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Modifying the Regioselectivity of Glycosynthase Reactions Through Changes in the Acceptor*

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Successful glycosynthase-mediated reactions have been performed on 6-*O*-benzyl-, 6-*O*-(4-nitrobenzyl)-, and 6-*O*-benzoyl-D-glucopyranose to give 1,2-β- and 1,3-β-D-glycosylated products; 4-*O*-benzyl-D-xylopyranose gave only a 1,2-β-glycosylated product. A rationale is presented for these rather unusual results.

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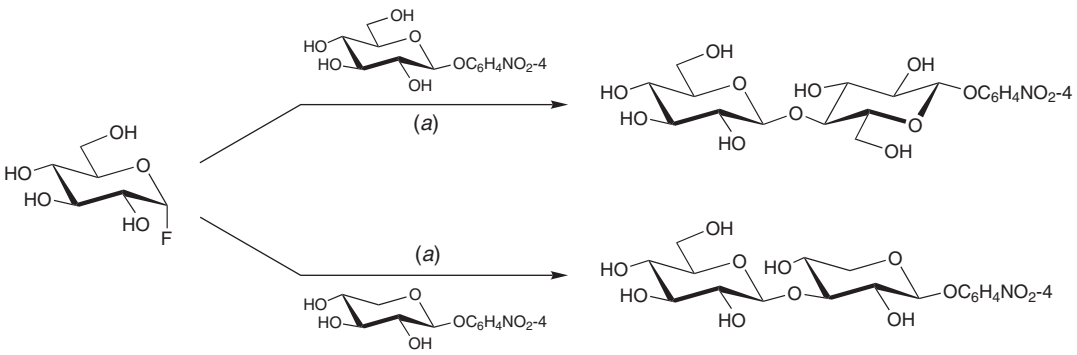
Introduction

Glycosynthases, which are mutant glycoside hydrolases that are capable of making but not breaking the glycosidic linkage, are currently available for the construction of β-1,3-, β-1,4-, β-1,6-, and α-1,4-linked oligosaccharides.[1–11] Generally, the choice of donor sugar in the glycosylation process is guided by the structure of the natural substrate for the original glycoside hydrolase, while the acceptor is often an aryl glycoside that exhibits good binding in both the +1 and +2 subsites of the original glycoside hydrolase (and glycosynthase).[1]

Like glycoside hydrolases, which are used in the *trans*-glycosylation mode for the construction of the glycosidic linkage, glycosynthases are capable of constructing linkages for which they were not designed. For example, although the glycosynthase (Abg E358S) derived from a β-glucosidase isolated from an *Agrobacterium* sp. predictably presided over the construction of a β-1,4-linkage when α-D-glucopyranosyl fluoride was treated with 4-nitrophenyl β-D-glucopyranoside, when the acceptor was changed to 4-nitrophenyl β-D-xylopyranoside, a β-1,3-linkage was obtained (Scheme 1).[3]

It occurred to us that it might be possible to increase this 'structural infidelity' of glycosynthases by employing aryl ethers or esters as acceptors, rather than aryl glycosides. Our premise was that a molecule such as 6-*O*-benzyl-D-glucopyranose would perhaps present an alternative hydroxyl group (from an aryl β-D-glucopyranoside) for functionalization by the glycosyl donor, that is, if it actually did bind to the active site of a glycosynthase (Scheme 2).

In order to test our hypothesis, we prepared 6-*O*-benzyl- **1**, 6-*O*-benzoyl- **2**, and 6-*O*-(4-nitrobenzyl)-D-glucopyranose **3** (Scheme 3). It was found that it was best to isolate the two ethers (**1** and **3**) as their tetraacetates (**4** and **5**), a feat which was not possible with ester **2**. To our delight, the treatment of all three of the free sugars (**1**, **2**, and **3**) with α-D-galactopyranosyl fluoride (to prevent higher saccharide formation[3]) in the presence of the glycosynthase Abg E358S resulted in the formation of mixtures of disaccharides that had either β-1,3- or β-1,2-linkages (Table 1) rather than the characteristic β-1,4-linkage observed when an aryl β-D-glucopyranoside was the acceptor.[3] Once again,



Scheme 1. (*a*) Abg E358S, NH_4HCO_3, H_2O.

* Dedicated to the memory of Christian Pedersen.

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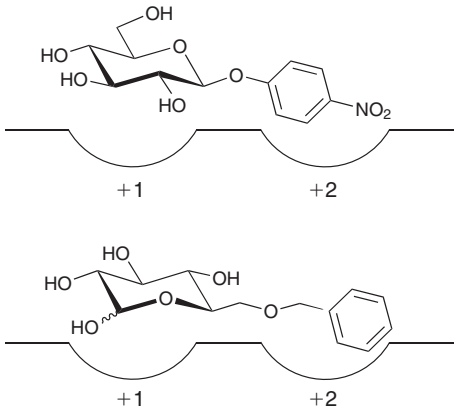
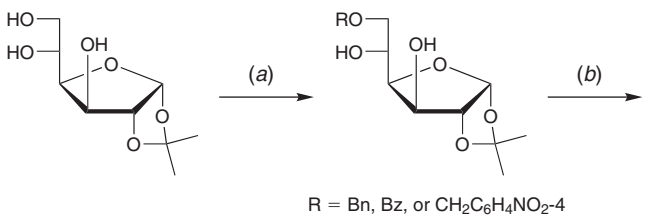
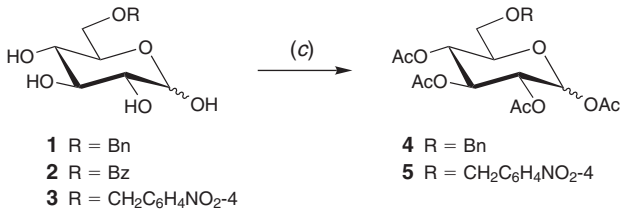
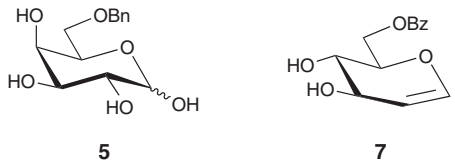


Scheme 2.





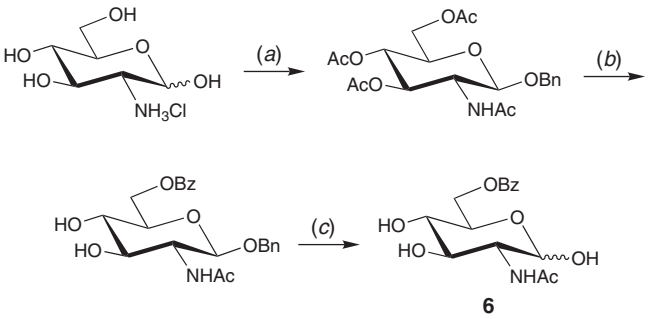
Scheme 3. (*a*) $(Bu_3Sn)_2O$, PhMe, then BnBr, BzCl or 4-$NO_2C_6H_4CH_2Br$; (*b*) CF_3CO_2H/H_2O (4 : 1); (*c*) Ac_2O, pyridine for **1** and **3**.



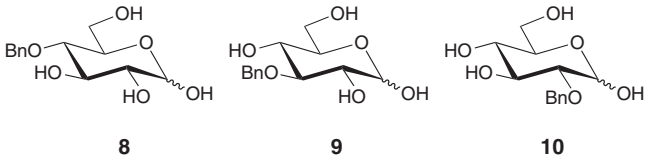
Scheme 4.

the disaccharides described here were best isolated as their per-acetates. Two of the reactions were repeated with α-D-glucopyranosyl fluoride as the donor and, again, the β-1,3- and β-1,2-linked disaccharides were the major products (Table 1).

