α-D-Galactopyranosyl-(1→6)-α-D-glucopyranosyl-(1↔2)-β-D-fructofuranoside (17)

(a) Sucrose (**14**, 342 mg, 1 mmol) and α-D-galactopyranosyl fluoride (**1**, 91 mg, 0.5 mmol) dissolved in potassium phosphate buffer (1 mL, 0.3 M, KH_2PO_4/K_2HPO_4, pH = 6.5) were incubated according to GP 1, version A. Workup and chromatography on Biogel P2 gave 38.8 mg (15.4%) of **17** as a colorless, amorphous solid.

(b) Sucrose (**14**, 342 mg, 1 mmol) and *p*-nitrophenyl α-D-galactopyranoside (**12**,[15] 150 mg, 0.5 mmol) dissolved in potassium phosphate buffer (1 mL, 0.1 M, KH_2PO_4/K_2HPO_4, pH = 6.5) were incubated according to GP 1, version B. The formed *p*-nitro-phenol was extracted with ethyl acetate (5 mL). Further workup and chromatography on Biogel P2 gave 42.9 mg (17%) of **17** as a colorless, amorphous solid.

(c) Sucrose (**14**, 342 mg, 1 mmol) and melibiose (**13**, 171 mg, 0.5 mmol) dissolved in potassium phosphate buffer (1 mL, 0.1 M, KH_2PO_4/K_2HPO_4, pH = 6.5) were incubated according to GP 1, version B. Workup and chromatography on Biogel P2 gave 17.6 mg (7%) of **17** as a colorless, amorphous solid.

(d) Sucrose (**14**, 427 mg, 1.25 mmol) and α-D-galactopyranosyl fluoride (**1**, 91 mg, 0.5 mmol) dissolved in potassium phosphate buffer (1 mL, 0.3 M, KH_2PO_4/K_2HPO_4, pH = 6.5) were incubated according to GP 2, version A. Workup and chromatography on Biogel P2 gave 8.6 mg (3.4%) of **17** as a colorless, amorphous solid.

(e) Sucrose (**14**, 427 mg, 1.25 mmol) and melibiose (**13**, 171 mg, 0.5 mmol) dissolved in potassium phosphate buffer (1 mL, 0.1 M, KH_2PO_4/K_2HPO_4, pH = 6.5) were incubated according to GP 2, version B. Workup and chromatography on Biogel P2 gave 22.7 mg (9%) of **17** as a colorless, amorphous solid.

$[\alpha]_D^{20}$ +73 (*c* 1.0, H_2O), lit.[14] +122.9 (*c* 2.0, H_2O); selected signals of **17**: ^{1}H NMR (D_2O, 500 MHz): δ 5.27 (d, 1H, $J_{1'',2''} = 3.8$ Hz, H-1″), 4.84 (d, 1H, $J_{1,2} = 3.5$ Hz, H-1), 3.41 (dd, 1H, $J_{1,2} = 3.8$, $J_{2,3} = 9.8$ Hz H-2), 3.26 (t, 1H, H-3 $J_{2,3} = J_{3,4} = 9.8$ Hz). ^{13}C NMR (D_2O, 100 MHz): δ 104.20 (C-2′), 98.87 (C-1″), 92.49 (C-1), 72.75 (C-2), 71.79 (C-3). Calcd for $C_{18}H_{32}O_{16}$ (504.44) MALDI-TOF *m*/*z* 527.51 [M+Na]$^+$, 543.45 [M+K]$^+$.

Peracetate **17Ac**: ^{1}H NMR ($CDCl_3$, 500 MHz): δ 6.08 (d, 1H, $J_{1'',2''} = 3.5$ Hz, H-1″), 5.97 (d, 1H, $J_{3'',4''} = 1.3$ Hz, H-4″), 5.95–5.92 (m, 2H, H-3″, H-6a), 5.87 (dd, 1H, $J_{5,6b} = 2.5$, $J_{6a,6b} = 11.9$ Hz, H-6b), 5.72 (d, 1H, $J_{3',4'} = 7.9$ Hz, H-3′), 5.68 (dd, 1H, $J_{1,2} = 3.8$, $J_{2,3} = 10.9$ Hz, H-2), 5.62–5.58 (m, 1H, H-5″), 5.43 (d, 1H, $J_{1,2} = 3.8$ Hz, H-1), 5.30 (t, 1H, $J_{2,3} = J_{3,4} = 10.9$ Hz, H-3), 5.10 (dd, 1H, $J_{1'',2''} = 3.8$, $J_{2'',3''} = 10.4$ Hz, H-2″), 4.82 (d, 1H, $J_{3',4'} = 7.9$ Hz, H-4′), 4.72–4.64 (m, 5H, H-1′a, H-1′b, H-6′a, H-6′b, H-5), 4.52–4.47 (m, 2H, H-6a, H-6b), 4.35 (dd, 1H, $J_{5',6'a} = 6.9$, $J_{5'6'b} = 11.1$ Hz, H-5′), 3.93 (dd, 1H, $J_{5'',6''a} = 6.9$, $J_{6''a,6''b} = 10.8$ Hz, H-6″a), 3.70 (dd, 1H, $J_{5',6''b} = 1.9$, $J_{6''a,6''b} = 10.8$ Hz, H-6″b), 2.07–1.96 (several s, 33H, CO-C*H*$_3$). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 171.06, 170.73, 170.61, 170.47, 170.16, 170.09, 169.81, 169.53 (*C*=O), 104.14 (C-2′), 96.55 (C-1″), 91.03 (C-1), 81.17 (C-5), 77.37 (C-3), 77.11 (C-3′), 76.15 (C-3″), 71.38 (C-2″), 70.40 (C-4′), 70.15 (C-5″), 69.83 (C-4″), 69.05 (C-4′), 68.55 (C-2), 67.23 (C-5′), 66.94 (C-6), 64.19 (C-1′), 62.27 (C-6′), 62.14 (C-6″), 20.77–20.31 (*C*H$_3$).

