Available online at www.sciencedirect.com
ScienceDirect
Carbohydrate Research 342 (2007) 1793–1804
Carbohydrate RESEARCH

# The synthesis of 2-, 3-, 4- and 6-*O*-α-D-glucopyranosyl-D-galactopyranose, and their evaluation as nutritional supplements for pre-term infants

Peter J. Meloncelli,[a] Tracey M. Williams,[b] Peter E. Hartmann[b] and Robert V. Stick[a,*]

[a]*Chemistry M313, School of Biomedical, Biomolecular and Chemical Sciences, University of Western Australia, Crawley, WA 6009, Australia*
[b]*Biochemistry M310, School of Biomedical, Biomolecular and Chemical Sciences, University of Western Australia, Crawley, WA 6009, Australia*

Received 30 March 2007; received in revised form 20 April 2007; accepted 25 April 2007
Available online 1 May 2007

**Abstract**—Four methods have been screened for the synthesis of some α-D-glucopyranosides, with the recently reported (Mukaiyama) combination of 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl iodide and triphenylphosphine oxide being the most successful, especially in the diastereoselectivity exhibited. The α-D-glucopyranosides so obtained have been deprotected to yield 2-, 3-, 4- and 6-*O*-α-D-glucopyranosyl-D-galactopyranose. Only the last disaccharide showed any hydrolysis by α-glycosidases but this success was not emulated by mucosal extracts from the small intestine of the pig.


*Keywords:* Disaccharides; Synthesis; Diastereoselectivity; Halide catalysis; Lactose; Supplement; Nutritional; Infants; Pre-term

## 1. Introduction

Lactose (4-*O*-β-D-galactopyranosyl-D-glucopyranose) is the disaccharide present in mammalian milk and, through the action of lactase, is digested by the infant to form D-galactose and D-glucose necessary for healthy development.[1] Pre-term infants lack the levels of lactase to utilize lactose and so must be supplemented with other sources of carbohydrate.[2–6] The direct feeding of monosaccharides is not possible as the subsequent increase in osmolality results in diarrhoea in the infant.[7,8] One of the present solutions is to provide nutritional carbohydrate in the form of maltodextrins, for example, Polyjoule (D-glucopyranose linked mainly α-(1→4)).[9] Pre-term infants obviously possess high enough levels of enzymes (the glucoamylase–maltase complex) that are able to cleave α-D-glucosidic linkages.[10,11] Unfortunately, these supplements provide the infant with only D-glucose, which often elicits a diabetic-like response.[12]

It occurred to us that an alternative source of carbohydrate for pre-term infants might be found in a diastereoisomer of lactose, namely 4-*O*-α-D-galactopyranosyl-D-glucopyranose; perhaps the enzymes present in the small intestine could accommodate and hydrolyze the α-D-galactosidic linkage.[13] An even better design would be 4-*O*-α-D-glucopyranosyl-D-galactopyranose, a molecule that should be stable in aqueous solution, capable of hydrolysis by enzymes present in the small intestine, and still able to provide the necessary D-glucose and D-galactose for the growing infant. It should be noted here that we never considered a disaccharide based solely on D-glucose as it is well recognized, though perhaps not completely understood, that D-galactose is an essential nutrient for development in the young.[14–16]

This paper therefore describes various methods for the synthesis of 4-*O*-α-D-glucopyranosyl-D-galactopyranose, as well as the regioisomeric 2-, 3- and 6-*O*-α-D-glucopyranosyl-D-galactopyranose. The paper concludes

* Corresponding author. E-mail: rvs@chem.uwa.edu.au

0008-6215/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carres.2007.04.022

with an investigation of the four isomers as potential candidates for use as nutritional supplements for preterm infants.

## 2. Discussion

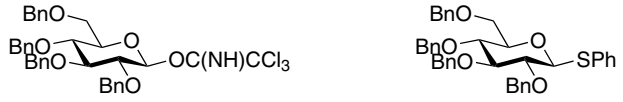
### 2.1. Synthesis of targets

The synthesis of the α-D-glycosidic linkage has occupied chemists for years, from Fischer's early successes with simple alcohol acceptors,[17] to the seminal work on 'halide catalysis' by Lemieux.[18] As new glycosyl donors were developed (trichloroacetimidates, thioglycosides), so were they applied to the synthesis of the α-D-linkage, with the common characteristic being the presence of a non-participating protecting group at O2. We were naturally attracted to the halide catalysis method and noted the recent significant improvements made by Lam and Gervay-Hague.[19] Therefore, we treated 6-*O*-acetyl-2,3,4-tri-*O*-benzyl-α-D-glucopyranosyl iodide **1** with the four D-*galacto* acceptors **2**–**5** (Scheme 1). Only alcohols **2** and **5** were sufficiently reactive, forming α-D-glucosides **6** and **7**, respectively (the actual α/β ratio was 97:3 in both instances), together with significant amounts of alkene **8** (Scheme 2). The formation of this alkene necessitated the use of an excess of donor **1** and generally detracted from the method.

With the failure of alcohols **3** and **4** to undergo glycosylation under the conditions of halide catalysis, we turned our attention to the more common methods of glycosylation, namely those employing trichloroacetimidates and thioglycosides. Thus, treatment of β-D-glucopyranosyl trichloroacetimidate **9** (Scheme 3) with alcohols **2**–**5** under standard conditions[20] gave disaccharide derivatives **10**–**13** (Scheme 4; Table 1).

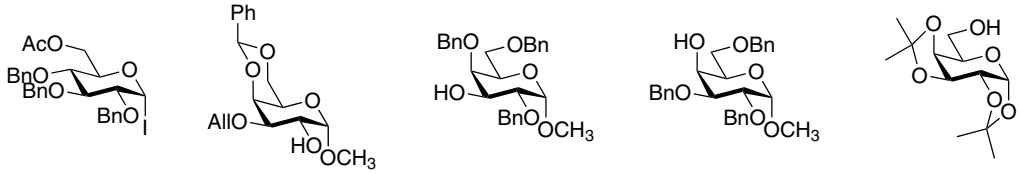
Treatment of thioglycoside **14** with alcohols **2**–**5** under conditions described by Crich[21] and by van Boom[22] again gave disaccharide derivatives **10**–**13** (Table 2).

At this stage of our work we noted two publications by Mukaiyama and Kobashi that announced even further improvements to the halide catalysis protocol, whereby triphenylphosphine oxide is added to the α-glycosyl iodide before the addition of the acceptor.[23,24] This combination purportedly forms a very reactive β-phosphonium ion, ensuring high α-selectivity in the subsequent glycosylation step; the phosphine oxide also acts



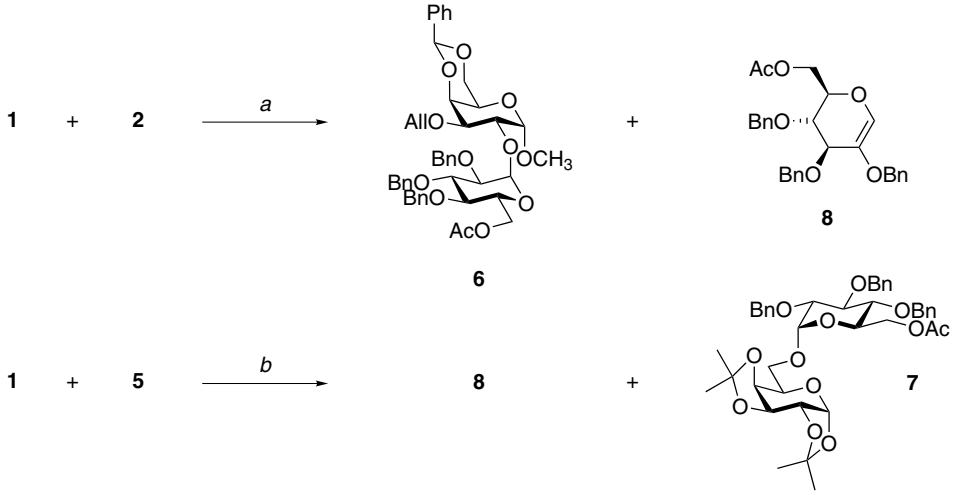

**9** **14**

**Scheme 3.**

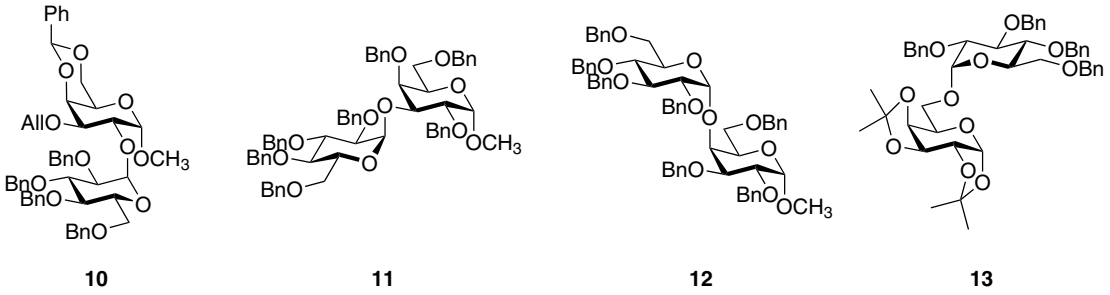



**1** **2** **3** **4** **5**

**Scheme 1.**




**Scheme 2.** Reagents: (a) $Bu_4NI$, $EtPr^i_2N$, 4 Å ms, benzene, 70%; (b) $Bu_4NI$, $EtPr^i_2N$, 4 Å ms, benzene, 90%.