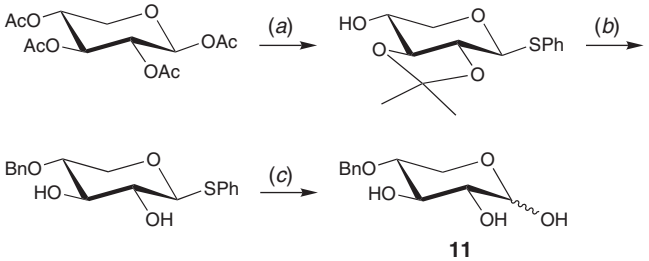
Encouraged by these early results, we prepared 6-*O*-benzyl-D-galactopyranose **5**[12] (Scheme 4), 2-acetamido-6-*O*-benzoyl-2-deoxy-D-glucopyranose **6** (Scheme 5), and 6-*O*-benzoyl-D-glucal **7**[13] (Scheme 4). Unfortunately, none of these molecules acted as acceptors in our glycosynthase reactions with α-D-galactopyranosyl fluoride. However, it still seemed worthwhile to investigate the ability of sugars functionalized at positions other than O1 and O6 to act as acceptors in glycosynthase reactions. Thus, we prepared



Scheme 5. (*a*) Et_2NH, EtOH, then Ac_2O, pyridine, then BnOH, TMSOTf, CH_2Cl_2; (*b*) Na, MeOH, then BzCl, pyridine, CH_2Cl_2, −20°C; (*c*) H_2, Pd/C, MeOH.



Scheme 6.



Scheme 7. (*a*) PhSH, Et_2OBF_3, CH_2Cl_2, then Na, MeOH, then 2-methoxypropene, CSA, DMF; (*b*) BnBr, NaH, DMF, then H_3O^+, MeOH; (*c*) NBS, H_3O^+, MeCN.

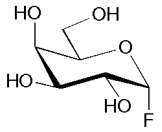
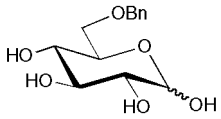
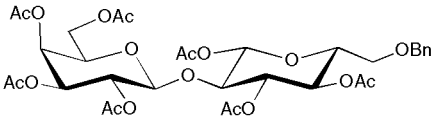
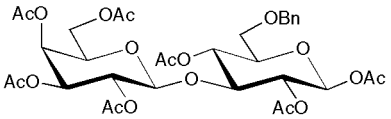
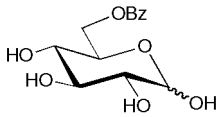
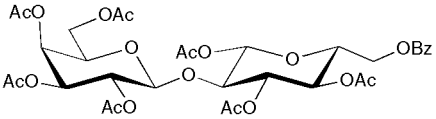
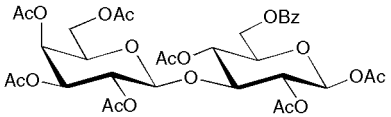
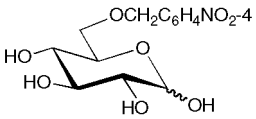
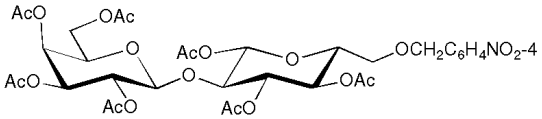
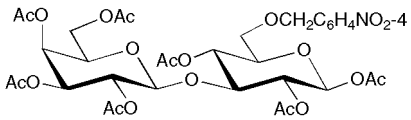
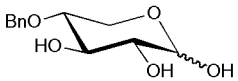
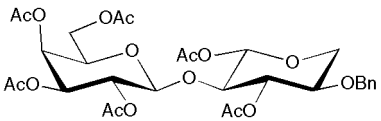
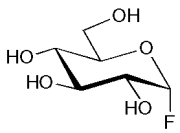
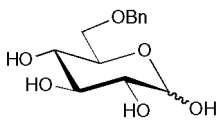
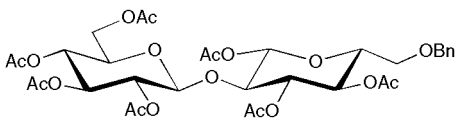
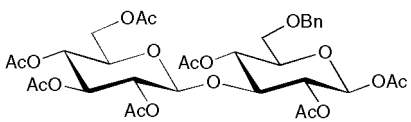
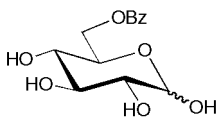
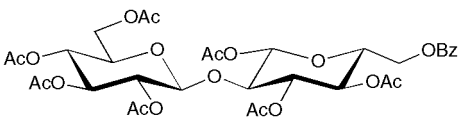
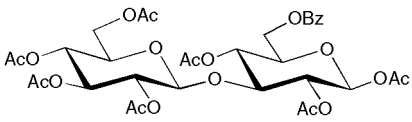
4-*O*-benzyl- **8**,[14] 3-*O*-benzyl- **9**,[15] and 2-*O*-benzyl-D-glucopyranose **10**[16] (Scheme 6), but once again, none of these free sugars acted as acceptors in glycosynthase-mediated reactions.

Finally, we prepared 4-*O*-benzyl-D-xylopyranose **11** (Scheme 7), which subsequently acted as an acceptor in the glycosynthase reaction to provide a single β-1,2-linked disaccharide derivative (Table 1).

Discussion

How can one rationalize the successful glycosynthase-mediated reactions reported here? For an acceptor such as 4-nitrophenyl β-D-glucopyranoside, binding to the active site of the glycosynthase is predictable and it is the 4-OH group that is correctly positioned for glycosylation to occur (Fig. 1*a*). For 4-nitrophenyl β-D-xylopyranoside, the mode of binding is likely to be similar (glycosyl moiety in the +1 site and aryl group in the +2 site), but the absence of a primary hydroxyl group allows a certain degree of positional freedom (through a 'flip' or translocation of the pyranose ring), and

Table 1. The glycosylation of various acceptors with glycosynthase Abg E358S

Donor	Acceptor		Products	Yield [%]
		**1**		53
				33
		**2**		41
				30
		**3**		47
				29
		**11**		56
		**1**		39
				32
		**2**		43
				33