4.3.5. α-D-Fucopyranosyl-(1→6)-α-D-glucopyranosyl-(1↔2)-β-D-fructofuranoside (18)

Sucrose (**14**, 342 mg, 1 mmol) and α-D-fucopyranosyl fluoride (**2**, 83 mg, 0.5 mmol) dissolved in potassium phosphate buffer (1 mL, 0.3 M, KH_2PO_4/K_2HPO_4, pH = 6.5) were incubated according to GP 1, version A. Workup and chromatography on Biogel P2 gave 5.1 mg (2%) of **18** as a colorless, amorphous solid. $[\alpha]_D^{20}$ +41 (*c* 0.2, MeOH); ^{1}H NMR (D_2O, 500 MHz): δ 5.38 (d, 1H, $J_{1'',2''} = 3.5$ Hz, H-1″), 4.82 (d, 1H, $J_{1,2} = 3.8$ Hz, H-1), 4.22 (m, 1H, H-3″), 4.16 (d, 1H, $J_{3',4'} = 8.4$ Hz, H-3′), 4.05–3.98 (m, 3H, H-4″, H-3, H-4), 3.93 (dd, 1H, $J_{1,2} = 3.5$, $J_{2,3} = 9.1$ Hz, H-2), 3.86–3.83 (m, 2H, H-2″, H-5″), 3.80 (m, 1H, H-5), 3.77–3.73 (m, 4H, H-1′a, H-1′b, H-6′a, H-6′b), 3.66–3.63 (m, 2H, H-6a, H-6b), 3.42 (t, 1H, $J_{3',4'} = 8.4$ Hz, H-4′), 1.16 (d, 3H, $J_{5'',6''} = 6.3$ Hz, 6″-CH$_3$). ^{13}C NMR (D_2O, 100 MHz): δ 104.53 (C-2′), 98.65 (C-1″), 96.83 (C-1), 81.71 (C-5′), 77.49 (C-5), 73.53 (C-3″), 72.37 (C-4′), 72.32 (C-3), 72.24 (C-3′), 72.15 (C-5″), 72.00 (C-2), 71.19 (C-2″), 69.84 (C-4″), 68.54 (C-6), 67.39 (C-4), 62.69 (C-6′), 61.76 (C-1′), 15.65 (C-6″). Calcd for $C_{18}H_{32}O_{15}$ (488.44) MALDI-TOF *m*/*z* 511.45 [M+Na]$^+$, 527.47 [M+K]$^+$.

4.3.6. α-D-Galactopyranosyl-(1→6)-α-D-glucopyranosyl-(1→6)-D-glucitol/mannitol (19)

(a) Palatinitol (**15**, 344 mg, 1 mmol) and α-D-galactopyranosyl fluoride (**1**, 91 mg, 0.5 mmol) dissolved in potassium phosphate buffer (1 mL, 0.3 M, KH_2PO_4/K_2HPO_4, pH = 6.5) were incubated according to GP 1, version A. Workup and chromatography on Biogel P2 gave 43.0 mg (17%) of **19** as a colorless, amorphous solid.

(b) Palatinitol (**15**, 344 mg, 1 mmol) and melibiose (**13**, 171 mg, 0.5 mmol) dissolved in potassium phosphate buffer (1 mL, 0.1 M, KH_2PO_4/K_2HPO_4, pH = 6.5) were incubated according to GP 1, version B. Workup and chromatography on Biogel P2 gave 22.8 mg (9%) of **19** as a colorless, amorphous solid.

(c) Palatinitol (**15**, 344 mg, 1 mmol) and α-D-galactopyranosyl fluoride (**1**, 91 mg, 0.5 mmol) dissolved in potassium phosphate buffer (1 mL, 0.3 M, KH_2PO_4/K_2HPO_4, pH = 6.5) were incubated according to GP 2, version A. Workup and chromatography on Biogel P2 gave 15.3 mg (6%) of **19** as a colorless, amorphous solid.

(d) Palatinitol (**15**, 344 mg, 1 mmol) and melibiose (**13**, 171 mg, 0.5 mmol) dissolved in potassium phosphate buffer (1 mL, 0.1 M, KH_2PO_4/K_2HPO_4, pH = 6.5) were incubated according to GP 1, version B. Workup and chromatography on Biogel P2 gave 30.5 mg (12%) of **19** as a colorless, amorphous solid.

$[\alpha]_D^{20}$ + 141 (*c* 1.0, H_2O); ^1H NMR (D_2O, 500 MHz): δ 4.94 (d, 1H, $J_{1'',2''}$ = 3.8 Hz, H-1″), 4.88 (m, 2H, C*H*$_2$-1). ^{13}C NMR (D_2O, 100 MHz): δ 98.70, 98.57 (C-1″), 98.53 (C-1), 73.68 (C-5″), 73.49 (C-3), 73.27 (C-3″), 72.15 (C-5), 71.97 (C-2), 71.88 (C-2″), 69.84 (C-4), 68.80 (C-6′), 69.58 (C-6), 68.89 (C-4″), 63.64 (C-1′, Man-ol), 62.79 (C-1′, Glc-ol), 61.61 (C-6″). Calcd for $C_{18}H_{34}O_{16}$ (506.18): C, 42.69; H, 6.77; found: C, 37.98; H, 6.66. MALDI-TOF *m*/*z* 529.35 $[M+Na]^+$, 545.53 $[M+K]^+$.

4.3.7. α-D-Galactopyranosyl-(1→6)-α-D-glucopyranosyl-(1→6)-β-D-fructofuranose (20)

(a) Isomaltulose (**16**, 342 mg, 1 mmol) and α-D-galactopyranosyl fluoride (**1**, 91 mg, 0.5 mmol) dissolved in potassium phosphate buffer (1 mL, 0.3 M, KH_2PO_4/K_2HPO_4, pH = 6.5) were incubated according to GP 1, version A. Workup and chromatography on Biogel P2 gave 30.3 mg (12%) of **20** as a colorless, amorphous solid.

(b) Isomaltulose (**16**, 342 mg, 1 mmol) and melibiose (**13**, 171 mg, 0.5 mmol) dissolved in potassium phosphate buffer (1 mL, 0.1 M, KH_2PO_4/K_2HPO_4, pH = 6.5) were incubated according to GP 1, version B. Workup and chromatography on Biogel P2 gave 20.2 mg (8%) of **20** as a colorless, amorphous solid.

(c) Isomaltulose (**16**, 427 mg, 1.25 mmol) and α-D-galactopyranosyl fluoride (**1**, 91 mg, 0.5 mmol) dissolved in potassium phosphate buffer (1 mL, 0.3 M, KH_2PO_4/K_2HPO_4, pH = 6.5) were incubated according to GP 2, version A. Workup and chromatography on Biogel P2 gave 17.7 mg (7%) of **20** as a colorless, amorphous solid.

(d) Isomaltulose (**16**, 427 mg, 1.25 mmol) and melibiose (**13**, 171 mg, 0.5 mmol) dissolved in potassium phosphate buffer (1 mL, 0.1 M, KH_2PO_4/K_2HPO_4, pH = 6.5) were incubated according to GP 2, version B. Workup and chromatography on Biogel P2 gave 32.8 mg (13%) of **20** as a colorless, amorphous solid.

$[\alpha]_D^{20}$ +145 (*c* 1.0, H_2O); ^1H NMR (D_2O, 500 MHz): δ 4.80 (d, 1H, $J_{1,2}$ = 3.5 Hz, H-1), 4.75 (d, 1H, $J_{1'',2''}$ = 3.1 Hz, H-1″), 3.98 (m, 1H, H-6a), 3.90 (m_c, 1H, H-6b). ^{13}C NMR (D_2O, 100 MHz): δ 102.11 (C-2′), 98.73 (C-1″), 98.42 (C-1), 69.88 (C-6).