**10** **11** **12** **13**

**Scheme 4.**

**Table 1.** Glycosylation of alcohols **2**–**5** with trichloroacetimidate **9** and trimethylsilyl trifluoromethanesulfonate in ether

| Acceptor | Product | Yield (%) | α:β |
|---|---|---|---|
| **2** | **10** | 82 | 85:15 |
| **3** | **11** | 72 | 75:25 |
| **4** | **12** | 72 | 65:35 |
| **5** | **13** | 71 | 80:20 |

**Table 2.** Glycosylation of alcohols **2**–**5** with thioglycoside **14** in dichloromethane, promoted by either 1-benzenesulfinylpiperidine (A) or diphenyl sulfoxide (B), in combination with 2,4,6-tri-*tert*-butylpyrimidine and trifluoromethanesulfonic anhydride

| Acceptor | Product | Yield (%) | | α:β | |
|---|---|---|---|---|---|
| | | A | B | A | B |
| **2** | **10** | 71 | 76 | 45:55 | 45:55 |
| **3** | **11** | 65 | 75 | 70:30 | 75:25 |
| **4** | **12** | 74 | 85 | 70:30 | 75:25 |
| **5** | **13** | 65 | 85 | 35:65 | 40:60 |

as a weak base to neutralize the liberated hydrogen iodide (Scheme 5). Treatment of 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl iodide **15** with alcohols **2**–**5** in the presence of triphenylphosphine oxide again formed disaccharide derivatives **10**–**13** in good yield but also with high diastereoselectivity (Table 3).

Although glycosyl iodide **15** is a little more tedious to prepare and handle,[25] it is more reactive than its counterpart **1**; also, the near neutral reaction conditions avoided the formation of any alkene by-product.

The diastereoselectivity for the formation of **6**, **7** and **10** was determined from an analysis of the $^1$H NMR spectrum of the reaction mixtures, and confirmed for **10** by isolation of the α- and β-anomers; for **11** and **12**, where the anomers were inseparable by chromatography, the diastereoselectivity was again initially determined by $^1$H NMR spectroscopy, and subsequently confirmed by debenzylation and acetylation of the reaction mixture, which facilitated the separation of the α- and β-anomers; for **13**, where the anomers were again inseparable by chromatography, the diastereoselectivity was determined by direct analysis of the $^1$H NMR spectrum.

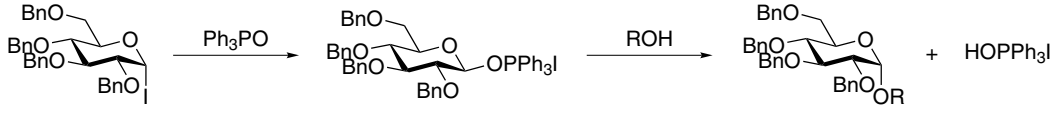
**Table 3.** Glycosylation of alcohols **2**–**5** with iodide **15** and triphenylphosphine oxide in chloroform

| Acceptor | Product | Yield (%) | α:β |
|---|---|---|---|
| **2** | **10** | 70 | 90:10 |
| **3** | **11** | 86 | 95:5 |
| **4** | **12** | 57 | 93:7 |
| **5** | **13** | 90 | 94:6 |

From an operational point of view, we chose to prepare disaccharides **6** and **7** using Gervay-Hague's halide catalysis protocol, and disaccharides **11** and **12** using the trichloroacetimidate method. For the latter method, it was always possible, at some stage, to separate the desired α-D-anomer from the smaller amount of contaminating β-D-anomer. In fact, having the pure undesired anomer helped to confirm the structure of the α-D-anomer. Finally, disaccharide derivatives **6**, **11**, **12** and **7** were converted into the target disaccharides by simple deprotection procedures (Schemes 6–9).

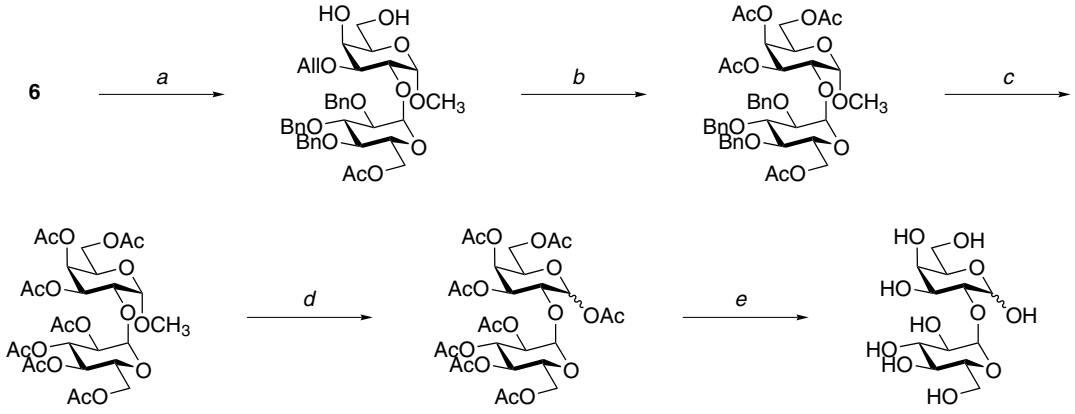
## 2.2. Evaluation of 2-, 3-, 4- and 6-*O*-α-D-glucopyranosyl-D-galactopyranose as nutritional supplements for pre-term infants

With the four synthetic disaccharides in hand, we were now in a position to examine their hydrolysis with a range of glycoside hydrolases. Initially, we chose commercially

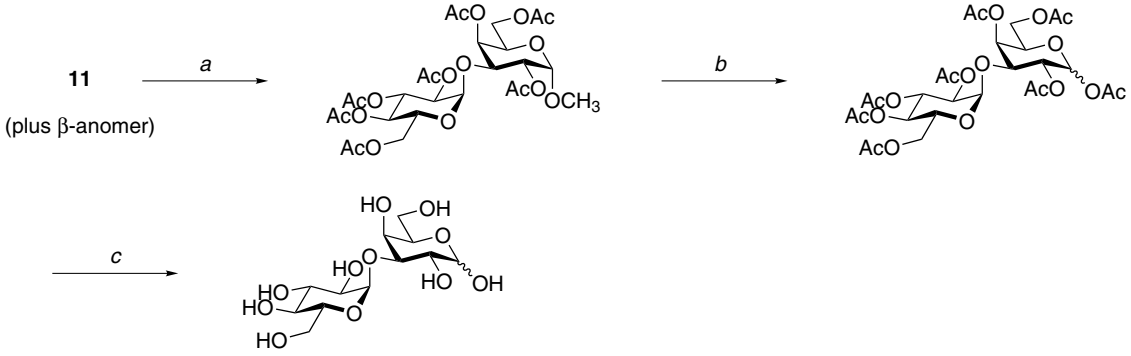


**15**

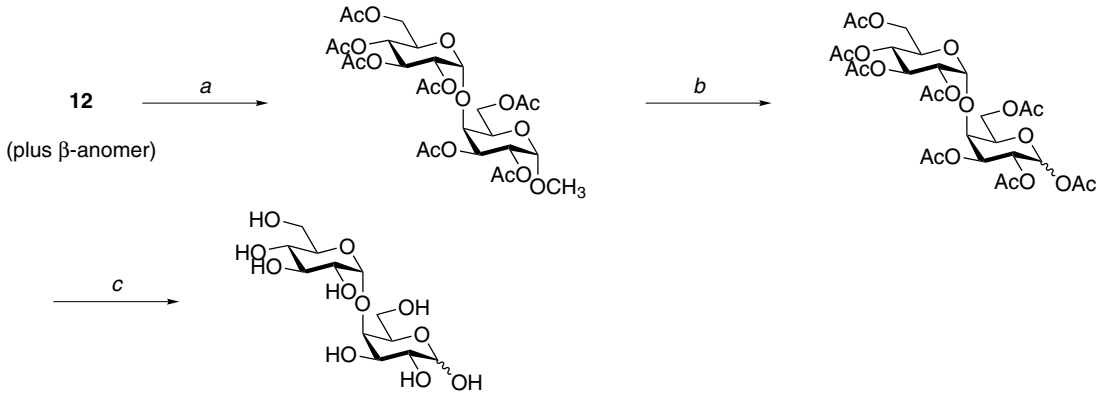
**Scheme 5.**



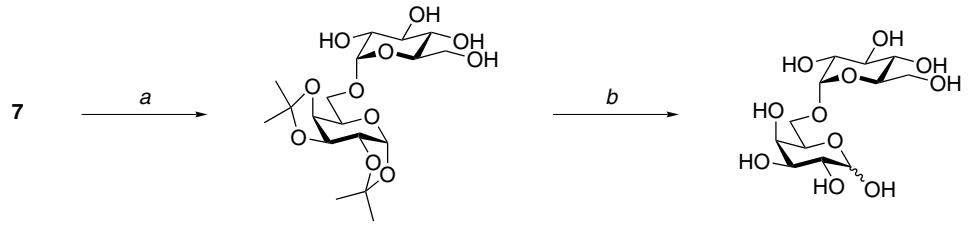
**Scheme 6.** Reagents: (a) AcOH/$H_2O$, 78%; (b) (i) $(Ph_3P)_3RhCl$, EtOH; (ii) 3 M HCl; (iii) $Ac_2O$, pyridine, DMAP, 84%; (c) (i) $H_2$, Pd/C, MeOH; (ii) $Ac_2O$, pyridine, DMAP, 90%; (d) $Ac_2O$, $H_2SO_4$, 95%; (e) NaOMe, MeOH.



**Scheme 7.** Reagents: (a) (i) $H_2$, Pd/C, MeOH; (ii) $Ac_2O$, pyridine, DMAP, 64%; (b) $Ac_2O$, $H_2SO_4$, 94%; (c) NaOMe, MeOH.



**Scheme 8.** Reagents: (a) (i) $H_2$, Pd/C, MeOH; (ii) $Ac_2O$, pyridine, DMAP, 55%; (b) $Ac_2O$, $H_2SO_4$; (c) NaOMe, MeOH.