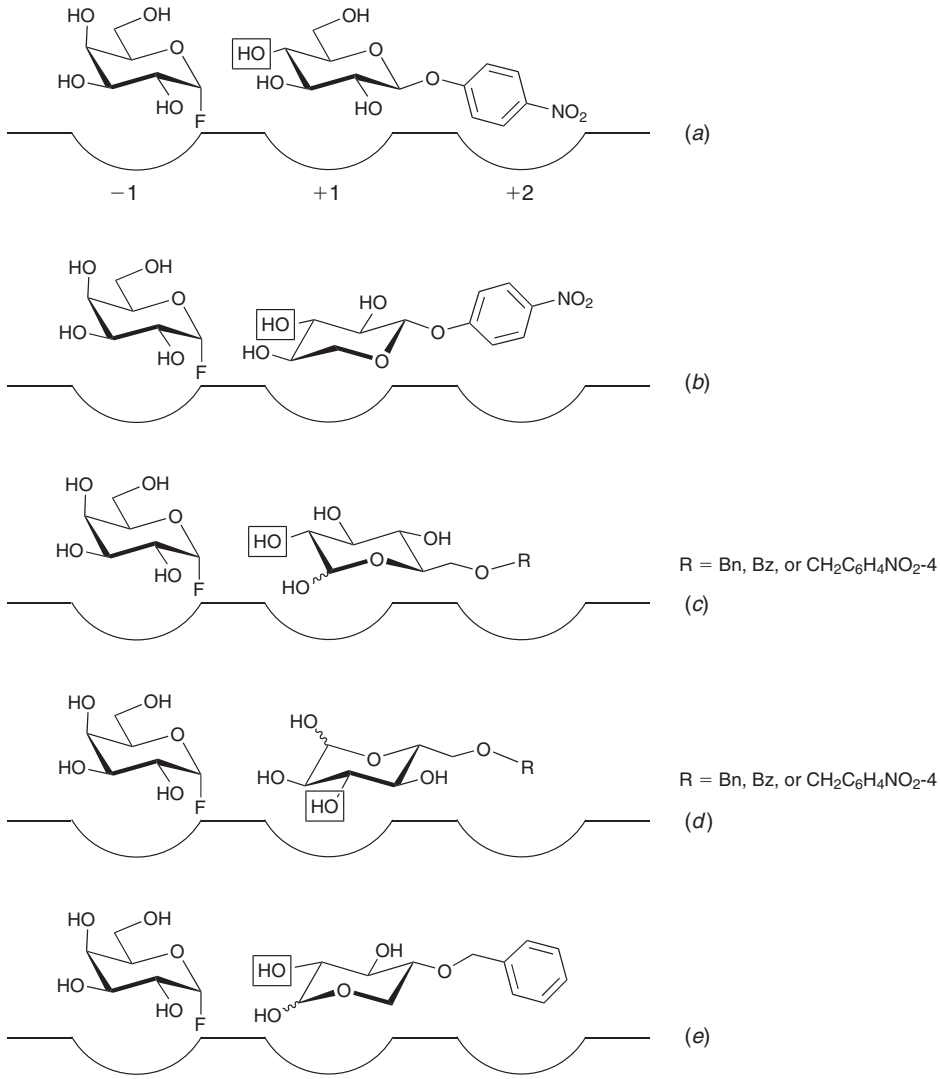


Fig. 1.

thus allows the 3-OH group to be presented for glycosylation (Fig. 1*b*).

The D-glucopyranoses **1**–**3** (Scheme 3) presumably bind in an alternative mode, whereby the functional group at O6 occupies the aglycon (+2) site, while the glycosyl moiety is rotated into the +1 site. Although these molecules have lost any contribution to binding that involves hydrogen bonding of the 6-OH group, compensation may be provided through hydrophobic interactions involving the aromatic ring in the +2 site. Therefore, the glycosyl moiety (which lacks a primary hydroxyl group) presents either the 2-OH group (Fig. 1*c*) or the 3-OH group (Fig. 1*d*) for glycosylation. The other positive result, namely the glycosylation of 4-*O*-benzyl-D-xylopyranose **11**, may be rationalized in similar terms, that is, the benzyl ether binds in the +2 site and so presents the 2-OH group of the sugar for glycosylation (Fig. 1*e*).

One final observation in the glycosynthase-mediated transformations described here is the lack of formation of higher oligosaccharides (tri- and tetra-saccharides). Normally, the initial product of glycosylation (usually a disaccharide) is a good (or even better) acceptor, and so further glycosylation occurs to give higher oligosaccharides. The 'unnatural' β-1,2- and β-1,3-linked products described here are obviously poor acceptors and so further glycosylation is discouraged, even in the presence of the 'natural' donor, α-D-glucopyranosyl fluoride.

Our next intention is to apply some of the principles established here to extend the scope of the recently developed thioglycoligases.[17]

Experimental

General experimental procedures have been given previously.[18]

Tetra-O-*acetyl*-6-O-*benzyl*-D-*glucopyranose* ***4***

(i) Bis(tributyltin) oxide (3.2 g, 5.4 mmol) was added to 1,2-*O*-isopropylidene-α-D-glucofuranose (1.0 g, 4.5 mmol) in toluene (50 mL) and the mixture was heated at reflux for 24 h. Benzyl bromide (1.1 g, 6.3 mmol) was then added and the solution was refluxed for a further 2 h. Concentration of the mixture left a brown residue that, after flash chromatography (EtOAc/light petroleum 2 : 3), furnished 6-*O*-benzyl-1,2-*O*-isopropylidene-α-D-glucofuranose (1.3 g, 92%) as an oil. The ^{1}H NMR spectrum of this oil was consistent with that reported.[19]

(ii) 6-*O*-Benzyl-1,2-*O*-isopropylidene-α-D-glucofuranose (1.3 g, 4.1 mmol) was added to a trifluoroacetic acid/H_2O (4 : 1, 15 mL) mixture and the solution was stirred at room temperature for 30 min. Concentration of the solution gave a brown residue that was dissolved in pyridine (20 mL). Acetic anhydride (20 mL) was then added and after 1 h the solution was quenched with MeOH (10 mL) and concentrated. Usual workup (EtOAc) followed by flash chromatography (EtOAc/light petroleum, 3 : 7) gave tetra-*O*-acetyl-6-*O*-benzyl-D-glucopyranose **4** (1.7 g, 92%) as an oil. The ^{1}H NMR spectrum of this oil was consistent with that reported.[20]

6-O-*Benzoyl-D-glucopyranose* ***2***

(i) Following the procedure described in (i) above, but using benzoyl chloride (890 mg) instead of benzyl bromide, gave 6-*O*-benzoyl-1,2-*O*-isopropylidene-α-D-glucofuranose[21] (87%) as an oil.

(ii) 6-*O*-Benzoyl-1,2-*O*-isopropylidene-α-D-glucofuranose (1.3 g, 4.0 mmol) was added to a trifluoroacetic acid/H_2O (4 : 1, 15 mL) mixture and the solution was stirred at room temperature for 30 min. Concentration of the mixture gave a brown residue that, upon flash chromatography (MeOH/$CHCl_3$, 3 : 7), gave tetrol **2** (1.1 g, 94%) as an oil. The ^{1}H NMR spectrum of this oil was consistent with that reported.[22]

Tetra-O-*acetyl-6*-O-*(4-nitrobenzyl)-D-glucopyranose* ***5***

(i) Following the procedure described in (i) above for **4**, but using 4-nitrobenzyl bromide (1.4 g) instead of benzyl bromide, gave 1,2-*O*-isopropylidene-6-*O*-(4-nitrobenzyl)-α-D-glucofuranose (91%) as an oil, $[\alpha]_D$ −1.9 (Found (FAB): *m/z* 356.1363. $C_{16}H_{21}NO_8$ requires $[M+H]^+$ 356.1345). δ_H (300 MHz) 8.21 and 7.50 (4H, AA′BB′, Ar), 5.95 (d, $J_{1,2}$ 3.7, H1), 4.70 (s, CH_2Ar), 4.54 (d, H2), 4.36 (d, $J_{3,4}$ 2.8, H3), 4.24 (ddd, $J_{4,5}$ 6.5, $J_{5,6}$ 5.7 and 3.3, H5), 4.13 (dd, H4), 3.82 (dd, $J_{6,6}$ 10.3, H6), 3.71 (dd, H6), 1.49 and 1.33 (6H, 2 s, Me). δ_C (75.5 MHz) 148.3, 145.1, 127.8, and 123.8 (6C, Ar), 111.8 (*C*Me_2), 104.9 (C1), 85.1, 79.5, 75.7, and 69.4 (C2, C3, C4, and C5), 72.3 and 71.8 (C6 and *C*H_2Ar), 26.8 and 26.1 (2C, Me).

