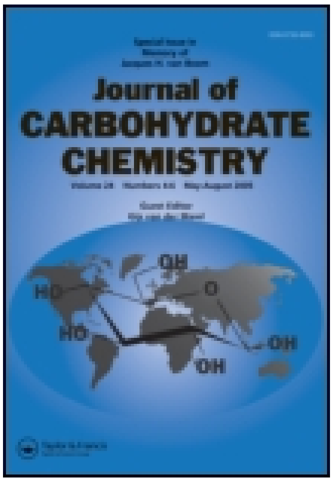

Journal of Carbohydrate Chemistry
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/lcar20

EFFICIENT SYNTHESIS OF POLYLACTOSAMINE STRUCTURES THROUGH REGIOSELECTIVE GLYCOSYLATIONS

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Published online: 16 Aug 2006.

J. CARBOHYDRATE CHEMISTRY, 20(7&8), 569–583 (2001)

EFFICIENT SYNTHESIS OF POLYLACTOSAMINE STRUCTURES THROUGH REGIOSELECTIVE GLYCOSYLATIONS[1]

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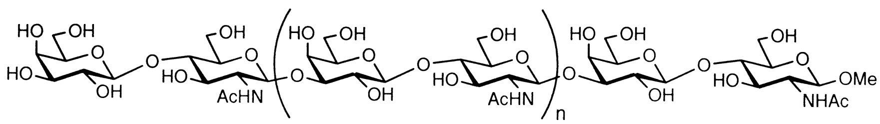
ABSTRACT

Di-, tri- and tetramers of β-(1→3)-linked *N*-acetyllactosamine residues have been synthesised as their methyl glycosides, to be used in ITC binding studies to various galectins. The synthetic strategy involves two types of regioselective glycosylations: couplings of a galactosyl donor to 3,4-diol *N*-tetrachlorophthalimido glucose acceptors to give the lactosamine monomer building blocks, and subsequent formation of the oligomers through consecutive couplings of lactosamine donors to 2′,3′,4′-lactosamine acceptors, with high selectivity for the desired products.

INTRODUCTION

Polylactosamines consist of β-(1→3)-linked *N*-acetyllactosamine chains attached to sphingolipids and proteins. They are the backbones of keratan sulfates and are present at the surface of mammalian cells with or without distal decorations (sulfate groups, single monosaccharides, and numerous oligosaccharide determinants).[2] A few chemical syntheses of polylactosamine structures have already been published, the tetramer by Alais and Veyrieres,[3–5] Srivastava and Hindsgaul[6] and Shimizu et al.,[7] the latter performed on solid phase, and the trimer by Nilsson and Norberg[8] as an intermediate in the synthesis of a trimeric Lewis x structure. Also enzymatic syntheses have been described.[9]



n=0 **17**, n=1 **20**

Figure 1.

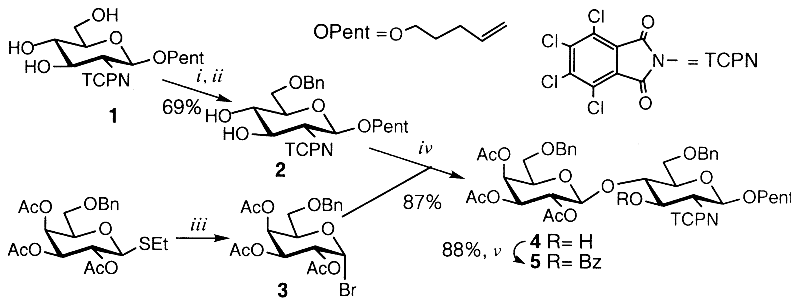
Galectins, named after their preferential binding to β-galactose motifs, are known to have a number of important biological functions,[10,11] and to further investigate these, the study of their binding to polylactosamine structures was important. Lactosamine oligomers with varying length were essential for these studies. Consequently, the di- and trimer of β-(1→3)-linked *N*-acetyllactoseamine residues (**17** and **20**, Figure 1) were synthesised. These will primarily be used in isothermal titration calorimetry (ITC) studies of their binding to galectins, to render possible the determination of the size of the binding site and the thermodynamics of the binding.