**Scheme 9.** Reagents: (a) $H_2$, Pd/C, MeOH, 89%; (b) $CF_3CO_2H$, $H_2O$.

available α-amylase, isomaltase, α-glucosidase and α-galactosidase, and a standard colorimetric glucose assay.[26] The four disaccharides were unaffected by α-amylase, α-glucosidase and α-galactosidase (the last as expected) but isomaltase caused some minor hydrolysis of 2-*O*-α-D-glucopyranosyl-D-galactopyranose and significant hydrolysis of 6-*O*-α-D-glucopyranosyl-D-galactopyranose. This last result was enough to encourage us to investigate the possible hydrolysis with mammalian enzymes; a porcine source was available and appropriate.[27–29]

Dahlqvist has developed a method, based on a colorimetric change in *o*-dianisidine, to determine the disaccharidase activity of intestinal mucosa samples.[30] We decided to adopt this method for our purposes but used ABTS [2,2′-azino-di(3-ethylbenzothiazoline-6-sulfonic acid)] as the colorimetric agent; other minor improvements were also made to the determination. Thus, the small intestinal mucosa of a 12 week old pig was collected at 50 cm intervals, to provide data of disaccharidase activity throughout the entire length of the small intestine. The mucosae were prepared as homogenates, following the method of Dahlqvist; Table 4 summarizes the results.

It is evident that there was significant maltase, sucrase and lactase activity along most of the small intestine; the same is true of isomaltase activity but fewer points were assayed. Most disappointing was the lack of significant hydrolysis of 6-*O*-α-D-glucopyranosyl-D-galactopyranose by any of the enzymes present in the small intestine. The small amount of hydrolysis that was observed was attributed to the action of sucrase.

The results here provide an overall picture of disaccharidase activity along the small intestine of the pig. Maltase activity appears to be quite high and constant throughout the length of the small intestine. Sucrase activity on the other hand peaks around the area of the jejunum and then declines to approximately 50% of its peak level. Lactase activity appears to peak quite early in the small intestine and then quickly declines to low levels. These observations were shown to have relevance in the human model with similar patterns observed by Raul et al.[5]

## 3. Conclusion

The synthesis of the four α-D-glucopyranosides was best approached through the halide catalysis procedure, utilizing an α-D-glucopyranosyl iodide as the donor, preferably in the presence of triphenylphosphine oxide. Although this method was operationally more difficult than those employing the more established trichloroacetimidate and thioglycoside donors, the compensation was in the excellent diastereoselectivity obtained.

Lactose still remains as the most easily hydrolyzed disaccharide in the mammalian small intestine for the supply of both D-glucose and D-galactose. The only synthetic disaccharide to show any promise as an alternative was 6-*O*-α-D-glucopyranosyl-D-galactopyranose.

## 4. Experimental

### 4.1. Synthesis of the four disaccharides

**4.1.1. General methods.** General experimental procedures have been described previously.[31]

**4.1.2. Methyl 3-*O*-allyl-4,6-*O*-benzylidene-α-D-galactopyranoside (2).** Methyl 3-*O*-allyl-α-D-galactopyranoside[32] (3.08 g, 13.2 mmol) in DMF (30 mL) was treated with benzaldehyde diethyl acetal (3.55 g, 19.7 mmol) and CSA (100 mg) and the solution stirred (35 °C, 36 h). The solution was neutralized with $Et_3N$ and concentrated; flash chromatography (EtOAc/petrol, 1:1) gave *alcohol* **2** (2.88 g, 68%) as a colourless solid, mp 159–161 °C. $[\alpha]_D$ +216.0; $^1$H NMR (500 MHz): $\delta_H$ 3.47 (s, $CH_3$), 3.66 (d, $J = 0.6$, H4), 3.75 (dd, $J_{2,3} = 10.0$, $J_{1,2}$ 3.7, H2), 4.09 (dd, $J_{6,6} = 12.4$, $J_{5,6} = 1.7$, H6), 4.16–4.31 (m, 5H, $CH_2O$, H3, H5, H6), 4.96 (d,

**Table 4.** Summary of pig intestinal disaccharidase activity

| Intestinal Section | Maltase[a] (U/g) | Sucrase (U/g) | Lactase (U/g) | Disaccharidase acting on α-Glc*p*-(1→6)-Gal (U/g) | Isomaltase (U/g) |
|---|---|---|---|---|---|
| 1 | 48 | 3.4 | 16.6 | 0.70 | |
| 2 | 136 | 20.4 | 48.9 | 1.20 | |
| 3 | 142 | 13.6 | 39.7 | 1.06 | |
| 4 | 143 | 32.7 | 48.9 | 1.51 | 19.8 |
| 5 | 146 | 36.4 | 41.9 | 1.86 | 17.6 |
| 6 | 145 | 39.9 | 22.0 | 1.34 | 27.3 |
| 7 | 152 | 27.2 | 18.1 | 1.50 | 39.9 |
| 8 | 155 | 31.0 | 10.1 | 1.36 | 10.1 |
| 9 | 151 | 24.4 | 9.3 | 1.08 | 3.7 |
| 10 | 91 | 19.9 | 1.5 | 0.42 | |
| 11 | 142 | 19.8 | 1.0 | 1.18 | |
| 12 | 141 | 22.1 | 5.4 | 1.12 | |

[a] Maltase activity (on maltose) of a mucosal homogenate, expressed as μmol $L^{-1}$ $min^{-1}$ $g^{-1}$ protein.

H1), 5.21–5.23, 5.32–5.36 (2 × m, C$H_2$CH), 5.56 (s, PhC$H$), 5.93–6.03 (m, $CH_2$C$H$), 7.32–7.39, 7.52–7.54 (2 × m, Ph); $^{13}$C NMR (125.8 MHz): $\delta_C$ 55.62 ($CH_3$), 62.82 (C5), 67.88, 73.64, 76.01 (C2, C3, C4), 69.50, 70.51 ($CH_2O$, C6), 100.18, 101.00 (Ph$C$H, C1), 117.54 ($C$H$_2$CH), 126.26–137.76 (Ph), 134.97 (CH$_2$$C$H). Anal. Calcd for $C_{17}H_{22}O_6$: C, 63.3; H, 6.9. Found: C, 63.4; H, 6.7.

**4.1.3. General procedure (A) for glycosylations with iodide 1.** The acceptor alcohol (0.32 mmol) in dry $C_6H_6$ (2 mL) was treated with EtNPr$^i_2$ (130 mg, 0.96 mmol), $Bu_4NI$ (350 mg, 0.96 mmol) and 4 Å molecular sieves (300 mg) and the mixture stirred (rt, 2 h). Iodide **1**[19] (800 mg, 1.28 mmol) in $C_6H_6$ (3 mL) was added and the mixture refluxed (6 h). The mixture was filtered, the filtrate concentrated and subjected to flash chromatography (EtOAc/petrol, 1:2) to give the disaccharide.

**4.1.4. General procedure (B) for glycosylations with trichloroacetimidate 9.** The trichloroacetimidate **9**[33] (37 mg, 0.055 mmol) and the acceptor alcohol (0.046 mmol) in dry ether (2 mL) were stirred with 4 Å molecular sieves (50 mg) (rt, 3 h). The mixture was cooled (−40 °C), trimethylsilyl trifluoromethanesulfonate (20 μL) added and the mixture allowed to warm slowly (rt). The mixture was then treated with $Et_3N$ (100 μL) and filtered, the filtrate was concentrated and subjected to flash chromatography (EtOAc/petrol, 1:2) to give the disaccharide as a colourless oil. The results of the four separate glycosidations are presented in Table 1.

**4.1.5. General procedure for glycosylations with thioglycoside 14.** (a) *Diphenyl sulfoxide/trifluoromethanesulfonic anhydride promotion.* Thioglycoside **14**[34] (55 mg, 0.095 mmol), 2,4,6-tri-*tert*-butylpyrimidine[35] (65 mg, 0.265 mmol) and $Ph_2SO$ (54 mg, 0.265 mmol) in dry $CH_2Cl_2$ (2 mL) were treated with $Tf_2O$ (45 μL, 0.265 mmol) and the solution was stirred (−60 °C, 10 min). The acceptor alcohol (0.142 mmol) in dry $CH_2Cl_2$ (1 mL) was added and the solution warmed (0 °C). The solution was then treated with $Et_3N$ (100 μL) and filtered, the filtrate concentrated and subjected to flash chromatography (EtOAc/petrol, 1:2) to give the disaccharide as a colourless oil. The results of the four separate glycosidations are presented in Table 2.

(b) *1-Benzenesulfinylpiperidine/trifluoromethanesulfonic anhydride promotion.* Thioglycoside **14** (84.7 mg, 0.146 mmol), 1-benzenesulfinylpiperidine (30.5 mg, 0.146 mmol) and 2,4,6-tri-*tert*-butylpyrimidine (65 mg, 0.265 mmol) in dry $CH_2Cl_2$ (2 mL) were treated with $Tf_2O$ (45 μL, 0.265 mmol) and the solution was stirred (−60 °C, 10 min). The acceptor alcohol (0.219 mmol) in dry $CH_2Cl_2$ (1 mL) was added and the solution warmed (0 °C). The solution was then treated with $Et_3N$ (100 μL) and filtered, the filtrate concentrated and subjected to flash chromatography (EtOAc/petrol, 1:2) to give the disaccharide as a colourless oil. The results of the four separate glycosidations are presented in Table 2.

**4.1.6. General procedure for glycosylations with the iodide 15/triphenylphosphine oxide.** 1-*O*-Acetyl-2,3,4,6-tetra-*O*-benzyl-D-glucopyranose[36] (290 mg, 0.51 mmol) in dry $CH_2Cl_2$ (5 mL) was stirred with 4 Å molecular sieves (300 mg) (0 °C, 1 h). Iodotrimethylsilane (122 mg, 0.608 mmol) was then added and the mixture stirred (30 min) before being concentrated and the excess reagent removed by azeotropic distillation with PhMe (4 × 10 mL). The residue, presumably iodide **15**, in dry $CHCl_3$ (2 mL) was treated with $Ph_3PO$ (280 mg, 1.0 mmol) and the acceptor alcohol (0.22 mmol) and the mixture stirred (rt, 14 h). The mixture was filtered, the filtrate concentrated and subjected to flash chromatography (EtOAc/petrol, 1:2) to give the disaccharide. The results of the four separate glycosidations are presented in Table 3.