(ii) Following the procedure described in (ii) above for **4**, 1,2-*O*-isopropylidene-6-*O*-(4-nitrobenzyl)-α-D-glucofuranose (1.5 g, 4.2 mmol) gave *tetra*-O-*acetyl-6*-O-*(4-nitrobenzyl)-D-glucopyranose* ***5*** (1.9 g, 93%) as an oil (Found: C 52.3, H 5.2, *m/z* 484.1435. $C_{21}H_{25}NO_{12}$ requires C 52.2, H 5.2%, $[M+H]^{+\bullet}$ 484.1455). δ_H (600 MHz) 8.19 and 7.49 (4H, AA′BB′, Ar), 6.33 (d, $J_{1,2}$ 3.7, H1α), 5.70 (d, $J_{1,2}$ 7.5, H1β), 5.47 (dd, $J_{3,4}$ 10.0, H3α), 5.27–5.20 (m, H3β, H4α, and H4β), 5.13 (dd, $J_{2,3}$ 9.9, H2β), 5.07 (dd, $J_{2,3}$ 10.0, H2α), 4.65 and 4.57 (AB, *J* 13.1, CH_2Ar), 4.07 (ddd, $J_{4,5}$ 9.2, $J_{5,6}$ 4.5 and 2.6, H5α), 3.82 (ddd, $J_{4,5}$ 9.3, $J_{5,6}$ 2.7 and 4.5, H5β), 3.68 (dd, $J_{6,6}$ 11.1, H6α), 3.64 (dd, H6α), 3.60–3.56 (m, H6β), 2.17, 2.10, 2.03, 2.02, 2.01, 1.98, and 1.97 (12H, 7 s, Me). δ_C (150.8 MHz) 170.1, 169.4, 169.2, and 169.0 (4C, C=O), 145.2, 145.2, 127.8, 123.6, and 123.6 (6C, Ar), 91.8 (C1β), 89.1 (C1α), 73.9 (C5β), 72.9 (C4α), 72.4 and 72.3 (*C*H_2Ar), 71.1 (C5α), 70.2 (C2β), 69.9 (C3α), 68.2 (C2α), 68.7 and 68.7 (C6α and C6β), 68.4 and 68.4 (C3β and C4β), 20.8, 20.8, 20.6, 20.6, 20.5, 20.5, and 20.4 (4C, Me).

2-Acetamido-6-O-*benzoyl-2-deoxy-D-glucopyranose* ***6***

(i) Benzoyl chloride (145 mg, 1.10 mmol) in DMF (5 mL) was added dropwise to benzyl 2-acetamido-2-deoxy-β-D-glucopyranoside[23] (250 mg, 0.810 mmol) and pyridine (0.10 mL, 1.1 mmol) in DMF (15 mL) at −30°C and the solution was stirred for 30 min. The mixture was quenched by the addition of MeOH (10 mL) and then concentrated. Flash chromatography of the residue (MeOH/$CHCl_3$, 1 : 9) gave *benzyl 2-acetamido-6*-O-*benzoyl-2-deoxy-β-D-glucopyranoside* (290 mg, 87%) as colourless crystals, mp 187–190°C (EtOH), $[\alpha]_D$ +26.1° (Found: C 63.3, H 6.2, *m/z* 416.1723. $C_{22}H_{25}NO_7$ requires C 63.6, H 6.1%, $[M+H]^{+\bullet}$ 416.1709). δ_H (500 MHz) 8.09–8.06, 7.63–7.59, 7.51–7.47, and 7.27–7.23 (10H, 4 m, Ph), 4.77 and 4.55 (AB, *J* 12.2, CH_2Ph), 4.69 (dd, $J_{5,6}$ 3.7, $J_{6,6}$ 12.3, H6), 4.51 (dd, $J_{5,6}$ 5.9, H6), 4.49 (d, $J_{1,2}$ 8.4, H1), 3.75 (ddd, $J_{2,3}$ 8.0, $J_{2,NH}$ 2.7, H2), 3.58 (ddd, $J_{4,5}$ 11.0, H5), 3.49 (m, H3 and H4), 1.95 (s, Me). δ_C (125.7 MHz) 173.7 and 167.9 (2C, C=O), 138.9, 134.4, 131.4, 130.6, 129.6, 129.3, 128.9, and 128.7 (Ph), 101.6 (C1), 75.8, 75.5, and 72.3 (C3, C4, and C5), 71.5 (*C*H_2Ph), 65.1 (C6), 57.4 (C2), 22.9 (Me).

(ii) The above benzoate (250 mg, 0.60 mmol) and palladium-on-carbon (20 mg of 10%) in MeOH (15 mL) were stirred vigorously under H_2 (1 atm) for 24 h. The mixture was then filtered through Celite, the filtrate was concentrated, and the residue was purified by flash chromatography (MeOH/$CHCl_3$, 1 : 4) to give hemiacetal **6** (195 mg) as an oil, $[\alpha]_D$ +37.7° (equilibrium) (Found: *m/z* 326.1224. $C_{15}H_{19}NO_7$ requires $[M+H]^{+\bullet}$ 326.1239). δ_H (600 MHz) 8.03–7.99, 7.60–7.57, and 7.48–7.43 (3 m, Ph), 5.13 (d, $J_{1,2}$ 3.4, H1α), 4.66 (m, H1β and H6α), 4.61 (dd, $J_{5,6}$ 1.9, $J_{6,6}$ 12.7, H6β), 4.47 (m, H6α and H6β), 4.14 (ddd, $J_{4,5}$ 10.0, $J_{5,6}$ 5.1, H5β), 3.91 (dd, $J_{2,3}$ 8.9, H2α), 3.78 (dd, $J_{3,4}$ 9.0, H3α), 3.67 (dd, $J_{1,2} \approx J_{2,3}$ 9.0, H2β), 3.64 (ddd, $J_{4,5}$ 9.9, $J_{5,6}$ 5.6 and 1.8, H5α), 3.51 (m, H3β, H4α, and H4β), 1.99 (s, Me). δ_C (150.8 MHz) 174.2, 173.7, 168.0, and 167.9 (2C, C=O), 134.4, 134.3, 132.1, 131.2, 130.5, 130.4, 129.5, and 128.9 (Ph), 97.0 (C1β), 92.6 (C1α), 75.8 (C3β), 75.4 (C5α), 72.5 (C3α and C4β), 72.1 (C4α), 70.8 (C5β), 65.3 (C6α), 65.2 (C6β), 58.7 (C2β), 55.8 (C2α), 22.9 and 22.7 (Me).

4-O-*Benzyl-D-xylose* ***11***

(i) Sodium hydride (60% dispersion in mineral oil, 120 mg, 2.8 mmol) and benzyl bromide (0.34 mL, 2.8 mmol) were added to phenyl 2,3-*O*-isopropylidene-1-thio-β-D-xyloside[24] (400 mg, 1.40 mmol) in DMF (10 mL). The reaction mixture was stirred at room temperature for 1 h before being quenched by the addition of MeOH (10 mL). Concentration of the mixture followed by a usual workup (EtOAc) and flash chromatography (EtOAc/light petroleum 17 : 83) of the crude product gave phenyl 4-*O*-benzyl-2,3-*O*-isopropylidene-1-thio-β-D-xyloside (530 mg, 89%) as a solid, $[\alpha]_D$ −32.9° (Found: *m/z* 373.1465. $C_{21}H_{24}O_4S$ requires $[M+H]^{+\bullet}$ 373.1473). δ_H (600 MHz) 7.56–7.54 and 7.34–7.30 (10H, 2 m, Ph), 4.81 (d, $J_{1,2}$ 9.4, H1), 4.80 and 4.59 (AB, *J* 11.9, CH_2Ph), 4.10 (dd, $J_{4,5}$ 5.1, $J_{5,5}$ 11.9, H5), 3.76 (ddd, $J_{3,4}$ 9.0, $J_{4,5}$ 8.7, H4), 3.66 (dd, $J_{2,3}$ 9.1, H3), 3.28 (dd, H5), 3.24 (dd, H2), 1.49 and 1.46 (6H, 2 s, Me). δ_C (150.8 MHz) 137.9, 132.8, 131.9, 128.8, 128.4, 128.0, 127.8, and 127.7 (Ph), 111.2 (*C*Me_2), 85.3 (C1), 82.5, 75.5, and 75.3 (C2, C3, and C4), 72.1 (*C*H_2Ph), 68.3 (C5), 26.7 and 26.6 (2C, Me).