Calcd for $C_{18}H_{36}O_{16}$ (504.44): C, 42.86; H, 6.39; found: C, 38.60; H, 6.53. MALDI-TOF *m*/*z* 527.45 $[M+Na]^+$, 543.35 $[M+K]^+$.

Peracetate **20Ac**: ^1H NMR (C_6D_6, 500 MHz): δ 5.85–5.76 (m, 2H, H-3″, H-3), 5.73 (d, 1H, $J_{3'',4''}$ = 2.2 Hz, H-4″), 5.58 (dd, 1H, $J_{1,2}$ = 3.5, $J_{2,3}$ = 9.6 Hz, H-2), 5.51 (m, 1H, H-5″), 5.20 (d, 1H, $J_{1,2}$ = 3.5 Hz, H-1), 5.13 (d, 1H, $J_{1'',2''}$ = 3.8 Hz, H-1″), 5.05 (dd, 1H, $J_{1'',2''}$ = 3.8, $J_{2'',3''}$ = 8.9 Hz, H-2′), 5.00 (d, 1H, $J_{3',4'}$ = 8.9 Hz, H-3′), 4.67 (t, 1H, $J_{3',4'} = J_{4',5'}$ = 8.9 Hz, H-4′), 4.58 (m, 1H, H-5′), 4.53 (m, 1H, H-6a), 4.45 (m, 1H, H-6b), 4.32–4.22 (m, 4H, H-1′a, H-1′b, H-6′a, H-6′b), 3.70 (m, 1H, H-5), 3.64–3.59 (m, H-6″a, H-6″b), 1.91–1.78, (several s, 33H, CO-C*H*$_3$). ^{13}C NMR (C_6D_6, 125 MHz): δ 170.95–169.55 (*C*=O), 103.56 (C-2′), 96.79 (C-1), 95. 97 (C-1″), 82.26 (C-5′), 79.87 (C-4′), 77.60 (C-5″), 74.84 (C-3′), 71.60 (C-5), 71.10 (C-4), 69.88 (C-3″), 69.20 (C-3), 69.07 (C-4″), 68.95 (C-2), 68.38 (C-2″), 67.39 (C-6), 62.38 (C-6′), 62.27 (C-1′), 61.96 (C-6″), 21.19–20.74 (*C*H$_3$).

4.3.8. (4-Pyridinyl) 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside (24)

2,3,4,6-Tetra-*O*-acetyl-α-D-galactopyranosyl bromide (**21**,[17] 2.88 g, 7.0 mmol) and silver 4-pyridoxide[18] (1.63 g, 8.07 mmol) were refluxed in toluene (30 mL) for 1 h. After cooling the mixture was filtered through Celite, washed with saturated aqueous sodium hydrogen carbonate and water, dried over Mg_2SO_4, and purified by column chromatography with petroleum ether (50–70)/ethyl acetate 1:1 to give 1.96 g (66%) of **24** as amorphous solid.

$[\alpha]_D^{20}$ +54 (*c* 0.5, $CHCl_3$); ^1H NMR ($CHCl_3$, 500 MHz): δ 8.51 (m, 2H, Ar-2, Ar-6), 6. 92 (m, 2H, Ar-3, Ar-5), 5.49 (dd,1H, $J_{1,2}$ = 7.6, $J_{2,3}$ = 10.7 Hz, H-2), 5.45 (m, 1H, H-4), 5.18 (d, 1H, $J_{1,2}$ = 7.6 Hz, H-1), 5.11 (dd, 1H, $J_{2,3}$ = 10.7, $J_{3,4}$ = 3.6 Hz, H-3), 4.20–4.09 (m, 3H, H-5, H-6a, H-6b), 2.16, 2.05, 2.04, 2.00 (4s, 12H, $COCH_3$). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 170.71, 170.52, 170.45, 169.66 (*C*=O), 163.54 (Ar-4), 151.05 (2 C, Ar-2, Ar-6), 112.27 (2C, Ar-3, Ar-5), 98.29 (C-1), 71.89 (C-5), 71.02 (C-3), 68.61 (C-2), 67.08 (C-4), 61.74 (C-6), 21.09, 21.05, 21.03, 20.96 ($COCH_3$).

4.3.9. (4-Pyridinyl) 2,3,4-tri-*O*-acetyl-β-D-fucopyranoside (25)

2,3,4-Tri-*O*-acetyl-α-D-fucopyranosyl bromide (**22**, 19 430 mg, 1.22 mmol) and silver 4-pyridoxide[18] (246 mg, 1.22 mmol) dissolved in acetonitrile (50 mL) and dimethyl formamide (5 mL) were heated at 80 °C under stirring for 5 h. After cooling the mixture was filtered through Celite, washed with saturated aqueous sodium hydrogen carbonate and water, dried over Mg_2SO_4, and purified by column chromatography with toluene/acetone 10:1 to give 183 mg (41%) of **25** as slightly yellow syrup. $[\alpha]_D^{20}$ +35.7 (*c* 0.3, $CHCl_3$); ^1H NMR (C_6D_6, 500 MHz): δ 8.48 (m, 2H, Ar-2, Ar-6), 6.92 (m, 2H, Ar-3, Ar-5), 5.49 (dd, 1H, $J_{1,2}$ = 7.8, $J_{2,3}$ = 10.4 Hz, H-2), 5.33 (d, 1H, $J_{3,4}$ = 3.4 Hz, H-4), 5.19 (d, 1H, $J_{1,2}$ = 7.8 Hz, H-1), 5.14 (dd, 1H, $J_{2,3}$ = 10.4, $J_{3,4}$ = 3.4 Hz, H-3), 4.05 (d, 1H, $J_{5,6}$ = 6.3 Hz, H-5), 2.20, 2.05, 2.02 (3s, 9H, $COCH_3$), 1.28 (d, 3H, $J_{5,6}$ = 6.3 Hz, 6-CH_3). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 170.53, 170.10, 169.34 (*C*=O), 151.14 (Ar), 111.62 (Ar), 97.78 (C-1), 71.05 (C-3), 69.91 (C-5), 69.82 (C-4), 68.37 (C-2), 20.69, 20.63, 20.59 ($COCH_3$), 16.08 (C-6).