**4.1.7. Methyl 2-*O*-(6-*O*-acetyl-2,3,4-tri-*O*-benzyl-α-D-glucopyranosyl)-3-*O*-allyl-4,6-*O*-benzylidene-α-D-galactopyranoside (6).** Using general procedure (A), acceptor alcohol **2** (100 mg) gave disaccharide **6** (172 mg, 70%) as a colourless solid and as predominantly the α-anomer (α:β, 97:3). Mp 134–136 °C; $[\alpha]_D$ +111.5; $^1$H NMR (60 MHz): $\delta_H$ 2.00 (s, $CH_3CO$), 3.43–3.45 (m, H5), 3.45 (s, $CH_3O$), 3.54 (dd, $J_{2,3} = 9.6$, $J_{1,2} = 3.6$, H2), 3.65 (s, H4), 3.92 (dd, $J_{5',6'} = 10.2$, 3.5, H5′), 4.06–4.09 (m, 2H, H3, H6), 4.16–4.19 (m, 3H, $CH_2O$, H6), 4.21–4.31 (m, 5H, H2′, H3′, H4′, H6′), 4.56, 4.85 (AB, $J = 11.2$, PhC$H_2$), 4.69, 4.78 (AB, $J = 12.0$, PhC$H_2$), 4.80, 5.00 (AB, $J = 10.8$, PhC$H_2$), 4.90 (d, H1), 4.96 (d, $J_{1',2'} = 3.4$, H1′), 5.11–5.15, 5.27–5.35 (2 × m, C$H_2$CH), 5.54 (s, PhC$H$), 5.91–5.94 (m, $CH_2$C$H$), 7.23–7.38, 7.49–7.51 (2 × m, Ph); $^{13}$C NMR (150.9 MHz): $\delta_C$ 20.55 ($C$H$_3$CO), 55.17 ($CH_3O$), 62.43 (C4), 62.94 ($CH_2O$), 68.53 (C2′), 69.26 (C6), 71.02 (C6′), 71.33, 74.02, 74.26 (C3′, C4′, C5′), 72.87, 74.38, 75.52 (3C, Ph$C$H$_2$), 77.40 (C5), 79.40 (C2), 81.81 (C3), 94.50 (C1), 97.60 (C1′), 101.02 (Ph$C$H), 117.14 ($C$H$_2$CH), 126.22–138.62 (Ph), 134.98 (CH$_2$$C$H), 170.82 (C=O); FAB-MS $m/z$ 795.3374 ($C_{46}H_{51}O_{12}$; $[M-H]^+$ requires 795.3380). A significant quantity (300 mg) of alkene **8** was also isolated, with $^1$H (300 MHz) and $^{13}$C (75.5 MHz) NMR spectral data consistent with those reported.[19]

**4.1.8. 6-*O*-(6-*O*-Acetyl-2,3,4-tri-*O*-benzyl-α-D-glucopyranosyl)-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (7).** Using general procedure (A), acceptor alcohol **5** (112 mg) gave disaccharide **7** (280 mg, 90%) as a colour-

less oil and as predominantly the α-anomer (α:β, 97:3). $[\alpha]_D$ +16.1; $^1$H NMR (600 MHz): $\delta_H$ 1.32, 1.33, 1.45, 1.55 (4 × s, 12H, $CH_3C$), 2.03 (s, $CH_3CO$), 3.51 (dd, $J_{4',5'} \approx J_{3',4'} = 9.8$, H4′), 3.56 (dd, $J_{2',3'} = 9.6$, $J_{1',2'} = 3.6$, H2′), 3.74 (dd, 1H, $J_{6,6} = 10.3$, $J_{5,6} = 7.3$, H6), 3.79 (dd, 1H, $J_{5,6} = 6.3$, H6), 3.93–3.96 (m, H5′), 4.01–4.06 (m, H3′, H5), 4.24 (dd, 1H, $J_{6',6'} = 11.9$, $J_{5',6'} = 1.9$, H6′), 4.31–4.37 (m, H2, H3, H4), 4.57, 4.88 (AB, $J = 10.8$, PhC$H_2$), 4.62 (dd, 1H, $J_{5',6'} = 1.3$, H6′), 4.71, 4.76 (AB, $J = 11.9$, PhC$H_2$), 4.82, 5.02 (AB, $J = 10.8$, PhC$H_2$), 4.96 (d, H1′), 5.53 (d, $J_{1,2} = 5.0$, H1), 7.25–7.40 (m, Ph); $^{13}$C NMR (150.9 MHz): $\delta_C$ 21.00 ($CH_3$CO), 24.75, 25.04, 26.18, 26.25 (4C, $CH_3$C), 63.20, 66.76 (C6, C6′), 66.00, 68.76 (C5, C5′), 70.70, 70.77, 70.99 (C2, C3, C4), 72.52, 74.99, 75.80 (3C, PhCH$_2$), 77.30 (C4′), 79.92 (C2′), 81.97 (C3′), 96.42, 97.12 (C1, C1′), 108.75, 109.37 (2C, $CH_3C$), 127.76-138.82 (Ph), 170.91 (C=O); FAB-MS *m*/*z* 734.3325 ($C_{41}H_{50}O_{12}$; $[M]^{+\cdot}$ requires 734.3302). A significant quantity (500 mg) of alkene **8** was again isolated.

**4.1.9. Methyl 3-*O*-allyl-4,6-*O*-benzylidene-2-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl)-α-D-galactopyranoside (10).** Using general procedure (B), acceptor alcohol **2** (65 mg) gave α-linked disaccharide **10** (105 mg, 63%) as a colourless oil, $[\alpha]_D$ +91.7. $^1$H NMR (500 MHz): $\delta_H$ 3.46 (s, $CH_3O$), 3.56–3.60 (m, 2H, H2′, H6′), 3.65–3.72 (m, 3H, H4′, H5, H6′), 3.94 (dd, $J_{2,3} = 10.3$, $J_{3,4} = 3.4$, H3), 4.04 (dd, $J_{3',4'} \approx J_{2',3'} = 9.4$, H3′), 4.08 (dd, $J_{6,6} = 12.6$, $J_{5,6} = 1.3$, H6), 4.15–4.24 (m, $CH_2O$, H5′), 4.25–4.31 (m, 2H, H4, H6), 4.32 (dd, $J_{1,2} = 3.4$, H2), 4.39, 4.57 (AB, $J = 12.1$, PhC$H_2$), 4.49, 4.79 (AB, $J = 11.3$, PhC$H_2$), 4.69, 4.81 (AB, $J = 12.0$, PhC$H_2$), 4.82, 4.96 (AB, $J = 10.8$, PhC$H_2$), 4.96 (d, $J_{1',2'} = 2.9$, H1′), 5.01 (d, H1), 5.07–5.13 (m, C$H_2$CH), 5.55 (s, PhC*H*), 5.88–5.97 (m, $CH_2$C*H*), 7.10–7.50 (m, Ph); $^{13}$C NMR (125.8 MHz): $\delta_C$ 55.41 ($CH_3O$), 62.73 (C5), 68.32, 69.47 (C6, C6′), 70.01, 71.18, 74.37, 74.83, 77.82, 79.57 (C2, C2′, C3, C4, C4′, C5′), 71.79 ($CH_2O$), 73.18, 73.35, 74.64, 75.72 (4C, PhC$H_2$), 82.17 (C3′), 94.77 (C1′), 97.84 (C1), 101.19 (PhCH), 117.43 (C$H_2$CH), 126.52–138.55 (Ph), 135.17 ($CH_2$CH); FAB-MS *m*/*z* 844.3731 ($C_{51}H_{56}O_{11}$ $[M]^{+\cdot}$ requires 844.3823).

Also obtained was the β-linked disaccharide (15 mg, 7%) as a colourless oil, $[\alpha]_D$ +98.2; $^1$H NMR (500 MHz): $\delta_H$ 3.44 (s, $CH_3O$), 3.55–3.75 (m, 7H, H2′, H3′, H4′, H5, H5′, H6′), 3.98 (dd, $J_{3,4} = 3.5$, $J_{2,3} = 10.3$, H3), 4.08–4.15 (m, 3H, $CH_2O$, H6), 4.25 (dd, $J_{1,2} = 3.5$, H2), 4.31 (dd, $J_{6,6} = 12.4$, $J_{5,6} = 1.4$, H6), 4.34 (dd, $J_{4,5} = 0.3$, H4), 4.52, 4.60 (AB, $J = 12.0$, PhC$H_2$), 4.56, 4.82 (AB, $J = 10.8$, PhC$H_2$), 4.66 (d, $J_{1',2'} = 7.7$, H1′), 4.74, 5.15 (AB, $J = 11.3$, PhC$H_2$), 4.77, 4.91 (AB, $J = 11.0$, PhC$H_2$), 5.04–5.09, 5.19–5.23 (2 × m, C$H_2$CH), 5.17 (d, H1), 5.58 (s, PhC*H*), 5.81–5.89 (m, $CH_2$C*H*), 7.15–7.58 (m, Ph); $^{13}$C NMR (125.8 MHz): $\delta_C$ 55.60 ($CH_3O$), 62.26 (C5), 69.01, 69.55 (C6, C6′), 70.59 ($CH_2O$), 73.41, 74.29, 74.92, 75.40 (4C, PhC$H_2$), 74.44 (C5′), 75.60, 76.58 (C2, C3, C4), 77.62, 82.02, 84.65 (C2′, C3′, C4′), 100.42 (C1′), 101.08 (PhC*H*), 104.95 (C1), 117.09 (C$H_2$CH), 126.32–138.90 (Ph), 135.23 ($CH_2$C*H*); FAB-MS *m*/*z* 844.3812.

**4.1.10. Methyl 2,4,6-tri-*O*-benzyl-3-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl)-α-D-galactopyranoside (11).** Using general procedure (B), acceptor alcohol **3**[32] (110 mg) gave an inseparable mixture of α-linked **11** and β-linked disaccharides (75:25) (233 mg, 72%) as a colourless oil.