(ii) Concentrated HCl (0.2 mL) was added to phenyl 4-*O*-benzyl-2,3-*O*-isopropylidene-1-thio-β-D-xyloside (500 mg, 1.3 mmol) in MeOH (10 mL) and the solution was stirred at room temperature for 1 h. The solution was concentrated and the residue was purified by flash chromatography (EtOAc/light petroleum 1 : 1) to give *phenyl 4*-O-*benzyl-1-thio-β-D-xyloside* (430 mg, 97%) as plates, mp 119–120°C (EtOAc/light petroleum), $[\alpha]_D$ −57.1° (Found: C 65.1, H 6.0, *m/z* 333.1141. $C_{18}H_{20}O_4S$ requires C 65.0, H 6.1%, $[M+H]^{+\bullet}$ 333.1160). δ_H (300 MHz) 7.51–7.47 and 7.35–7.25 (10H, 2 m, Ph), 4.73 and 4.60 (AB, *J* 11.7, CH_2Ph), 4.54 (d, $J_{1,2}$ 9.4, H1), 3.99 (dd, $J_{4,5}$ 4.9, $J_{5,5}$ 11.3, H5), 3.50 (dd, $J_{2,3}$ 8.8, H2), 3.38 (ddd, $J_{3,4}$ 8.7, $J_{4,5}$ 8.6, H4), 3.22–3.18 (m, H3 and H5). δ_C (75.5 MHz) 139.8, 134.7, 133.1, 129.9, 129.3, 128.9, 128.7, and 128.5 (Ph), 89.9 (C1), 78.6, 78.4, and 74.0 (C2, C3, and C4), 73.7 (*C*H_2Ph), 68.3 (C5).

(iii) *N*-Bromosuccinimide (170 mg, 0.94 mmol) was added to a solution of phenyl 4-*O*-benzyl-1-thio-β-D-xyloside (310 mg, 0.94 mmol) and concentrated HCl (0.1 mL) in CH_3CN/H_2O (19 : 1, 16 mL). The mixture was then stirred for 30 min and subsequently quenched with pyridine (10 mL). Evaporation of the solvents followed by flash chromatography (MeOH/$CHCl_3$, 1 : 9) of the residue gave hemiacetal **11** (180 mg, 80%) as colourless crystals. The ^{13}C NMR spectrum was consistent with that reported.[25]

General Procedure for the Glycosynthase (E358S)-Mediated Glycosylation of 6-O-*Benzyl-D-glucopyranose* ***1*** *and 6*-O-*(4-Nitrobenzyl)-D-glucopyranose* ***3***

A few drops of NaOMe in MeOH (1.5 M) were added to a mixture of tetra-*O*-acetyl-α-D-galactopyranosyl or -glucopyranosyl fluoride and tetra-*O*-acetyl-6-*O*-benzyl-D-glucopyranose **4** or tetra-*O*-acetyl-6-*O*-(4-nitrobenzyl)-D-glucopyranose **5** in MeOH and the solution was stirred for 30 min. The mixture was quenched with resin (Amberlite IR-120, H^+), filtered, and the filtrate was concentrated. The residue was dissolved in NH_4HCO_3 solution (3 mL of 150 mM), Abg E358S (2 mg)

was added, and the solution was kept at 25°C for 7 days. The solvent was then removed under reduced pressure, the residue was dissolved in Ac_2O (5 mL) that contained NaOAc (50 mg), and the solution was heated at reflux for 10 min. The reaction was quenched by the addition of ice/water. Usual workup (CH_2Cl_2) followed by flash chromatography (EtOAc/light petroleum, 1 : 4 to 1 : 1) gave the appropriate per-acetylated disaccharides.

1,3,4-Tri-O-*acetyl-6*-O-*benzyl-2*-O-*(tetra*-O-*acetyl-β-D-galactopyranosyl)-β-D-glucose and 1,2,4-Tri*-O-*acetyl-6*-O-*benzyl-3*-O-*(tetra*-O-*acetyl-β-D-galactopyranosyl)-β-D-glucose*

Utilizing the above general procedure, tetra-*O*-acetyl-α-D-galactopyranosyl fluoride (275 mg, 0.79 mmol) and tetra-*O*-acetyl-6-*O*-benzyl-D-glucopyranose **4** (230 mg, 0.52 mmol) firstly gave 1,3,4-tri-*O*-acetyl-6-*O*-benzyl-2-*O*-(tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-glucose (200 mg, 53%) as an oil (Found: *m/z* 727.2385. $C_{33}H_{42}O_{18}$ requires $[M+H]^{+\bullet}$ 727.2449). δ_H (600 MHz) 7.31–7.25 (m, Ph), 5.63 (d, $J_{1,2}$ 8.0, H1), 5.33 (dd, $J_{4',5'}$ 1.1, $J_{3',4'}$ 3.5, H4′), 5.17 (dd, $J_{2,3}$ 9.3, $J_{3,4}$ 10.0, H3), 5.10 (dd, $J_{4,5}$ 9.8, H4), 5.08 (dd, $J_{1',2'}$ 8.0, $J_{2',3'}$ 10.4, H2′), 4.91 (dd, H3′), 4.58 (d, H1′), 4.55 and 4.40 (AB, *J* 12.2, CH_2Ph), 4.11 (dd, $J_{5',6'}$ 6.1, $J_{6',6'}$ 11.8, H6′), 4.06 (dd, $J_{5',6'}$ 9.9, H6′), 3.92 (ddd, H5′), 3.81 (dd, H2), 3.70 (ddd, $J_{5,6}$ 3.9 and 2.6, H5), 3.56 (dd, $J_{6,6}$ 11.1, H6), 3.47 (dd, H6), 2.12, 2.09, 2.06, 2.02, 1.99, 1.94, and 1.84 (21H, 7 s, Me). δ_C (150.8 MHz) 170.3, 170.1, 170.1, 169.9, 169.6, 169.3, and 168.8 (7C, C=O), 137.4, 128.3, 127.9, and 127.7 (Ph), 101.1 (C1′), 91.7 (C1), 76.9 (C2), 75.0 (C5′), 74.7 (C3), 73.5 (C5), 73.4 (*C*H_2Ph), 70.9 (C3′), 68.6 and 68.5 (C4 and C2′), 67.3 (C6), 66.8 (C4′), 61.2 (C6′), 20.9, 20.7, 20.7, 20.5, 20.4, 20.4, and 20.4 (7C, Me).

Next to elute was 1,2,4-tri-*O*-acetyl-6-*O*-benzyl-3-*O*-(tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-glucose (120 mg, 33%), which was isolated as an oil (Found: *m/z* 727.2445. $C_{33}H_{42}O_{18}$ requires $[M+H]^{+\bullet}$ 727.2449). δ_H (600 MHz) 7.31–7.23 (m, Ph), 5.60 (d, $J_{1,2}$ 8.2, H1), 5.32 (dd, $J_{3',4'}$ 3.5, $J_{4',5'}$ 1.0, H4′), 5.09 (dd, $J_{2,3}$ 7.9, H2), 5.05 (dd, $J_{1',2'}$ 8.0, $J_{2',3'}$ 10.4, H2′), 4.99 (dd, $J_{3,4}$ 7.9, $J_{4,5}$ 9.8, H4), 4.91 (dd, H3′), 4.54 (d, H1′), 4.51 and 4.49 (AB, *J* 12.0, CH_2Ph), 4.11 (dd, $J_{5',6'}$ 6.8, $J_{6',6'}$ 11.0, H6′), 4.06 (dd, $J_{5',6'}$ 8.8, H6′), 4.00 (ddd, H5′), 3.86 (dd, H3), 3.75 (ddd, $J_{5,6}$ 4.9 and 2.9, H5), 3.56 (dd, $J_{6,6}$ 11.0, H6), 3.49 (dd, H6), 2.11, 2.07, 2.05, 2.03, 1.99, 1.94, and 1.93 (21H, 7 s, Me). δ_C (150.8 MHz) 170.3, 170.1, 170.1, 170.1, and 168.7 (7C, C=O), 137.5, 128.2, 127.8, and 127.6 (Ph), 101.1 (C1′), 91.8 (C1), 78.4 (C5′), 74.1 (C5), 73.4 (*C*H_2Ph), 72.0 (C2), 70.9 (C3′), 70.4 (C3), 68.5 and 68.5 (C4 and C2′), 68.3 (C6), 66.7 (C4′), 60.9 (C6′), 20.9, 20.7, 20.7, 20.5, 20.4, 20.4, and 20.3 (7C, Me).