4.3.10. (4-Pyridinyl) 2,3,4-tri-*O*-acetyl-α-L-arabinopyranoside (26)

2,3,4-Tri-*O*-acetyl-β-L-arabinopyranosyl bromide (**23**,[20] 4.41 g, 13.2 mmol) and silver 4-pyridoxide[18] (2.6 g, 13.2 mmol) dissolved in acetonitrile (200 mL) and dimethyl formamide (60 mL) were heated at 80 °C under stirring for 5 h. After cooling the mixture was filtered through Celite, washed with saturated aqueous sodium hydrogen carbonate and water, dried over Mg_2SO_4, and purified by column chromatography with toluene/acetone 10:1 to give 1.76 g (38%) of **26** as slightly yellow syrup. $[\alpha]_D^{20}$ +115.0 (*c* 1.0, $CHCl_3$); ^1H NMR (C_6D_6, 500 MHz): δ 8.49 (m, 2H, Ar-2, Ar-6), 6.98 (m, 2H, Ar-3, Ar-5), 5.41 (dd, 1H, $J_{1,2}$ = 5.7, $J_{2,3}$ = 8.2 Hz, H-2), 5.34 (ddd, 1H, $J_{3,4}$ = 3.5, $J_{4,5a}$ = 4.7, $J_{4,5b}$ = 2.2 Hz, H-4), 5.25 (d, 1H, $J_{1,2}$ = 5.7 Hz, H-1), 5.19 (dd, 1H, $J_{2,3}$ = 8.2, $J_{3,4}$ = 3.5 Hz, H-3), 4.10 (dd, 1H, $J_{4,5a}$ = 4.7, $J_{5a,5b}$ = 12.6 Hz, H-5a), 3.79 (dd, 1H, $J_{4,5b}$ = 2.2, $J_{5a,5b}$ = 12.6 Hz, H-5b), 2.14, 2.09, 2.05 (3s, 9H, $COCH_3$). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 170.10, 169.99, 169.30 (*C*=O), 151.39 (Ar), 111.61 (Ar), 96.91 (C-1), 69.24 (C-3), 68.58 (C-4), 66.57 (C-2), 62.27 (C-5), 20.84, 20.72, 20.68 ($COCH_3$).

4.3.11. (4-Pyridinyl) β-D-galactopyranoside (27)

Compound **24** (1.24 g, 2.91 mmol) dissolved in anhydrous methanol (70 mL) was treated with NH_3 in methanol (1.5 mL) for 6 h at room temperature then concentrated and purified by column chromatography (ethyl acetate/methanol 6:1) to give 717 mg (96%) of **27** as amorphous solid. $[\alpha]_D^{20}$ +94.0 (*c* 0.1, MeOH); ^1H NMR (DMSO-d_6, 400 MHz): δ 8.43 (m, 2H, Ar-2, Ar-6), 7.04 (m, 2H, Ar-3, Ar-5), 5.02 (d, 1H, $J_{1,2}$ = 8.1 Hz, H-1), 3.72 (d, 1H, $J_{3,4}$ = 3.5 Hz, H-4), 3.65 (dt, 1H, $J_{3,4}$ = 3.5, $J_{5,6a}$ = 5.7, $J_{5,6b}$ = 6.6 Hz, H-5), 3.60 (dd, 1H, $J_{1,2}$ = 8.1, $J_{2,3}$ = 9.5 Hz, H-2), 3.55 (dd, 1H, $J_{5,6a}$ = 5.7, $J_{6a,6b}$ = 11.0 Hz, H-6a), 3.49 (dd, 1H, $J_{5,6b}$ = 6.6, $J_{6a,6b}$ = 11.0 Hz, H-6b), 3.41 (dd, 1H, $J_{2,3}$ = 9.5, $J_{3,4}$ = 3.5 Hz, H-3). ^{13}C NMR (100.6 MHz, DMSO-d_6): δ 163.59 (Ar-4), 151.30 (Ar-2, Ar-6), 111.97 (Ar-3, Ar-5), 100.14 (C-1), 76.06 (C-5), 73.56 (C-3), 70.35 (C-2), 68.43 (C-4), 60.66 (C-6).

4.3.12. (4-Pyridinyl) β-D-fucopyranoside (28)

Compound **25** (183 mg, 0.5 mmol) dissolved in anhydrous methanol (10 mL) was treated with sodium methoxide in methanol (0.1 M, 0.5 mL) for 6 h at room temperature, then neutralized with Amberlite IR 120 (H^+) to give 110 mg (92%) of **28** as slightly yellow, amorphous solid. $[\alpha]_D^{20}$ +124.0 (*c* 0.2, H_2O); ^1H NMR (MeOH-d_4, 500 MHz): δ 8.41 (m, 2H, Ar), 7.05 (m, 2H, Ar), 5.03 (d, 1H, $J_{1,2}$ = 7.5 Hz, H-1), 3.84 (m, 1H, H-5), 3.57 (dd, 1H, $J_{1,2}$ = 7.5, $J_{2,3}$ = 9.8 Hz, H-2), 3.51 (d, 1H, $J_{3,4}$ = 3.2 Hz, H-4), 3.44 (dd, 1H, $J_{2,3}$ = 9.8, $J_{3,4}$ = 3.2 Hz, H-3), 1.18 (d, 3H, $J_{5,6}$ = 6.3 Hz, 6-CH_3). ^{13}C NMR (MeOH-d_4, 100.6 MHz): δ 151.29 (Ar), 111.86 (Ar), 99.75 (C-1), 73.67 (C-3), 71.19 (C-4), 70.86 (C-5), 69.98 (C-2), 16.83 (C-6).

Calcd for $C_{11}H_{15}NO_5$ (241.24): C, 54.77; H, 6.27; N, 5.81; found: C, 54.26; H, 6.39; N, 5.49.

4.3.13. (4-Pyridinyl) α-L-arabinopyranoside (29)

Compound **26** (1.76 g, 5.0 mmol) dissolved in anhydrous methanol (100 mL) was treated with sodium methoxide in methanol (0.1 M, 5.0 mL) for 6 h at room temperature, then neutralized with Amberlite IR 120 (H^+) to give 932 mg (89%) of **29** as orange, amorphous solid. $[\alpha]_D^{20}$ +5.0 (*c* 0.15, H_2O); ^1H NMR (MeOH-d_4, 500 MHz): δ 8.42 (m, 2H, Ar), 7.05 (m, 2H, Ar), 5.03 (d, 1H, $J_{1,2}$ = 6.9 Hz, H-1), 3.76–3.71 (m, 2H, H-4, H-5a), 3.68–3.62 (m, 2H, H-2, H-5b), 3.48 (m, 1H, H-3). ^{13}C NMR (MeOH-d_4, 100.6 MHz): δ 151.31 (Ar), 111.90 (Ar), 99.81 (C-1), 72.67 (C-3), 70.34 (C-4), 67.74 (C-2), 66.10 (C-5).

Calcd for $C_{10}H_{13}NO_5$ (227.27): C, 52.86; H, 5.77; N, 6.16; found: C, 51.98; H, 5.89; N, 6.29.