**4.1.11. Methyl 2,3,6-tri-*O*-benzyl-4-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl)-α-D-galactopyranoside (12).** Using general procedure (B), acceptor alcohol **4**[37] (105 mg) gave an inseparable mixture of α-linked **12** and β-linked disaccharides (65:35) (223 mg, 72%) as a colourless oil.

**4.1.12. 1,2:3,4-Di-*O*-isopropylidene-6-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl)-α-D-galactopyranose (13).** Using general procedure (B), acceptor alcohol **5** (70 mg) gave an inseparable mixture of α-linked **13** and β-linked disaccharides (80:20) (210 mg, 71%) as a colourless oil. Partial $^1$H NMR (600 MHz); $\delta_H$ 1.32, 1.33, 1.34, 1.46, 1.54 (5 × s, $CH_3$), 3.60 (dd, $J_{2',3'} = 9.6$, $J_{1',2'} = 3.6$, H2′α), 4.00 (dd, $J_{3',4'} = 9.6$, H3′α), 4.44 (d, $J_{1',2'} = 7.5$, H1′β), 5.01 (d, H1′α), 5.54 (d, $J_{1,2} = 5.0$, H1α), 5.58 (d, $J_{1,2} = 5.0$, H1β); Partial $^{13}$C NMR (150.9 MHz): $\delta_C$ 24.35, 24.54, 24.82, 24.93, 25.90, 25.94, 25.96, 26.06 (4C, $CH_3$), 65.63 (C5α), 66.11, 68.28, 68.64, 69.60 (C6α, C6′α, C6β, C6′β), 79.70 (C2′α), 81.53 (C2′β), 81.84 (C3′α), 84.43 (C3′β), 96.20 (C1α), 96.28 (C1β), 96.88 (C1′α), 104.26 (C1′β), 108.52, 109.15, 109.30 ($CH_3C$).

**4.1.13. 2-*O*-(α-D-Glucopyranosyl)-D-galactopyranose.** (i) Disaccharide **6** (246 mg) was stirred in AcOH/$H_2O$ (4:1, 3 mL) (1 h, 50 °C). The solution was then concentrated and subjected to flash chromatography (EtOAc/petrol, 1:1) to give methyl 2-*O*-(6-*O*-acetyl-2,3,4-tri-*O*-benzyl-α-D-glucopyranosyl)-3-*O*-allyl-α-D-galactopyranoside (168 mg, 78%) as a colourless oil. $[\alpha]_D$ +80.4; $^1$H NMR (600 MHz): $\delta_H$ 2.00 (s, $CH_3CO$), 3.43 (s, $CH_3O$), 3.49 (dd, $J = 9.5$, 9.3, H3′), 3.55 (dd, $J_{2,3} = 9.6$, $J_{1,2} = 3.5$, H2), 3.80–3.85 (m, 3H, H4, H5, H6), 3.95 (dd, $J_{6,6} = 10.9$, $J_{5,6} = 5.2$, H6), 4.05–4.08 (m, H2′, H3), 4.12–4.25 (m, 6H, $CH_2O$, H4′, H5′, H6′), 4.58, 4.80 (AB, $J = 11.1$, PhC$H_2$), 4.68, 4.78 (AB, $J = 12.6$, PhC$H_2$), 4.86, 5.01 (AB, $J = 10.6$, PhC$H_2$), 4.87 (d, H1), 4.89 (d, $J_{1',2'} = 3.4$, H1′), 5.13–5.17, 5.57–5.31 (2 × m, C$H_2$CH), 5.89–5.96 (m, $CH_2$C*H*), 7.26–7.37 (m, Ph); $^{13}$C NMR (150.9 MHz): $\delta_C$ 20.95 ($CH_3$CO), 55.20 ($CH_3O$), 63.07, 63.17 (C6, C6′), 68.61, 68.63

(C4′, C5′), 69.10, 75.76 (C4, C5), 71.15 (C2′), 71.76 ($CH_2O$), 73.13, 74.73, 75.79 (3C, Ph$CH_2$), 77.38 (C3′), 79.46 (C2), 82.09 (C3), 94.54 (C1), 96.95 (C1′), 118.34 ($CH_2CH$), 127.77–138.55 (Ph), 134.22 ($CH_2CH$), 170.88 (C=O); FAB-MS *m/z* 708.3177 ($C_{39}H_{48}O_{12}$ $[M]^{+\cdot}$ requires 708.3146).

(ii) The diol from (i) (170 mg) in EtOH (20 mL) was treated with Wilkinson's catalyst (27 mg) and the mixture refluxed (12 h); the mixture was then treated with hydrochloric acid (3 M, 1 mL) and refluxed (1 h). The mixture was neutralized with $Et_3N$ (1 mL) and concentrated to give a pale coloured oil that was dissolved in $CH_2Cl_2$ (3 mL) and treated with pyridine (8 mL), $Ac_2O$ (3 mL) and DMAP (10 mg) and the solution stirred (rt, 10 h). The solution was then treated with MeOH (4 mL) (1 h, rt); concentration of the mixture followed by flash chromatography (EtOAc/petrol, 1:1) gave methyl 3,4,6-tri-*O*-acetyl-2-*O*-(6-*O*-acetyl-2,3,4-tri-*O*-benzyl-α-D-glucopyranosyl)-α-D-galactopyranoside (160 mg, 84%) as a colourless oil. $[\alpha]_D$ +81.3; $^1$H NMR (600 MHz): $\delta_H$ 2.01, 2.05, 2.06, 2.11 (4 × s, 12H, $CH_3CO$), 3.42 (s, $CH_3O$), 3.48 (dd, $J = 9.8$, 9.3, H4′), 3.52 (dd, $J_{2',3'} = 9.6$, $J_{1',2'} = 3.5$, H2′), 3.95–4.00 (m, H3′, H5), 4.07 (dd, $J_{2,3} = 10.7$, $J_{1,2} = 3.5$, H2), 4.09–4.10 (m, 2H, H6), 4.17–4.20 (m, 2H, H5′, H6′), 4.24 (dd, $J_{6',6'} = 12.0$, $J_{5',6'} = 3.8$, H6′), 4.56, 4.86 (AB, $J = 11.1$, PhC$H_2$), 4.66, 4.78 (AB, $J = 11.9$, PhC$H_2$), 4.80, 4.98 (AB, $J = 10.7$, PhC$H_2$), 4.84 (d, H1′), 4.91 (d, H1), 5.36 (dd, $J_{3,4} = 2.7$, H3), 5.46 (d, H4), 7.22–7.36 (m, Ph); $^{13}$C NMR (150.9 MHz): $\delta_C$ 20.78, 20.86, 20.96 ($CH_3CO$), 55.56 ($CH_3O$), 62.03, 62.89 (C6, C6′), 66.37 (C5′), 68.50 (C4), 68.80 (C3), 69.19 (C5), 70.69 (C2), 73.32, 74.94, 75.80 (3C, Ph$CH_2$), 77.07 (C4′), 79.60 (C2′), 81.78 (C3′), 95.50 (C1′), 97.32 (C1), 127.85–138.67 (Ph), 169.87, 170.21, 170.61, 170.79 (4C, C=O). Anal. Calcd for $C_{42}H_{50}O_{15}$: C, 63.5; H, 6.3. Found: C, 63.3; H, 6.6.

(iii) The tetra-acetate from (ii) (140 mg) in MeOH (10 mL) was stirred with Pd/C (10%, 10 mg) and $H_2$ (1 atm, 12 h, 35 °C). The mixture was filtered, the filtrate concentrated and treated with pyridine (3 mL), $Ac_2O$ (1 mL) and DMAP (10 mg) (rt, 5 h). Treatment with MeOH (5 mL) (rt, 1 h), followed by concentration of the mixture and flash chromatography (EtOAc/petrol, 1:1), gave methyl 3,4,6-tri-*O*-acetyl-2-*O*-(tetra-*O*-acetyl-α-D-glucopyranosyl)-α-D-galactopyranoside (103 mg, 90%) as a colourless oil. $[\alpha]_D$ +108; $^1$H NMR (600 MHz): $\delta_H$ 1.98, 1.99, 1.99, 2.02, 2.06, 2.11 (6 × s, 21H, $CH_3CO$), 3.38 (s, $CH_3O$), 4.01 (dd, $J_{2,3} = 10.6$, $J_{1,2} = 3.6$, H2), 4.04–4.11 (m, 4H, H5′, H6, H6′), 4.13 (dd, $J_{5,6} = 6.8$, 6.4, H5), 4.22 (dd, $J_{6',6'} = 12.3$, $J_{5',6'} = 3.9$, H6′), 4.71 (dd, $J_{2',3'} = 10.1$, $J_{1',2'} = 3.7$, H2′), 4.83 (d, H1), 5.02 (dd, $J = 9.9$, 9.8, H4′), 5.26 (dd, $J_{3,4} = 3.5$, H3), 5.27 (d, H1′), 5.40 (dd, H3′), 5.42 (d, H4); $^{13}$C NMR (150.9 MHz): $\delta_C$ 20.68, 20.72, 20.77 ($CH_3CO$), 55.49 ($CH_3O$), 61.67, 61.84 (C6, C6′), 66.26 (C5), 67.65 (C5′), 68.28, 68.37 (C4, C4′), 68.86 (C3), 70.02 (C3′), 71.34, 71.60 (C2, C2′), 94.02 (C1), 97.15 (C1′), 169.67–170.65 (7C, C=O); FAB-MS *m/z* 651.2145 ($C_{27}H_{39}O_{18}$ $[M+H]^+$ requires 651.2136).