1,3,4-Tri-O-*acetyl-6*-O-*(4-nitrobenzyl)-2*-O-*(tetra*-O-*acetyl-β-D-galactopyranosyl)-β-D-glucose and 1,2,4-Tri*-O-*acetyl-6*-O-*(4-nitrobenzyl)-3*-O-*(tetra*-O-*acetyl-β-D-galactopyranosyl)-β-D-glucose*

Utilizing the above general procedure, tetra-*O*-acetyl-α-D-galactopyranosyl fluoride (275 mg, 0.79 mmol) and tetra-*O*-acetyl-6-*O*-(4-nitrobenzyl)-D-glucopyranose **5** (250 mg, 0.52 mmol) firstly gave 1,3,4-tri-*O*-acetyl-6-*O*-(4-nitrobenzyl)-2-*O*-(tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-glucose (190 mg, 47%) as an oil (Found: *m/z* 772.2224. $C_{33}H_{41}NO_{20}$ requires $[M+H]^{+\bullet}$ 772.2300). δ_H (600 MHz) 8.19 and 7.46 (4H, AA′BB′, Ar), 5.65 (d, $J_{1,2}$ 8.0, H1), 5.35 (dd, $J_{3',4'}$ 3.5, $J_{4',5'}$ 1.1, H4′), 5.24 (dd, $J_{2,3}$ 8.9, $J_{3,4}$ 9.3, H3), 5.14 (dd, $J_{1',2'}$ 8.0, $J_{2',3'}$ 9.9, H2′), 5.06 (dd, $J_{4,5}$ 8.4, H4), 4.94 (dd, H3′), 4.65 and 4.63 (AB, *J* 13.2, CH_2Ar), 4.59 (d, H1′), 4.13 (dd, $J_{5',6'}$ 3.4, $J_{6',6'}$ 10.9, H6′), 4.05 (dd, $J_{5',6'}$ 6.4, H6′), 3.98 (ddd, H5′), 3.84 (dd, H2), 3.68 (dd, $J_{5,6}$ 2.4, $J_{6,6}$ 11.1, H6), 3.65 (ddd, $J_{5,6}$ 4.1, H5), 3.56 (dd, H6), 2.11, 2.09, 2.08, 2.05, 2.01, 1.99, and 1.95 (21H, 7 s, Me). δ_C (150.8 MHz) 170.4, 170.3, 170.1, 170.1, 169.9, 169.3, and 168.8 (7C, C=O), 147.4, 145.2, 127.8, and 123.6 (6C, Ar), 101.3 (C1′), 91.8 (C1), 75.3 (C5), 74.4 (C5′), 73.5 (C3), 72.3 (*C*H_2Ar), 70.9 (C3′), 69.1 (C4), 68.6 (C2′), 68.5 (C6), 67.8 (C2), 66.8 (C4′), 61.1 (C6′), 20.8, 20.7, 20.6, 20.5, and 20.4 (7C, Me).

Next to elute was 1,2,4-tri-*O*-acetyl-6-*O*-(4-nitrobenzyl)-3-*O*-(tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-glucose (115 mg, 29%), which was isolated as an oil (Found: *m/z* 772.2276. $C_{33}H_{41}NO_{20}$ requires $[M+H]^{+\bullet}$ 772.2300). δ_H (600 MHz) 8.16 and 7.47 (4H, AA′BB′, Ar), 5.59 (d, $J_{1,2}$ 8.4, H1), 5.33 (dd, $J_{3',4'}$ 3.5, $J_{4',5'}$ 1.0, H4′), 5.08–5.05 (m, H2 and H2′), 5.00 (dd, $J_{3,4}$ 9.5, $J_{4,5}$ 9.9, H4), 4.94 (dd, $J_{2',3'}$ 10.4, H3′), 4.60 and 4.58 (AB, *J* 13.4, CH_2Ar), 4.55 (d, $J_{1',2'}$ 8.0, H1′), 4.15 (dd, $J_{5',6'}$ 3.9, $J_{6',6'}$ 11.1, H6′), 4.04 (dd, $J_{5',6'}$ 6.4, H6′), 3.95 (dd, $J_{2,3}$ 9.4, H3), 3.90 (ddd, H5′), 3.76 (ddd, $J_{5,6}$ 2.6 and 2.5, $J_{6,6}$ 11.3, H5), 3.62 (dd, H6), 3.56 (dd, H6), 2.16, 2.11, 2.08, 2.02, 2.00, 1.99, and 1.94 (21H, 7 s, Me). δ_C (150.8 MHz) 170.3, 170.1, 170.1, 170.0, 169.3, 169.1, and 168.9 (7C, C=O), 147.3, 145.3, 127.8, and 123.5 (6C, Ar), 101.1 (C1′), 91.8 (C1), 78.3 (C3), 74.2 (C5), 72.3 (*C*H_2Ar), 70.9 (C3′), 70.5 (C5′), 69.0 (C6), 71.3, 68.7, and 68.3 (C2, C4, and C2′), 66.7 (C4′), 60.9 (C6′), 20.8, 20.7, 20.7, 20.5, 20.4, 20.4, and 20.3 (7C, Me).

1,3,4-Tri-O-*acetyl-6*-O-*benzyl-2*-O-*(tetra*-O-*acetyl-β-D-glucopyranosyl)-β-D-glucose and 1,2,4-Tri*-O-*acetyl-6*-O-*benzyl-3*-O-*(tetra*-O-*acetyl-β-D-glucopyranosyl)-β-D-glucose*

Utilizing the above general procedure, tetra-*O*-acetyl-α-D-glucopyranosyl fluoride (180 mg, 0.52 mmol) and tetra-*O*-acetyl-6-*O*-benzyl-D-glucopyranose **4** (230 mg, 0.52 mmol) firstly gave 1,3,4-tri-*O*-acetyl-6-*O*-benzyl-2-*O*-(tetra-*O*-acetyl-β-D-glucopyranosyl)-β-D-glucose (147 mg, 39%) as an oil (Found: *m/z* 727.2455. $C_{33}H_{42}O_{18}$ requires $[M+H]^{+\bullet}$ 727.2449). δ_H (600 MHz) 7.31–7.25 (m, Ph), 5.61 (d, $J_{1,2}$ 8.1, H1), 5.17–5.16 (m, H3 and H3′), 5.10–5.07 (m, H4 and H4′), 4.89 (dd, $J_{1',2'}$ 8.0, $J_{2',3'}$ 9.9, H2′), 4.55 (d, H1′), 4.45 and 4.40 (AB, *J* 12.5, CH_2Ph), 4.12 (dd, $J_{5',6'}$ 2.7, $J_{6',6'}$ 11.9, H6′), 4.06 (dd, $J_{5',6'}$ 4.4, H6′), 3.83 (dd, $J_{2,3}$ 9.9, H2), 3.71 (ddd, $J_{4,5}$ 10.0, $J_{5,6}$ 3.6 and 2.8, H5), 3.64 (ddd, $J_{4',5'}$ 8.2, H5′), 3.58 (dd, $J_{6,6}$ 11.0, H6), 3.46 (dd, H6), 2.14, 2.11, 2.07, 2.02, 1.99, 1.96, and 1.94 (21H, 7 s, Me). δ_C (150.8 MHz) 170.3, 170.1, 169.9, 169.2, and 168.6 (7C, C=O), 137.2, 128.1, 127.5, and 127.3 (Ph), 100.3 (C1′), 91.9 (C1), 76.8 (C2), 75.2, 68.9, 68.2, and 68.1 (C3, C4, C3′, and C4′), 73.9 (C5), 73.1 (*C*H_2Ph), 71.8 (C5′), 71.0 (C2′), 67.1 (C6), 61.6 (C6′), 20.7, 20.7, 20.6, 20.5, 20.4, 20.4, and 20.2 (7C, Me).