4.3.14. Methyl β-D-galactopyranosyl-(1→3)-α-D-glucopyranoside (32)

(a) Methyl α-D-glucopyranoside (**4**, 194 mg, 1.0 mmol) and (4-pyridinyl)β-D-galactopyranoside (**27**, 129 mg, 0.5 mmol) dissolved in potassium phosphate buffer (1 mL, 0.1 M, KH_2PO_4/K_2HPO_4, pH = 6.5) were incubated according to GP 3. Workup and chromatography on Biogel P2 gave 47.9 mg (27%) of **32** asa colorless, amorphous solid.

(b) Methyl α-D-glucopyranoside (**4**, 194 mg, 1.0 mmol) and *p*-nitrophenyl β-D-galactopyranoside (**30**,[24] 150 mg, 0.5 mmol) dissolved in potassium phosphate buffer (1 mL, 0.1 M, KH_2PO_4/K_2HPO_4, pH = 6.5) were incubated according to GP 3. The formed *p*-nitrophenol was extracted with ethyl acetate (5 mL). Further workup and chromatography on Biogel P2 gave 40.8 mg (23%) of **32** as a colorless, amorphous solid.

(c) Methyl α-D-glucopyranoside (**4**, 242 mg, 1.25 mmol) and lactose (**31**, 171 mg, 0.5 mmol) dissolved in potassium phosphate buffer (1 mL, 0.1 M, KH_2PO_4/K_2HPO_4, pH = 6.5) were incubated according to GP 3. Workup and chromatography on Biogel P2 gave 23.1 mg (13%) of **32** as colorless, amorphous solid.

$[\alpha]_D^{20}$ +97 (*c* 1.0, H_2O); selected signals of **32**: ^1H NMR (D_2O, 500 MHz): δ 4.77 (d, 1H, $J_{1',2'}$ = 3.8 Hz, H-1), 4.56 (d, 1H, $J_{1',2'}$ = 7.9 - Hz, H-1′), 3.87 (d, 1H, $J_{3',4'}$ = 3.1 Hz, H-4′), 3.81 (m, 1H, H-3), 3.82 (dd, 1H, $J_{2',3'}$ = 9.8, $J_{3',4'}$ = 3.1 Hz, H-3′), 3.55 (dd, 1H, $J_{1',2'}$ = 7.9, $J_{2',3'}$ = 9.8 Hz, H-2′). ^{13}C NMR (D_2O, 125 MHz): δ 103.28 (C-1′), 99.41 (C-1), 82.98 (C-3), 71.72 (C-2′), 71.31 (C-3′), 71.06 (C-4).

Calcd for $C_{13}H_{24}O_{11}$ (356.33): C, 43.82; H, 6.79; found: C, 40.72; H, 6.85.

Peracetate **32Ac**: ^1H NMR (C_6D_6, 500 MHz): δ 5.48 (dd, 1H, $J_{1',2'}$ = 8.0, $J_{2',3'}$ = 10.7 Hz, H-2′), 5.40 (d, 1H, $J_{3',4'}$ = 3.1 Hz, H-4′), 5.16 (t, 1H, $J_{2,3}$ = $J_{3,4}$ = 10.1 Hz, H-3), 5.06 (dd, 1H, $J_{2',3'}$ = 10.7, $J_{3',4'}$ = 3.1 Hz, H-3′), 5.01 (dd, 1H, $J_{1,2}$ = 3.8, $J_{2,3}$ = 10.1 Hz, H-2), 4.78 (d, 1H, $J_{1,2}$ = 3.8 Hz, H-1), 4.62 (d, 1H, $J_{1',2'}$ = 8.0 Hz, H-1′), 4.35 (dd, 1H, $J_{2,3}$ = $J_{3,4}$ = 10.1 Hz, H-4), 4.25 (dd, 1H, $J_{5,6a}$ = 5.0, $J_{6a,6b}$ = 12.1 Hz, H-6a), 4.16 (dd, 1H, $J_{5,6b}$ = 2.5, $J_{6a,6b}$ = 12.1 Hz, H-6b), 4.08 (dd, 1H, $J_{5',6'a}$ = 6.5, $J_{6'a,6'b}$ = 11.0 Hz, H-6′a), 4.01 (dd, 1H, $J_{5',6'b}$ = 6.5 Hz, $J_{6'a,6'b}$ = 11.0 Hz, H-6′b), 3.95 (ddd, 1H, $J_{4,5}$ = 2.5, $J_{5,6a}$ = 5.0, $J_{5,6b}$ = 12.1 Hz, H-5), 3.27 (t, 1H, $J_{5',6'a}$ = $J_{5',6'b}$ = 6.5 Hz, H-5′), 3.35 (s, 3H, OCH_3), 2.16, 2.14, 2.10, 2.06, 2.04, 1.98, 1.96 (7s, each 3H, CO-CH_3). ^{13}C NMR (C_6D_6, 125 MHz): δ 170.62, 170.58, 170.31, 170.10, 169.94, 169.83, 169.55 (C═O), 101.69 (C-1′), 97.24 (C-1), 76.59 (C-4), 73.65 (C-2), 71.89 (C-3′), 71.14 (C-5′), 69.76 (C-2′), 69.27 (C-3), 68.24 (C-5), 67.30 (C-4′), 62.70 (C-6), 60.75 (C-6′), 54.96 (OCH_3), 20.71, 20.44, 20.21, 20.03, 20.00, 19.98, 19.95 (CO-CH_3). Calcd for **32Ac**: $C_{27}H_{38}O_{18}$ (650.20). MALDI-TOF: *m*/*z* 673.17 $[M+Na]^+$, 689.25 $[M+K]^+$.

4.3.15. β-D-Galactopyranosyl-(1→3)-α-D-glucopyranosyl-(1↔2)-β-D-fructofuranoside (33)

(a) Sucrose (**14**, 342 mg, 1 mmol) and (4-pyridinyl) β-D-galactopyranoside (**27**, 78 mg, 0.3 mmol) dissolved in potassium phosphate buffer (1 mL, 0.1 M, KH_2PO_4/K_2HPO_4, pH = 6.5) were incubated according to GP 3. Workup and chromatography on Biogel P2 gave 46.9 mg (31%) of **33** as a colorless, amorphous solid.

(b) Sucrose (**14**, 342 mg, 1 mmol) and lactose (**31**, 171 mg, 0.5 mmol) dissolved in potassium phosphate buffer (1 mL, 0.1 M, KH_2PO_4/K_2HPO_4, pH = 6.5) were incubated according to GP 3. Workup and chromatography on Biogel P2 gave 30.2 mg (12%) of **33** as colorless, amorphous solid.