(iv) The hepta-acetate from (iii) (103 mg) was stirred with $Ac_2O$ (3 mL) and $H_2SO_4$ (50 μL) (6 h, 0 °C). The solution was then poured onto ice and kept (rt, 12 h). The mixture was extracted with EtOAc, the extracts were washed with water, saturated $NaHCO_3$ and dried. Concentration of the organic extract gave 1,3,4,6-tetra-*O*-acetyl-2-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl)-D-galactopyranose (102 mg) as a colourless oil and as a mixture of anomers (α:β, 4:1); $^1$H NMR (600 MHz): $\delta_H$ 1.97–2.19 ($CH_3CO$), 4.02–4.20 (m, H2β, H5′, H5β, H6, H6′), 4.19 (dd, $J_{2,3} = 10.6$, $J_{1,2} = 3.6$, H2α), 4.24 (dd, $J_{6,6} = 12.2$, $J_{5,6} = 3.5$, H6α), 4.25–4.28 (m, H6β), 4.32 (dd, $J_{5,6} = 7.3$, H5α), 4.75 (dd, $J_{2,3} = 10.3$, $J_{1,2} = 3.9$, H2′β), 4.92 (dd, $J_{2',3'} = 10.4$, $J_{1',2'} = 3.5$, H2′α), 5.03–5.05 (m, H3β, H4′β), 5.07–5.09 (m, H4′α), 5.10 (d, H1′α), 5.31 (dd, $J_{2,3} = 10.6$, $J_{3,4} = 2.5$, H3α), 5.33–5.35 (m, H3′β), 5.35 (dd, $J_{3',4'} = 10.3$, H3′α), 5.40–5.45 (m, H1′β, H4β), 5.52 (d, $J_{3,4} = 2.5$, H4α), 5.65 (d, $J_{1,2} = 8.1$, H1β), 6.29 (d, H1α); $^{13}$C NMR (150.9 MHz): $\delta_C$ 20.65–21.10 ($CH_3$), 61.11, 61.27, 61.61 (C6, C6′), 66.99–72.12 (C2, C2′, C3, C3′, C4, C4′, C5, C5′), 89.21 (C1α), 93.75 (C1β), 95.17 (C1′β), 95.72 (C1′α), 169.08–170.73 (C=O); FAB-MS *m/z* 619.1857 ($C_{26}H_{35}O_{17}$ $[M-OAc]^+$ requires 619.1874).

(v) A small piece of Na in MeOH (1 mL) was added to the octa-acetate from (iv) (90 mg) in MeOH (3 mL) and the solution stirred (rt, 4 h). The solution was neutralized with resin (Amberlite IR-120, $H^+$), filtered and the filtrate concentrated to give 2-*O*-(α-D-glucopyranosyl)-D-galactopyranose (41 mg) as a colourless oil and as a mixture of anomers (α:β, 1:1.4). $[\alpha]_D$ +129.0 ($H_2O$); $^1$H NMR (600 MHz, $D_2O$): $\delta_H$ 3.41–3.45 (m, H5), 3.52 (m, H2, H2′β), 3.67–3.92, 4.01–4.04, 4.07–4.11 (3 × m, H2′α, H3, H3′, H4, H4′, H5′β, H6, H6′), 3.96 (dd, $J = 10.2$, 3.3, H5′α), 4.69–4.71 (m, H1β), 5.09 (d, $J_{1,2} = 3.7$, H1α), 5.37 (d, $J_{1',2'} = 3.8$, H1′β), 5.46 (d, $J_{1',2'} = 3.5$, H1′α); $^{13}$C NMR (150.9 MHz, $D_2O$): $\delta_C$ 63.12, 63.66 (C6β, C6′β), 63.15, 63.82 (C6α, C6′α), 70.34–79.71 (C2, C2′, C3, C3′, C4, C4′, C5, C5′), 92.35 (C1′α), 98.91 (C1α), 99.33 (C1β), 100.64 (C1′β); FAB-MS *m/z* 343.1253 ($C_{12}H_{23}O_{11}$ $[M+H]^+$ requires 343.1240).

**4.1.14. 3-*O*-(α-D-Glucopyranosyl)-D-galactopyranose.** (i) Disaccharide **11** (156 mg), containing the β-anomer, in MeOH (20 mL) was stirred with Pd/C (10%, 15 mg) and $H_2$ (1 atm, 12 h). The mixture was filtered, the filtrate concentrated and treated with pyridine (2 mL), $Ac_2O$ (1 mL) and DMAP (5 mg) and stirred (rt, 8 h). Treatment with MeOH (2 mL), followed by concentration of the mixture and flash chromatography (EtOAc/

petrol, 1:2), gave first methyl 2,4,6-tri-*O*-acetyl-3-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl)-α-D-galactopyranoside (65 mg, 64%) as a colourless oil. $[\alpha]_D$ +154.2; $^1$H NMR (600 MHz): $\delta_H$ 1.98, 2.01, 2.05, 2.05, 2.12, 2.14, 2.14 (7 × s, 21H, $CH_3CO$), 3.36 (s, $CH_3O$), 4.06–4.10 (m, 4H, H5, H6, H6′), 4.27–4.29 (m, H5′), 4.30 (dd, $J_{3,4} = 3.0$, $J_{2,3} = 10.5$, H3), 4.34 (dd, $J_{6',6'} = 12.2$, $J_{5',6'} = 1.5$, H6′), 5.00–5.04 (m, H2′, H4′), 5.05 (d, $J_{1,2} = 3.5$, H1), 5.08 (dd, H2), 5.19 (d, $J_{1',2'} = 3.4$, H1′), 5.36 (dd, $J_{3',4'} \approx J_{2',3'} = 9.8$, H3′), 5.40 (d, $J = 2.8$, H4); $^{13}$C NMR (150.9 MHz): $\delta_C$ 20.44, 20.49, 20.53, 20.58, 20.61 ($CH_3CO$), 55.25 ($CH_3O$), 61.68, 61.81 (C6, C6′), 65.68 (C4), 66.19 (C5), 67.74, 68.07 (C3, C5′), 68.66, 69.18 (C2′, C4′), 69.31 (C2), 69.69 (C3′), 91.88 (C1′), 96.98 (C1), 169.39, 169.76, 169.91, 170.06, 170.32 (C=O); FAB-MS *m*/*z* 651.2179 ($C_{27}H_{39}O_{18}$ $[M+H]^+$ requires 651.2136).

Further elution gave methyl 2,4,6-tri-*O*-acetyl-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-α-D-galactopyranoside (25 mg, 24%) as a colourless oil, $[\alpha]_D$ +69.0 (lit.[38] +75.0). The $^1$H (600 MHz) and $^{13}$C (150.9 MHz) NMR spectral data were in good agreement with those reported.[39]

(ii) The α-hepta-acetate (46 mg) was stirred with $Ac_2O$ (2 mL) and $H_2SO_4$ (100 μL) (0 °C, 6 h). The solution was then poured onto ice and stirred (12 h), the mixture extracted with EtOAc, the extract washed with saturated $NaHCO_3$ and brine and then dried. Concentration of the organic extract followed by flash chromatography (EtOAc/petrol, 1:3) gave 1,2,4,6-tetra-*O*-acetyl-3-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl)-α-D-galactopyranose (45 mg, 94%) as a colourless oil, containing some 5% of the β-D-galactose anomer. (α-D-anomer) $^1$H NMR (600 MHz): $\delta_H$ 1.98, 2.00, 2.04, 2.06, 2.07, 2.11, 2.14, 2.27 (8 × s, 24H, $CH_3CO$), 4.05–4.25 (m, 6H, H5, H5′, H6, H6′), 4.27 (dd, $J_{3,4} = 3.0$, $J_{2,3} = 10.8$, H3), 5.02 (dd, $J_{2',3'} = 10.0$, $J_{1',2'} = 3.3$, H2′), 5.09 (dd, $J_{4',5'} \approx J_{3',4'} = 9.4$, H4′), 5.22 (d, H1′), 5.30 (dd, $J_{1,2} = 3.6$, H2), 5.35 (dd, H3′), 5.45 (d, $J = 2.1$, H4), 6.42 (d, H1); $^{13}$C NMR (150.9 MHz): $\delta_C$ 20.56, 20.71, 20.76, 20.79, 20.83, 20.89, 21.01, 21.05 (8C, $CH_3$), 61.19, 61.58 (C6, C6′), 65.28 (C4), 67.51, 68.52, 68.75, 68.83, 69.17, 69.52 (C2, C2′, C3, C4′, C5, C5′), 67.69 (C3′), 89.64 (C1), 92.57 (C1′), 168.94, 169.51, 169.54, 170.01, 170.13, 170.15, 170.52, 170.71 (8C, C=O); FAB-MS *m*/*z* 619.1835 ($C_{26}H_{35}O_{17}$ $[M-OAc]^+$ requires 619.1874).

(iii) The (mainly) α-D-anomer from (ii) (39 mg) in MeOH (3 mL) was treated with NaOMe in MeOH (1 mL) and the solution stirred (rt, 4 h). The solution was neutralized with resin (Amberlite IR-120, $H^+$), filtered and the filtrate concentrated to give 3-*O*-(α-D-glucopyranosyl)-D-galactopyranose (20 mg) as a colourless oil and as a mixture of anomers (α:β, 1:1.4). $[\alpha]_D$ +146.2 ($H_2O$); $^1$H NMR (600 MHz, $D_2O$): $\delta_H$ 3.42–3.46, 3.56–3.62, 3.67–3.86, 3.92–3.96 (4 × m, H2α, H2′, H3, H3′, H4′, H5, H5′, H6, H6′), 4.07 (dd, $J_{1,2} = 7.0$, $J_{2,3} = 6.1$, H2β), 4.15 (d, $J = 2.5$ Hz, H4β), 4.21 (s, H4α), 4.62 (d, H1β), 5.10 (d, $J_{1',2'} = 3.5$, H1′β), 5.12 (d, $J_{1',2'} = 3.6$, H1′α), 5.28 (d, $J_{1,2} = 1.4$, H1α); $^{13}$C NMR (150.9 MHz, $D_2O$): $\delta_C$ 63.03, 63.08 (C6β, C6′β), 63.14, 63.87 (C6α, C6′α), 67.74 (C4β), 68.31 (C4α), 69.42–76.82 (C2, C2′, C3α, C3′, C4′, C5α, C5′), 77.57 (C5β), 80.20 (C3β), 95.04 (C1α), 97.69 (C1′α), 97.98 (C1′β), 99.10 (C1β); FAB-MS *m*/*z* 343.1230 ($C_{12}H_{23}O_{11}$ $[M+H]^+$ requires 343.1240).