Next to elute was 1,2,4-tri-*O*-acetyl-6-*O*-benzyl-3-*O*-(tetra-*O*-acetyl-β-D-glucopyranosyl)-β-D-glucose (120 mg, 32%), which was isolated as an oil (Found: *m/z* 727.2445. $C_{33}H_{42}O_{18}$ requires $[M+H]^{+\bullet}$ 727.2449). δ_H (600 MHz) 7.38–7.25 (m, Ph), 5.62 (d, $J_{1,2}$ 8.2, H1), 5.14 (dd, $J_{2',3'}$ 10.0, $J_{3',4'}$ 10.0, H3′), 5.10–5.08 (m, H2 and H4′), 4.99 (dd, $J_{3,4}$ 7.9, $J_{4,5}$ 9.9, H4), 4.88 (dd, $J_{1',2'}$ 7.9, H2′), 4.59 (d, H1′), 4.48 and 4.44 (AB, *J* 12.3, CH_2Ph), 4.19 (dd, $J_{5',6'}$ 2.9, $J_{6',6'}$ 11.3, H6′), 4.05 (dd, $J_{5',6'}$ 4.3, H6′), 3.84 (dd, $J_{2,3}$ 8.2, H3), 3.73 (ddd, $J_{5,6}$ 4.9 and 2.7, H5), 3.65 (ddd, $J_{4',5'}$ 8.8, H5′), 3.53 (dd, $J_{6,6}$ 11.2, H6), 3.51 (dd, H6), 2.09, 2.06, 2.05, 2.01, 1.99, 1.96, and 1.94 (21H, 7 s, Me). δ_C (150.8 MHz) 170.8, 170.4, 170.2, 169.9, 169.6, 169.0, and 168.6 (7C, C=O), 137.4, 128.8, 128.0, and 127.1 (Ph), 100.7 (C1′), 91.9 (C1), 74.3 (C5), 73.3 (*C*H_2Ph), 70.9 (C3), 70.5 (C2′), 68.6 (C4), 68.4 (C6), 68.2 (C3′), 72.2 and 67.9 (C2, C4′, and C5′), 61.8 (C6′), 20.8, 20.7, 20.6, 20.5, 20.4, 20.3, and 20.2 (7C, Me).

*General Procedure for the Glycosynthase (E358S)-Mediated Glycosylation of Hemiacetals **2** and **11***

A few drops of NaOMe in MeOH (1.5 M) were added to tetra-*O*-acetyl-α-D-galactopyranosyl or -glucopyranosyl fluoride in MeOH (5 mL) and the resultant solution was stirred for 30 min. The mixture was quenched with resin (Amberlite IR-120, H^+), filtered, and the filtrate was concentrated. The residue was dissolved in NH_4HCO_3 solution (3 mL of 150 mM), the hemiacetal and Abg E358S (2 mg) were added, and the solution was kept at 25°C for 7 days. The solution was then concentrated, the residue was dissolved in Ac_2O (5 mL) that contained NaOAc (50 mg), and the solution was heated at reflux for 10 min. The reaction was quenched by the addition of ice/water. Usual workup (CH_2Cl_2) followed by flash chromatography (EtOAc/light petroleum, 1 : 4 to 1 : 1) gave the appropriate per-acetylated disaccharide(s).

1,3,4-Tri-O-*acetyl-6*-O-*benzoyl-2*-O-*(tetra*-O-*acetyl-β-D-galactopyranosyl)-β-D-glucose and 1,2,4-Tri*-O-*acetyl-6*-O-*benzoyl-3*-O-*(tetra*-O-*acetyl-β-D-galactopyranosyl)-β-D-glucose*

Utilizing the above general procedure, tetra-*O*-acetyl-α-D-galactopyranosyl fluoride (270 mg, 0.77 mmol) and 6-*O*-benzoyl-D-glucopyranose **2** (140 mg, 0.51 mmol) firstly gave 1,3,4-tri-*O*-acetyl-6-*O*-benzoyl-2-*O*-(tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-glucose (87 mg, 41%) as an oil (Found: *m/z* 741.2275. $C_{33}H_{40}O_{19}$ requires $[M+H]^{+\bullet}$ 741.2242). δ_H (600 MHz) 8.04–8.00, 7.57–7.54, and 7.45–7.43 (3 m, Ph), 5.72 (d, $J_{1,2}$ 7.9, H1), 5.35 (dd, $J_{3',4'}$ 3.5, $J_{4',5'}$ 1.1, H4′), 5.25 (dd, $J_{2,3}$ 9.3, $J_{3,4}$ 9.3, H3), 5.13 (dd, $J_{1',2'}$ 8.0, $J_{2',3'}$ 9.0, H2′), 5.09 (dd, $J_{4,5}$ 9.3, H4), 4.94 (dd, H3′), 4.60 (d, H1′), 4.45 (dd, $J_{5,6}$ 2.3, $J_{6,6}$ 12.4, H6), 4.38 (dd, $J_{5,6}$ 4.4, H6), 4.16 (dd, $J_{5',6'}$ 6.6, $J_{6',6'}$ 11.3, H6′), 4.06 (dd, $J_{5',6'}$ 6.8, H6′), 3.96 (ddd, H5), 3.87 (ddd, H5′), 3.85 (dd, H2), 2.18, 2.15, 2.06, 2.03, 2.02, 2.01, and 1.96 (21H, 7 s, Me). δ_C (150.8 MHz) 170.4, 170.2, 170.1, 169.8, 169.6, 169.0, 168.7, and 166.0 (8C, C=O), 133.2, 129.7, 129.5, and 128.4 (Ph), 101.2 (C1′), 92.0 (C1), 77.0 (C2), 74.5 (C3), 72.4 (C5), 70.9 (C3′), 70.6 (C5′), 68.6 (C4), 68.3 (C2′), 66.8 (C4′), 62.2 (C6), 61.1 (C6′), 20.7, 20.6, 20.6, 20.6, 20.5, 20.4, and 20.4 (7C, Me).

Next to elute was 1,2,4-tri-*O*-acetyl-6-*O*-benzoyl-3-*O*-(tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-glucose (64 mg, 30%), which was isolated as an oil (Found: *m/z* 741.2235. $C_{33}H_{40}O_{19}$ requires $[M+H]^{+\bullet}$ 741.2242). δ_H (600 MHz) 8.05–8.03, 7.58–7.54, and 7.46–7.43 (3 m, Ph), 5.65 (d, $J_{1,2}$ 7.9, H1), 5.34 (dd, $J_{3',4'}$ 3.5, $J_{4',5'}$ 1.1, H4′), 5.14 (dd, $J_{3,4}$ 9.4, $J_{4,5}$ 8.3, H4), 5.09 (dd, $J_{2,3}$ 9.1, H2), 5.05 (dd, $J_{1',2'}$ 8.0, $J_{2',3'}$ 8.8, H2′), 4.95 (dd, H3′), 4.57 (d, H1′), 4.50 (dd, $J_{5,6}$ 2.6, $J_{6,6}$ 12.1, H6), 4.29 (dd, $J_{5,6}$ 4.4, H6), 4.24 (dd, $J_{5',6'}$ 5.9, $J_{6',6'}$ 11.0, H6′), 4.19 (ddd, $J_{5',6'}$ 3.0, H5′), 4.05 (dd, H3), 4.01 (dd, H6′), 3.93 (ddd, H5), 2.15, 2.13, 2.06, 2.03, 2.02, 1.99, and 1.95 (21H, 7 s, Me). δ_C (150.8 MHz) 170.3, 170.2, 170.1, 169.3, 169.1, 169.0, 168.8, and 166.1 (8C, C=O), 133.1, 129.8, 129.5, and 128.4 (Ph), 101.2 (C1′), 91.8 (C1), 78.4 (C3), 72.8 (C5), 71.9 (C4), 71.0 (C3′), 70.2 (C5′), 68.7 (C2′), 68.1 (C2), 66.8 (C4′), 62.4 (C6), 60.9 (C6′), 20.7, 20.6, 20.5, 20.5, 20.5, 20.5, and 20.4 (7C, Me).