$[\alpha]_D^{20}$ +49 (*c* 1.0, H_2O), lit.:[4] +45 (*c* 0.5, H_2O); selected signals of **30**: ^1H NMR (D_2O, 500 MHz): δ 5.46 (d, 1H, $J_{1,2}$ = 4.0 Hz, H-1), 4.67 (d, 1H, $J_{1'',2''}$ = 7.9 Hz, H-1″), 4.24 (d, 1H, $J_{2',3'}$ = $J_{3',4'}$ = 8.7 Hz, H-3′), 4.08 (t, 1H, $J_{3',4'}$ = $J_{4',5'}$ = 8.7 Hz, H-4′), 4.02 (t, 1H, $J_{2,3}$ = $J_{3,4}$ = 9.3 Hz, H-3), 3.93–3.89 (m, 1H, H-5), 3.84 (m, 2H, H-6′a, H-6′b), 3.79 (d, 1H, $J_{1,2}$ = 4.0 Hz, H-2), 3.65 (dd, 1H, $J_{1'',2''}$ = 7.9, $J_{2'',3''}$ = 9.3 Hz, H-2″). ^{13}C NMR (D_2O, 125 MHz): δ 104.07 (C-1″), 103.56 (C-2), 92.50 (C-1), 82.14 (C-4′), 81.77 (C-3), 76.94 (C-3′), 74.40 (C-5′), 62.74 (C-6′). Calcd for $C_{18}H_{32}O_{16}$ (504.44). MALDI-TOF *m*/*z* 527.33 $[M+Na]^+$, 543.45 $[M+K]^+$.

Peracetate **33Ac**: ^1H NMR (C_6D_6, 500 MHz): δ 5.93 (d, 1H, $J_{1,2}$ = 3.8 Hz, H-1), 5.88 (d, 1H, $J_{3',4'}$ = 6.3 Hz, H-3′), 5.75 (t, 1H, $J_{3',4'}$ = $J_{4',5'}$ = 6.3 Hz, H-4′), 5.65 (dd, 1H, $J_{1'',2''}$ = 8.0, $J_{2'',3''}$ = 10.4 Hz, H-2″), 5.60 (d, 1H, $J_{3'',4''}$ = 2.8 Hz, H-4″), 5.30 (dd, 1H, $J_{2'',3''}$ = 10.4, $J_{3'',4''}$ = 2.8 Hz, H-3″), 5.24 (t, 1H, $J_{3,4}$ = $J_{4,5}$ = 10.0 Hz, H-4), 5.16 (dd, 1H, $J_{1,2}$ = 3.8, $J_{2,3}$ = 10.0 Hz, H-2), 4.94 (d, 1H, $J_{1'',2''}$ = 8.0 Hz, H-1″), 4.62–4.56 (m, 3H, H-5, H-6a, H6b), 4.54 (t, 1H, $J_{2,3}$ = $J_{3,4}$ = 10.0 Hz, H-3), 4.50–4.41 (m, 4H, H-1′a, H-1′b, H-6′a, H-6′b), 4.34 (dd, 1H, H-5′, $J_{5',6'a}$ = 6.9, $J_{5',6'b}$ = 11.3 Hz, H-5′), 4.29 (dd, 1H, $J_{5'',6''a}$ = 6.9, $J_{6''a,6''b}$ = 11.0 Hz, H-6″a), 4.24 (dd, 1H, $J_{5'',6''b}$ = 11.3, $J_{6''a,6''b}$ = 11.0 Hz, H-6″b), 3.67 (dd, 1H, $J_{5'',6''a}$ = 6.9, $J_{5',6'b}$ = 11.3 Hz, H-5″), 2.02–2.68 (several s, 33H, CO-CH_3). ^{13}C NMR (C_6D_6, 125 MHz): δ 170.57, 170.55, 170.48, 170.29, 170.25, 170.06, 169.94, 169.83, 169.35, 169.12 (C═O), 104.98 (C-2′), 102.01 (C-1″), 91.21 (C-1), 79.68 (C-5′), 77.01 (C-3′), 76.46 (C-3), 76.10 (C-4′), 73.26 (C-2), 72.00 (C-3″), 71.18 (C-5″), 70.00 (C-2″), 69.63 (C-5), 69.25 (C-4), 67.71 (C-4″), 64.43 (C-1′), 63.76 (C-6″), 62.75 (C-6′), 61.40 (C-6), 20.85, 20.65, 20.51, 20.43, 20.35, 20.22, 20.17, 20.05, 19.98, 19.95, 19.91 (CO-CH_3).

4.3.16. β-D-Fucopyranosyl-(1→3)-α-D-glucopyranosyl-(1↔2)-β-D-fructofuranoside (34)

Sucrose (**14**, 342 mg, 1 mmol) and (4-pyridinyl) β-D-fucopyranoside (28, 121 mg, 0.5 mmol) dissolved in potassium phosphate buffer (1 mL, 0.1 M, KH_2PO_4/K_2HPO_4, pH = 6.5) were incubated according to GP 3. Workup and chromatography on Biogel P2 gave 6.5 mg (2.6%) of **34** as colorless, amorphous solid. $[\alpha]_D^{20}$ +45 (*c* 0.2, H_2O); ^1H NMR (D_2O, 500 MHz): δ 5.39 (d, 1H, $J_{1,2}$ = 3.8 Hz, H-1), 4.56 (d, 1H, $J_{1'',2''}$ = 7.9 Hz, H-1″), 4.15 (d, 1H, $J_{3',4'}$ = 8.8 Hz, H-3′), 4.01 (t, 1H, $J_{3',4'}$ = $J_{4',5'}$ = 8.8 Hz, H-4′), 3.95 (dd, 1H, $J_{1,2}$ = 3.8, $J_{2,3}$ = 9.2 Hz, H-2), 3.90 (t, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.8 Hz, H-4), 3.83 (m, 1H, H-5), 3.74–3.73 (m, 5H, H-3″, H-1′a, H-1′b, H-6′a, H-6′b), 3.71 (m, 1H, H-5′), 3.67 (d, 1H, $J_{3'',4''}$ = 2.1 Hz, H-4″), 3.63–3.60 (m, 3H,

H-5″, H-6a, H-6b), 3.57 (dd, 1H, $J_{1″,2″}$ = 7.9, $J_{2″,3″}$ = 10.2 Hz, H-2″), 3.50 (t, 1H, $J_{2,3} = J_{3,4}$ = 9.2 Hz, H-3), 1.19 (d, 3H, $J_{5″,6″}$ = 6.5 Hz, 6″-CH_3). ^{13}C NMR (D_2O, 100 MHz): δ 104.43 (C-2′), 103.08 (C-1″), 92.43 (C-1), 82.57 (C-3), 81.71 (C-5′), 76.79 (C-3′), 74.30 (C-4′), 73.09 (C-3″), 72.43 (C-5), 71.63 (C-5″), 71.27 (C-2), 71.23 (C-2″), 71.08 (C-4), 70.91 (C-4″), 62.69 (C-1′), 61.79 (C-6′), 60.43 (C-6), 15.84 (C-6″).