**4.1.15. 4-*O*-(α-D-Glucopyranosyl)-D-galactopyranose.** (i) Disaccharide **12** (150 mg), containing the β-anomer, in MeOH (20 mL) was stirred with Pd/C (10%, 15 mg) and $H_2$ (12 h, 1 atm). The mixture was filtered, the filtrate concentrated and treated with pyridine (2 mL), $Ac_2O$ (1 mL) and DMAP (5 mg) and the solution stirred (rt, 8 h). Treatment with MeOH (2 mL), followed by concentration of the mixture and flash chromatography (EtOAc/petrol, 1:2), gave methyl 2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl)-α-D-galactopyranoside (57 mg, 55%) as a colourless oil, $[\alpha]_D$ +32.2 (lit.[36] +36.0). The $^1$H (600 MHz) and $^{13}$C (150.9 MHz) NMR spectral data were in good agreement with those reported.[36]

Further elution gave methyl 2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-α-D-galactopyranoside (20 mg, 20%) as a colourless oil, $[\alpha]_D$ +8.5 (lit.[39] +7.0). The $^1$H (600 MHz) and $^{13}$C (150.9 MHz) NMR spectral data were in good agreement with those reported.[39]

(ii) The α-hepta-acetate from (i) (40 mg) was stirred with $Ac_2O$ (2 mL) and $H_2SO_4$ (100 μL) (rt, 6 h). The solution was poured onto ice and stirred (6 h); the mixture was then extracted with EtOAc, the extract washed with saturated $NaHCO_3$, brine and then dried. Concentration of the organic extract gave 1,2,3,6-tetra-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl)-D-galactopyranose (40 mg) as a colourless oil and as a mixture of anomers (α:β, 5:1). $^1$H NMR (600 MHz): $\delta_H$ 1.98–2.15 (24H, $CH_3$), 3.93 (dd, $J_{5,6} = 6.6$, 6.4, H5β), 4.09–4.29 (m, H4, H5α, H5′, H6, H6′), 4.36 (dd, $J_{6,6} = 11.1$, $J_{5,6} = 6.7$, H6α), 4.41 (dd, $J_{6,6} = 11.4$, H6β), 4.84 (dd, $J_{3,4} = 2.6$, $J_{2,3} = 10.7$, H3β), 4.93–4.96 (m, H1′, H3α), 5.14–5.17 (m, H2′, H4′), 5.30 (dd, $J_{1,2} = 8.1$, H2β), 5.44 (dd, $J_{2,3} = 11.2$, $J_{1,2} = 3.7$, H2α), 5.47 (dd, $J_{3',4'} \approx J_{2',3'} = 9.6$, H3′α), 5.48 (dd, $J_{3',4'} \approx J_{2',3'} = 9.7$, H3′β), 5.71 (d, H1β), 6.36 (d, H1α); $^{13}$C NMR (150.9 MHz): $\delta_C$ 20.65–21.19 ($CH_3$), 61.17, 62.04 (C6β, C6′β), 61.30, 61.96 (C6α, C6′α), 66.07 (C2α), 67.74 (C2β), 68.12, 68.74, 69.64, 70.09, 70.48, 71.12 (C2′α, C3α, C3′α, C4′α, C5α, C5′α), 68.18, 68.47, 70.26, 71.18, 72.83, 73.07 (C2′β, C3β, C3′β, C4′β, C5β, C5′β), 77.94 (C4β), 78.43 (C4α), 89.99 (C1′α), 92.05 (C1′β), 99.43 (C1α), 99.54 (C1β),

168.98–170.81 (C=O); FAB-MS *m/z* 619.1847 ($C_{26}H_{35}O_{17}$ $[M-OAc]^+$ requires 619.1874).

(iii) The octa-acetate from (ii) (37 mg) in MeOH (4 mL) was stirred with NaOMe in MeOH (1 mL) (rt, 1 h). The solution was neutralized with resin (Amberlite IR-120, $H^+$), filtered and the filtrate concentrated to give 4-*O*-(α-D-glucopyranosyl)-D-galactopyranose (18 mg) as a colourless oil and as a mixture of anomers (α:β, 0.4:1). $[\alpha]_D$ +116.5 ($H_2O$); $^1H$ NMR (600 MHz, $D_2O$): $\delta_H$ 3.45 (dd, $J = 9.7$, 9.7, H3′β), 3.50–3.55, 3.70–3.93, 4.09–4.15 (3 × m, H2, H2′, H3, H3′α, H4′, H5, H5′, H6, H6′), 4.00 (d, $J = 2.9$, H4β), 4.07 (d, $J = 2.7$, H4α), 4.64 (d, $J_{1,2} = 7.8$, H1β), 4.92–4.93 (m, H1′), 5.29 (d, $J_{1,2} = 3.8$, H1α); $^{13}C$ NMR (150.9 MHz, $D_2O$): $\delta_C$ 62.79, 62.82, 62.87, 63.08 (C6, C6′), 71.17–77.81 (C2, C2′, C3, C3′, C4′α, C5, C5′), 80.09 (C4β), 81.39 (C4α), 95.08 (C1α), 99.35 (C1β), 102.70 (C1′β), 102.90 (C1′α); FAB-MS *m/z* 342.1235 ($C_{12}H_{23}O_{11}$ $[M+H]^+$ requires 343.1240).

**4.1.16. 6-*O*-(α-D-Glucopyranosyl)-D-galactopyranose.** (i) Disaccharide **7** (139 mg) in MeOH (25 mL) was stirred with Pd/C (10%, 20 mg) and $H_2$ (2 d, 1 atm). The mixture was filtered, the filtrate concentrated and subjected to flash chromatography (EtOAc/petrol, 2:1) to give 6-*O*-(α-D-glucopyranosyl)-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (60 mg, 89%) as a colourless oil. $^1H$ NMR (600 MHz): $\delta_H$ 1.30, 1.32, 1.41, 1.52 (4 × s, 12H, $CH_3$), 3.53–3.58, 3.62–3.63, 3.73–3.76 (3 × m, 5H, H2′, H3′, H4′, H5, H6), 3.69 (dd, $J_{6',6'} = 10.8$, $J_{5',6'} = 5.2$, H6′), 3.80 (dd, $J_{5',6'} = 7.2$, H6′), 3.84–3.86 (m, 1H, H6), 3.96 (m, H5′), 4.25 (dd, $J_{4,5} = 1.5$, $J_{3,4} = 8.0$, H4), 4.29 (dd, $J_{2,3} = 2.4$, $J_{1,2} = 4.9$, H2), 4.59 (dd, H3), 4.86 (d, $J_{1',2'} = 3.5$, H1′), 5.51 (d, H1); $^{13}C$ NMR (150.9 MHz): $\delta_C$ 24.64, 25.02, 26.09, 26.13 (4C, *C*$H_3$C), 61.35, 67.25 (C6, C6′), 66.65, 69.62 (C5, C5′), 70.56 (C2), 70.71 (C3), 71.24 (C4), 71.99, 72.11, 74.24 (C2′, C3′, C4′), 96.34 (C1′), 99.38 (C1), 108.96, 109.58 (2C, $CH_3$*C*); FAB-MS *m/z* 423.1873 ($C_{18}H_{31}O_{11}$ $[M+H]^+$ requires 423.1866).

(ii) The tetrol from (i) (26 mg) was stirred in $CF_3COOH/H_2O$ (4:1, 2 mL) (0 °C, 1 h). The solution was then concentrated, applied to a Sephadex (IR 120) column and eluted with $H_2O$. Concentration of the eluant gave 6-*O*-(α-D-glucopyranosyl)-D-galactopyranose (18.5 mg) as a colourless oil and as a mixture of anomers (α:β, 0.6:1); $[\alpha]_D$ +119.0 ($H_2O$); $^1H$ NMR (600 MHz, $D_2O$): $\delta_H$ 3.39 (m, H3′), 3.48 (dd, $J_{2,3} = 9.8$, $J_{1,2} = 7.9$, H2β), 3.54–3.56 (m, H2′), 3.65 (dd, $J_{3,4} = 3.4$, H3β), 3.67–3.77, 3.82–3.88 (2 × m, H3α, H4′, H5β, H5′, H6, H6′), 3.79 (dd, $J_{2,3} = 10.3$, $J_{1,2} = 3.9$, H2α), 3.97 (d, H4β), 4.02 (d, $J_{3,4} = 3.0$, H4α), 4.26 (dd, $J_{5,6} = 6.4$, 5.9, H5α), 4.58 (d, H1β), 4.93–4.95 (m, H1′), 5.25 (d, H1α); $^{13}C$ NMR (150.9 MHz, $D_2O$): $\delta_C$ 63.22 (C6′), 69.25, 69.55 (C6), 71.04 (C2α), 71.25 (C5α), 71.50, 71.79, 72.05, 72.26 (C4, C4′, C5β, C5′), 74.00, 74.03 (C2′), 74.52, 74.55, 75.44, 75.70, 75.75 (C2β, C3, C3′), 95.07 (C1β), 99.17 (C1α), 100.95, 100.98 (C1′); FAB-MS *m/z* 343.1239 ($C_{12}H_{23}O_{11}$ $[M+H]^+$ requires 343.1240).

## 4.2. Evaluation of the four disaccharides

**4.2.1. General methods.** α-Amylase (human saliva), α-galactosidase (green coffee beans) and α-glucosidase and isomaltase (baker's yeast) were purchased from Sigma Chemicals. The 'glucose oxidase reagent 1' consisted of glucose oxidase (10.4 U/mL), peroxidase (3.1 U/mL) and ABTS (300 μg/mL); 'glucose oxidase reagent 2' consisted of glucose oxidase (20.8 U/mL), peroxidase (12.2 U/mL), tris buffer (pH 7.0, 0.5 M) and ABTS (600 μg/mL).