1,3,4-Tri-O-*acetyl-6*-O-*benzoyl-2*-O-*(tetra*-O-*acetyl-β-D-glucopyranosyl)-β-D-glucose and 1,2,4-Tri*-O-*acetyl-6*-O-*benzoyl-3*-O-*(tetra*-O-*acetyl-β-D-glucopyranosyl)-β-D-glucose*

Utilizing the above general procedure, tetra-*O*-acetyl-α-D-glucopyranosyl fluoride (180 mg, 0.51 mmol) and 6-*O*-benzoyl-D-glucopyranose **2** (140 mg, 0.51 mmol) firstly gave 1,3,4-tri-*O*-acetyl-6-*O*-benzoyl-2-*O*-(tetra-*O*-acetyl-β-D-glucopyranosyl)-β-D-glucose (91 mg, 43%) as an oil (Found: *m/z* 741.2239. $C_{33}H_{40}O_{19}$ requires $[M+H]^{+\bullet}$ 741.2242). δ_H (600 MHz) 8.03–7.99, 7.58–7.54, and 7.44–7.42 (3 m, Ph), 5.71 (d, $J_{1,2}$ 7.8, H1), 5.47 (dd, $J_{2,3}$ 9.4, $J_{3,4}$ 9.5, H3), 5.16 (dd, $J_{2',3'}$ 9.6, $J_{3',4'}$ 9.5, H3′), 5.12 (dd, $J_{4,5}$ 8.2, H4), 5.08 (dd, $J_{4',5'}$ 8.6, H4′), 4.89 (dd, $J_{1',2'}$ 7.9, H2′), 4.59 (d, H1′), 4.46 (dd, $J_{5,6}$ 2.2, $J_{6,6}$ 12.1, H6), 4.39 (dd, $J_{5,6}$ 4.5, H6), 4.09–4.05 (3H, m, H5 and H6′), 3.86 (dd, H2), 3.64 (ddd, $J_{5',6'}$ 4.4 and 2.8, H5′), 2.15, 2.11, 2.06, 2.03, 1.99, 1.97, and 1.96 (21H, 7 s, Me). δ_C (150.8 MHz) 170.7, 170.3, 170.2, 169.8, 169.6, 169.2, 168.6, and 166.0 (8C, C=O), 133.1, 129.6, 129.5, and 128.3 (Ph), 100.9 (C1′), 92.0 (C1), 78.3 (C3), 72.7 (C5), 72.0 (C4), 71.8 (C5′), 70.9 (C2′), 68.2 (C2), 68.2 (C3′), 68.0 (C4′), 62.3 (C6), 61.5 (C6′), 20.7, 20.65, 20.5, 20.5, 20.5, 20.4, and 20.3 (7C, Me).

Next to elute was 1,2,4-tri-*O*-acetyl-6-*O*-benzoyl-3-*O*-(tetra-*O*-acetyl-β-D-glucopyranosyl)-β-D-glucose (70 mg, 33%), which was isolated as an oil (Found: *m/z* 741.2237. $C_{33}H_{40}O_{19}$ requires $[M+H]^{+\bullet}$ 741.2242). δ_H (600 MHz) 8.05–8.03, 7.55–7.53, and 7.47–7.43 (3 m, Ph), 5.64 (d, $J_{1,2}$ 8.1, H1), 5.16–5.14 (m, H3′ and H4), 5.10–5.08 (m, H2 and H4′), 4.88 (dd, $J_{1',2'}$ 8.0, $J_{2',3'}$ 9.4, H2′), 4.58 (d, H1′), 4.50 (dd, $J_{5,6}$ 2.6, $J_{6,6}$ 12.2, H6), 4.29 (dd, $J_{5,6}$ 4.4, H6), 4.21 (dd, $J_{5',6'}$ 2.9, $J_{6',6'}$ 11.3, H6′), 4.10–4.06 (2H, m, H3 and H6′), 3.93 (ddd, $J_{4,5}$ 8.3, H5), 3.63 (ddd, $J_{4',5'}$ 8.5, $J_{5',6'}$ 4.4, H5′), 2.12, 2.09, 2.04, 2.03, 2.02, 1.99, and 1.95 (21H, 7 s, Me). δ_C (150.8 MHz) 170.4, 170.3, 170.1, 169.2, 169.0, 169.0, 168.7, and 166.2 (8C, C=O), 133.2, 129.8, 129.7, and 128.4 (Ph), 100.8 (C1′), 92.0 (C1), 78.4 (C3), 72.7 (C5), 71.9 (C5′), 71.9 (C4), 70.8 (C2′), 68.1 (C2 and C3′), 67.8 (C4′), 62.7 (C6), 61.5 (C6′), 20.7, 20.5, 20.5, 20.5, 20.4, 20.3, and 20.2 (7C, Me).

1,3-Di-O-*acetyl-4*-O-*benzyl-2*-O-*(tetra*-O-*acetyl-β-D-galactopyranosyl)-β-D-xylose*

Utilizing the above general procedure, tetra-*O*-acetyl-α-D-galactopyranosyl fluoride (110 mg, 0.31 mmol) and 4-*O*-benzyl-D-xylose **11** (50 mg, 0.20 mmol) gave 1,3-di-*O*-acetyl-4-*O*-benzyl-2-*O*-(tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-xylose (76 mg, 56%) as an oil (Found: *m/z* 655.2229. $C_{30}H_{38}O_{16}$ requires $[M+H]^{+\bullet}$ 655.2238). δ_H (600 MHz) 7.35–7.28 and 7.25–7.33 (2 m, Ph), 5.59 (d, $J_{1,2}$ 7.3, H1), 5.33 (dd, $J_{3',4'}$ 3.4, $J_{4',5'}$ 1.1, H4′), 5.18 (dd, $J_{2,3}$ 9.0, $J_{3,4}$ 8.9, H3), 5.10 (dd, $J_{1',2'}$ 9.8, $J_{2',3'}$ 10.5, H2′), 4.92 (dd, H3′), 4.55 (d, H1′), 4.57 and 4.49 (AB, J 12.2, CH_2Ph), 4.14 (dd, $J_{5',6'}$ 6.9, $J_{6',6'}$ 11.2, H6′), 4.04 (dd, $J_{5',6'}$ 6.5, H6′), 3.95 (dd, $J_{4,5}$ 4.9, $J_{5,5}$ 11.7, H5), 3.85 (ddd, H5′), 3.66 (dd, H2), 3.52 (ddd, $J_{4,5}$ 9.8, H4), 3.42 (dd, H5), 2.13, 2.73, 2.06, 2.04, 2.03, and 1.96 (18H, 6 s, Me). δ_C (150.8 MHz) 170.4, 170.2, 169.4, 169.4, and 168.8 (6C, C=O), 137.5, 128.5, 128.0, and 127.7 (Ph), 101.2 (C1′), 92.8 (C1), 76.8 (C2), 74.6 (C4), 74.4 (C3), 72.4 (*C*H$_2$Ph), 70.9 (C3′), 70.5 (C5′), 68.5 (C2′), 66.8 (C4′), 64.0 (C5), 61.1 (C6′), 21.0, 20.9, 20.9, 20.7, 20.6, and 20.5 (6C, Me).

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