Calcd for $C_{18}H_{32}O_{15}$ (488.17). MALDI-TOF *m*/*z* 510.25 $[M+Na]^+$, 526.51 $[M+K]^+$.

4.3.17. α-L-Arabinopyranosyl-(1→3)-α-D-glucopyranosyl-(1↔2)-β-D-fructofuranoside (35)

Sucrose (**14**, 342 mg, 1 mmol) and (4-pyridinyl) α-L-arabinopyranoside (**29**, 113 mg, 0.5 mmol) dissolved in potassium phosphate buffer (1 mL, 0.1 M, KH_2PO_4/K_2HPO_4, pH = 6.5) were incubated according to GP 3. Workup and chromatography on Biogel P2 gave 7.2 mg (3.0%) of **35** as colorless, amorphous solid. $[\alpha]_D^{20}$ +100 (*c* 1.0, H_2O); ^{1}H NMR (D_2O, 500 MHz): δ 5.36 (d, 1H, $J_{1,2}$ = 3.8 Hz, H-1), 4.53 (d, 1H, $J_{1″,2″}$ = 6.5 Hz, H-1″), 4.15 (d, 1H, $J_{3′,4′}$ = 8.9 Hz, H-3′), 3.98 (t, 1H, $J_{3′,4′} = J_{4′,5′}$ = 8.9 Hz, H-4′), 3.92–3.87 (m, 2H, H-3, H-4″), 3.82 (m, 2H, H-5, H-5′), 3.77–3.74 (m, 4H, H-1′a, H-1′b, H-6′a, H-6′b), 3.67 (dd, 1H, $J_{1,2}$ = 3.8, $J_{2,3}$ = 9.8 Hz, H-2), 3.46 (dd, 1H, $J_{2″,3″}$ = 7.8, $J_{3″,4″}$ = 2.5 Hz, H-3″), 3.62–3.58 (m, 4H, H-5″a, H-5″b, H-6a, H-6b), 3.54 (dd, 1H, $J_{1″,2″}$ = 6.5, $J_{2″,3″}$ = 7.8 Hz, H-2″), 3.49 (t, 1H, $J_{3,4} = J_{4,5}$ = 9.5 Hz, H-4). ^{13}CNMR (D_2O, 100 MHz): δ 104.02 (C-2′), 103.77 (C-1″), 92.48 (C-1), 82.52 (C-3), 82.10 (C-5′), 76.85 (C-3′), 74.32 (C-4′), 72.60 (C-5″), 72.52 (C-5), 71.56 (C-2″), 71.51 (C-2), 70.98 (C-4), 68.61 (C-4″), 66.53 (C-3″), 62.69 (C-1′), 61.80 (C-6), 60.46 (C-6′). Calcd for $C_{17}H_{30}O_{15}$ (474.15). MALDI-TOF *m*/*z* 497.25 $[M+Na]^+$, 513.09 $[M+K]^+$.

4.3.18. β-D-Galactopyranosyl-(1→3)-α-D-glucopyranosyl-(1→6)-glucitol/mannitol (36)

(a) Palatinitol (**15**, 344 mg, 1 mmol) and (4-pyridinyl) β-D-galactopyranoside (**27**, 78 mg, 0.3 mmol) dissolved in potassium phosphate buffer (1 mL, 0.1 M, KH_2PO_4/K_2HPO_4, pH = 6.5) were incubated according to GP 3. Workup and chromatography on Biogel P2 gave 17.0 mg (11.2%) of **36** as a colorless, amorphous solid.

(b) Palatinitol (**15**, 344 mg, 1 mmol) and lactose (**31**, 171 mg, 0.5 mmol) dissolved in potassium phosphate buffer (1 mL, 0.1 M, KH_2PO_4/K_2HPO_4, pH = 6.5) were incubated according to GP 3. Workup and chromatography on Biogel P2 gave 22.2 mg (8.8%) of **36** as a colorless, amorphous solid. $[\alpha]_D^{20}$ +54 (*c* 1, H_2O); lit.:[4] +52 (*c* 0.6, H_2O); ^{1}H NMR (D_2O, 500 MHz): δ 4.90 (m, 2H, CH_2-1), 4.45 (d, 1H, $J_{1″,2″}$ = 7.8 Hz, H-1″). ^{13}C NMR (D_2O, 100 MHz): δ 103.68 (C-1″), 98.47 (C-1), 82.37 (C-3), 75.43 (C-5″), 72.88 (C-3″), 71.40 (C-2), 71.13 (C-2″), 69.91/69.63 (C-4/4″), 68.99/68.94 (C-6′), 63.63 (C-1′, Man-ol), 62.78 (C-1′, Glc-ol), 61.36 (C-6″), 60.86 (C-6). Calcd for $C_{18}H_{34}O_{16}$ (506.18). MALDI-TOF *m*/*z* 529.13 $[M+Na]^+$, 545.34 $[M+K]^+$.

4.3.19. β-D-Galactopyranosyl-(1→3)-α-D-glucopyranosyl-(1→6)-β-D-fructofuranose (37)

(a) Isomaltulose (**16**, 342 mg, 1 mmol) and (4-pyridinyl) β-D-galactopyranoside (**27**, 78 mg, 0.3 mmol) dissolved in potassium phosphate buffer (1 mL, 0.1 M, KH_2PO_4/K_2HPO_4, pH = 6.5) were incubated according to GP 3. Workup and chromatography on Biogel P2 gave 19.7 mg (13.2%) of **37** as a colorless, amorphous solid.

(b) Isomaltulose (**16**, 342 mg, 1 mmol) and lactose (**31**, 171 mg, 0.5 mmol) dissolved in potassium phosphate buffer (1 mL, 0.1 M, KH_2PO_4/K_2HPO_4, pH = 6.5) were incubated according to GP 3. Workup and chromatography on Biogel P2 gave 19.5 mg (7.9%) of **37** as a colorless, amorphous solid. $[\alpha]_D^{20}$ +44 (*c* 0.97, H_2O); Lit.:[4] +43 (*c* 1.2, H_2O). Calcd for $C_{18}H_{32}O_{18}$ (504.15). MALDI-TOF *m*/*z* 527.45 $[M+Na]^+$, 543.35 $[M+K]^+$. Selected signals of **34**: ^{1}H NMR (D_2O, 500 MHz): δ 4.92 (d, 1H, $J_{1,2}$ = 3.8 Hz, H-1), 4.57 (d, 1H, $J_{1″,2″}$ = 7.9 Hz, H-1″). ^{13}C NMR (D_2O, 100 MHz): δ 103.72 (C-1″), 103.25 (C-2′), 98.57 (C-1), 82.72 (C-3), 68.31 (C-6′).