**4.2.2. Method for glucose measurement.** The enzyme in phosphate buffer (Table 5; 50 μL, 4–8 U/mL, pH 5.0–6.9) was added to samples and standards in doubly-deionized (DDI) water (5 μL, 0–3 mM) in the wells of a microtiter plate, and the plate mixed on a plate shaker. The plate was incubated at 37 °C for 60 min. After this time the 'glucose oxidase reagent 1' in phosphate buffer (150 μL) was added to each well and the plate mixed. Absorbance of the ABTS was monitored at 405 nm every 5 min until peak absorbance reached a maximum.

**4.2.3. Processing of pig intestinal samples.** Intestinal mucosa samples were taken from a freshly euthanased 19.5 kg pig. The pig was restricted from feed for 12 h prior to death to minimize the quantity of undigested material. Sampling commenced 20 cm into the small intestine, with an interval of 50 cm between samples. At each interval a 20 cm portion of small intestine was removed, the sample was washed with isosmotic saline, cut open, patted dry with filter paper and the mucosa scraped using a glass microscope slide. Approximately 1 g of the mucosa was suspended in DDI water (20 mL) and the sample homogenized by sonication.

**Table 5.** The enzymes used in glucose measurement

| Enzyme | Enzyme concentration (U/mL) | Buffer system (concn) | pH | Additional reagent |
|---|---|---|---|---|
| α-Amylase | 8 | Potassium phosphate (20 mM) | 6.9 | NaCl (67 mM) |
| α-Galactosidase | 4 | Potassium phosphate (100 mM) | 5.0 | |
| α-Glucosidase | 8 | Potassium phosphate (100 mM) | 5.0 | |
| Isomaltase | 8 | Potassium phosphate (50 mM) | 6.7 | |

**Table 6.** The disaccharide reagents used in disaccharidase assays

| Maltose reagent | Sucrose reagent | Lactose reagent | Isomaltose reagent | α-Glc*p*-(1→6)-Gal reagent |
|---|---|---|---|---|
| Maleate buffer, pH 6.9, 0.1 M Maltose, 0.1 M | Maleate buffer, pH 6.9, 0.1 M Sucrose, 0.1 M | Maleate buffer, pH 6.9, 0.1 M Lactose, 0.1 M | Maleate buffer, pH 6.9, 0.1 M Isomaltose, 0.1 M | Maleate buffer, pH 6.9, 0.1 M α-Glc*p*-(1→6)-Gal, 0.1 M |

**Table 7.** The pig intestinal homogenates used in disaccharidase assays

| Enzyme | Reagent volume (μL) | Homogenate volume (μL) | Incubation time (min) | Blank incubation time (min) |
|---|---|---|---|---|
| Maltase | 90 | 10 | 120 | 60 |
| Sucrase | 50 | 50 | 120 | 60 |
| Lactase | 50 | 50 | 120 | 60 |
| Isomaltase | 50 | 50 | 120 | 60 |
| Disaccharidase acting on α-Glc*p*-(1→6)-Gal | 50 | 50 | 360 | 180 |

The volume was made up to 50 mL with DDI water and the sample centrifuged to remove cellular debris (2000 rpm, 10 min). The supernatant was collected and stored (−20 °C) until required for testing.

**4.2.4. Protein assay.** Protein concentration of each sample was determined using the Bio-Rad assay dye reagent concentrate. The assay was carried out according to the 'Bio-Rad Protein Assay' technical instructions provided. The dye concentrate was diluted in DDI water (1:4) and was filtered prior to use. Standards were prepared using bovine serum albumin, with concentrations of 0–1.03 mg/mL. In each well 5 μL of sample was treated with 250 μL of the diluted Bio-Rad reagent and incubated for 30 min at 37 °C. The absorbance was measured at 620 nm and compared to a protein standard curve.

**4.2.5. Disaccharidase assays.** The disaccharide reagent (Table 6) was treated with the intestinal homogenate and incubated at 37 °C; a blank was prepared by treatment of the disaccharide reagent with the intestinal homogenate and incubated (Table 7). At the end of the incubation, DDI water (900 μL) was added to both samples and the solutions were transferred to a Pyrex test tube. The solutions were then heated in a water bath at 90 °C for 4 min and subsequently cooled in an ice bath. An aliquot (100 μL) of each solution was treated with the 'glucose oxidase reagent 2′ (150 μL) and the absorbance measured (405 nm). Glucose concentration was measured by comparison of absorbance against a set of glucose standards.

## Acknowledgement

We thank the Women and Infants Research Foundation for financial assistance.

## References

1. Lawrence, R. A.; Lawrence, R. M. *Breastfeeding: A Guide for the Medical Profession*; Mosby: St. Louis, MO, 2005.
2. Shulman, R. J.; Schanler, R. J.; Lau, C.; Heitkemper, M.; Ou, C. N.; Smith, E. O. *J. Pediatr.* **1998**, *133*, 645–649.
3. Griffin, M. P.; Hansen, J. W. *J. Pediatr.* **1999**, *135*, 587–592.
4. Antonowicz, I.; Lebenthal, E. *Gastroenterology* **1977**, *72*, 1299–1303.
5. Raul, F.; Larcroix, B.; Aprahamian, M. *Early Hum. Dev.* **1986**, *13*, 225–234.
6. Shulman, R. J.; Feste, A.; Ou, C. *J. Pediatr.* **1995**, *127*, 626–631.
7. De Curtis, M.; Candusso, M.; Pieltain, C.; Rogo, J. *Arch. Dis. Child Fetal Neonatal Ed.* **1999**, *81*, 141F–143F.
8. Fenton, T. R.; Belik, J. *J. Pediatr. Gastroenterol. Nutr.* **2002**, *35*, 298–302.
9. Neeser, J. R.; Golliard, M.; Del Vedovo, S. D. *J. Dairy Sci.* **1991**, *74*, 2860–2871.
10. Hiromi, K.; Ohnishi, M.; Tanaka, A. *Mol. Cell. Biochem.* **1983**, *51*, 79–95.
11. Shulman, R. J.; Kerzner, B.; Sloan, H. R.; Boutton, T. W.; Wong, W. W.; Nichols, B. L.; Klein, P. D. *Pediatr. Res.* **1986**, *20*, 740–743.
12. Zarif, M.; Pildes, R. S.; Vidyasagar, D. *Diabetes* **1976**, *25*, 428–433.
13. Hertel, S.; Heinz, F.; Vogel, M. *Carbohydr. Res.* **2000**, *326*, 264–276.
14. Kliegman, R. M.; Sparks, J. W. *J. Pediatr.* **1985**, *107*, 831–839.
15. Kliegman, R. M.; Miettinen, E. L.; Morton, S. *Science* **1983**, *220*, 302–304.
16. Walker, D. G.; Khan, H. N. *Biochem. J.* **1968**, *108*, 169–175.
17. Fischer, E. *Chem. Ber.* **1893**, *26*, 2412–2415.
18. Lemieux, R. U.; Hendriks, K. B.; Stick, R. V.; James, K. *J. Am. Chem. Soc.* **1975**, *97*, 4056–4062.
19. Lam, S. N.; Gervay-Hague, J. *Carbohydr. Res.* **2002**, *337*, 1953–1965.
20. Wegmann, B.; Schmidt, R. R. *J. Carbohydr. Chem.* **1987**, *6*, 357–375.
21. Crich, D.; Smith, M. *J. Am. Chem. Soc.* **2001**, *123*, 9015–9020.
22. Codée, J. D. C.; Litjens, R. E. J. N.; den Heeten, R.; Overkleeft, H. S.; van Boom, J. H.; van der Marel, G. A. *Org. Lett.* **2003**, *5*, 1519–1522.

23. Kobashi, Y.; Mukaiyama, T. *Chem. Lett.* **2004**, *33*, 874–875.
24. Mukaiyama, T.; Kobashi, Y. *Chem. Lett.* **2004**, *33*, 10–11.
25. Thiem, J.; Meyer, B. *Chem. Ber.* **1980**, *113*, 3075–3085.
26. Bergmeyer, H. U. *Methods of Enzymatic Analysis*, 2nd ed.; Verlag Chemie and Academic Press: Weinheim and New York, 1974; pp 1205–1212.
27. Douglas, W. R. *Origins Life Evol. B.* **1972**, *3*, 226–234.
28. Sun, H.-F. S.; Ernst, C. W.; Yerle, M.; Pinton, P.; Rothschild, M. F.; Chardon, P.; Rogel-Gaillard, C.; Tuggle, C. K. *Cytogenet. Cell Genet.* **1999**, *85*, 273–278.
29. Calvo, J. H.; Lopez-Corrales, N. L.; Osta, R.; Skinner, T. M.; Anderson, S. I.; Rodellar, C.; Zaragoza, P.; Archibald, A. L. *Cytogenet. Cell Genet.* **2000**, *90*, 236–237.
30. Dahlqvist, A. *Anal. Biochem.* **1964**, *7*, 18–25.
31. Scaffidi, A.; Skelton, B. W.; Stick, R. V.; White, A. H. *Aust. J. Chem.* **2006**, *59*, 426–433.
32. Kong, F.; Lu, D. *Carbohydr. Res.* **1990**, *198*, 141–148.
33. Rathore, H.; Hashimoto, T.; Igarashi, K.; Nukaya, H.; Fullerton, D. S. *Tetrahedron* **1985**, *41*, 5427–5438.
34. France, R. R.; Rees, N. V.; Wadhawan, J. D.; Fairbanks, A. J.; Compton, R. G. *Org. Biomol. Chem.* **2004**, *2*, 2188–2194.
35. Crich, D.; Smith, M.; Yao, Q.; Picione, J. *Synthesis* **2001**, 323–326.
36. Schmidt, R. R.; Michel, J. *J. Org. Chem.* **1981**, *46*, 4787–4788.
37. Garegg, P. J.; Hultberg, H.; Wallin, S. *Carbohydr. Res.* **1982**, *108*, 97–101.
38. Wozney, Y. V.; Backinowsky, L. V.; Kochetkov, N. K. *Carbohydr. Res.* **1979**, *73*, 282–286.
39. Müller, M.; Huchel, U.; Geyer, A.; Schmidt, R. R. *J. Org. Chem.* **1999**, *64*, 6190–6201.