Peracetate **37Ac**: ^{1}H NMR (C_6D_6, 500 MHz): δ 5.59 (d, 1H, $J_{3″,4″}$ = 2.5 Hz, H-4″), 5.56 (dd, 1H, $J_{2″,3″}$ = 9.2, $J_{3″,4″}$ = 2.5 Hz, H-3″), 5.49 (m, 1H, H-4), 5.25 (t, 1H, $J_{3′,4′} = J_{4′,5′}$ = 6.7 Hz, H-4′), 5.04 (d, 1H, $J_{1,2}$ = 3.8 Hz, H-1), 4.76 (dd, 1H, $J_{1″,2″}$ = 7.8, $J_{2″,3″}$ = 9.2 Hz, H-2″), 4.62 (ddd, 1H, $J_{4′,5′}$ = 6.7, $J_{5′,6′a}$ = 6.2, $J_{5′,6′b}$ = 3.3 Hz, H-5′), 4.58 (dd, 1H, $J_{1,2}$ = 3.8, $J_{2,3}$ = 8.2 Hz, H-2), 4.52 (d, 1H, $J_{1″,2″}$ = 7.8 Hz, H-1″), 4.46 (t, 1H, $J_{2,3} = J_{3,4}$ = 8.2 Hz, H-3), 4.40–4.36 (m, 2H, H-3′, H-5), 4.30 (m, 1H, H-5″), 4.23–4.18 (m, 4H, H-6a, H-6b, H-6″a, H-6″b), 4.10 (m, 1H, H-1′a), 4.05 (m, 1H, H-1′b), 3.79 (dd, 1H, $J_{5′,6′a}$ = 6.2, $J_{6′a,6′b}$ = 10.7 Hz, H-6′a), 3.73 (dd, 1H, $J_{5′,6′b}$ = 3.3, $J_{6′a,6′b}$ = 10.7 Hz, H-6′b), 2.23–2.00 (several s, 33H, CO-CH_3). ^{13}C NMR (C_6D_6, 100 MHz): δ 170.52, 170.45, 170.41, 170.37, 170.27, 170.21, 170.17, 170.14, 170.07, 170.01, 169.95 (C=O), 102.88 (C-2′), 101.71 (C-1″), 96.60 (C-1), 83.12 (C-3), 81.54 (C-5′), 79.33 (C-5″), 78.39 (C-3″), 76.90 (C-4′), 76.79 (C-3′), 76.31 (C-3″), 73.61 (C-4), 73.53 (C-5), 71.89 (C-4″), 71.75 (C-2), 71.71 (C-2″), 68.55 (C-6′), 61.75 (C-6″), 61.26 (C-1′), 61.14 (C-6), 20.78, 20.74, 20.72, 20.67, 20.63, 20.58, 20.56, 20.48, 20.42 (CO-CH_3).

Acknowledgements

Partial support of this study by the Federal Ministry of Economics and Technology (BMWi) via German Federation of Industrial Research Associations (AIF) and Research Association of the German Food Industry (FEI) within Project No. 12401N is gratefully acknowledged.

References

1. Onishi, N.; Yamashiro, A.; Yokozeki, K. *Appl. Environ. Microbiol.* **1995**, *61*, 4022–4025.
2. Fransen, C. T. M.; van Laere, K. M. J.; van Wijk, A. A. C.; Brüll, L. P.; Dignum, M.; Thomas-Oates, J. E.; Haverkamp, J.; Schols, H. A.; Voragen, A. G. J.; Kamerling, J. P.; Vliegenthart, J. F. G. *Carbohydr. Res.* **1998**, *314*, 101–114.
3. Farkas, E.; Schmidt, U.; Thiem, J.; Kowalczyk, J.; Kunz, M.; Vogel, M. *Synthesis* **2003**, 699–706.
4. Schröder, S.; Thiem, J.; Kowalczyk, J.; Kunz, M.; Vogel, M. *Tetrahedron* **2004**, *11*, 2601–2608.
5. Foucault, M.; Watzlawick, H.; Mattes, R.; Haser, R.; Gouet, P. *Acta Crystallogr.* **2006**, *62*, 100–103.
6. Fridjonsson, O.; Watzlawick, H.; Mattes, R. *Extremophiles* **2000**, *4*, 23–33.
7. Spangenberg, P.; André, C.; Dion, M.; Rabiller, C.; Mattes, R. *Carbohydr. Res.* **2000**, *329*, 65–73.
8. Barnett, J. E. G. *Carbohydr. Res.* **1969**, *9*, 21–31.
9. Steinmann, A.; Thimm, J.; Matwiejuk, M.; Thiem, J. *Macromolecules* **2010**, *43*, 3606–3612.
10. Schuttle, J. B.; de Jong, J.; van Weerden, E. J.; Tamenga, S. *Br. J. Nutr.* **1992**, *1*, 195–207.
11. Osaki, S.; Kimura, T.; Sugimoto, T.; Hizukuri, S.; Iritani, N. J. *Nutrition* **2001**, *3*, 796–799.
12. Nisikawa, Y.; Yoshikawa, G.; Tanaka, H.; Morimoto, A.; Mukai, K. Unitika Ltd, WO0157230A1, 2001; *Chem. Abstr.* **2001**, *135*, 136725.
13. Kent, P. W.; Barnett, J. E. G. *J. Chem. Soc.* **1964**, 6196–6204.
14. Kuhn, R.; Grassner, H. *Liebigs Ann. Chem.* **1957**, 55–67.
15. Helferich, B.; Jung, K.-H. *Liebigs Ann. Chem.* **1954**, *589*, 77–81; Helferich, B.; Jung, K.-H. *Liebigs Ann. Chem.* **1955**, *595*, 242.
16. Yasukochi, T.; Inaba, C.; Fukase, K.; Kusumoto, S. *Tetrahedron Lett.* **1999**, *40*, 6585–6589.
17. Lemieux, R. U. *Methods Carbohydr. Chem.* **1963**, *2*, 221–222.
18. Hanessian, S.; Saavedra, O. M.; Mascitti, V.; Marterer, W.; Oehrlein, R.; Mak, C.-P. *Tetrahedron* **2001**, *57*, 3267–3280.
19. Nicolaou, K. C.; Schreiner, E. P.; Stahl, W. *Angew. Chem., Int. Ed.* **1991**, *30*, 585–590.
20. Smiatacz, Z.; Zygfryd, M. *Carbohydr. Res.* **1990**, *196*, 167–174.
21. Kröger, L.; Scudlo, A.; Thiem, J. *Adv. Synth. Catal.* **2006**, *348*, 1217–1227.
22. Distler, J. J.; Jourdan, G. W. *J. Biol. Chem.* **1973**, *248*, 6772–6780.
23. Hedbys, L.; Johannson, E.; Mosbach, K.; Larsson, P.-O.; Gunnarsson, A.; Svensson, S. *Carbohydr. Res.* **1989**, *186*, 217–223.
24. Heyworth, R.; Walker, P. G. *Biochem. J.* **1962**, *83*, 331–335.