RESULTS AND DISCUSSION

Although a number of syntheses of 2-amino-2-deoxylactose from a disaccharide precursor have been published,[3,12–14] the alternative to start from monosaccharides is still a relevant option. The "drawback" of having to perform an extra glycosylation reaction is balanced by the advantage of the easy access to monosaccharide precursors, protected in a suitable way for subsequent reactions. Especially with phthalimido-protected derivatives the latter approach is feasible, since it has been found that the coupling between galactosyl donors and, easily obtainable, 3,4-diol *N*-phthalimido glucose acceptors is high-yielding and very regioselective for the 4-position due to the bulkiness of the phthalimido group.[15–17] This regioselectivity is even more pronounced for tetrachlorophthalimido compounds.[18]

Regioselective benzylation of the tin-activated pentenyl glycoside **1**[19] gave directly the 3,4-diol **2** in 69% yield (Scheme 1). This procedure was found to be more convenient than the two-step one, involving 4,6-*O*-benzylidene formation and reductive opening used earlier.[20] The silver triflate-promoted coupling between **2** and galactosyl donor **3** (obtained from the known corresponding thioethyl glycoside[21] by bromine treatment) proceeded in high yield (87%) and with regiospecificity. The 1→4-linkage in the product **4** was proven by the downfield shift of the H-3 proton in the benzoylated derivative **5**. To allow for more flexibility in the synthetic strategy, thioglycoside donors were also synthesised (Scheme 2). Hence, derivatives **10** and **12** were synthesised in a parallel fashion from the same galactosyl donor (**3**) and the 3,4-diol thioglycoside acceptors **7** and **8**,[17] respectively. Once more the glycosylations gave high regioselectivity and yield of products.

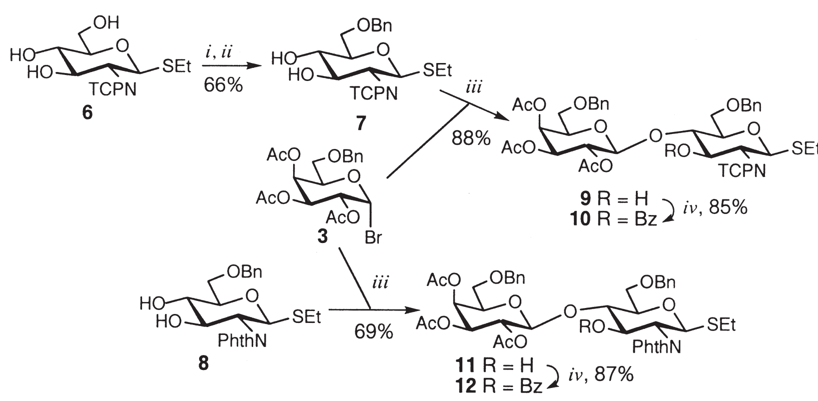
Since the primary use of the target compounds was to perform ITC-studies of



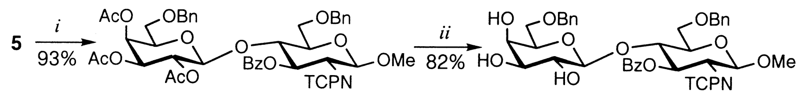
Scheme 1. *i*: $(Bu_3Sn)_2O$, MeOH; *ii*: BnBr, QBr, 90 °C ; *iii*: Br_2, CH_2Cl_2; *iv*: AgOTf, CH_2Cl_2, 3Å MS, −40°C; *v*: BzCl, pyridine.

their binding to various animal lectins, and accordingly no conjugate formation was necessary, it was decided to synthesise them as their methyl β-glycosides. Therefore, the pentenyl glycoside **5** was transformed into the methyl glycoside **13** (93%) by treatment with 5 equiv of MeOH in the presence of NIS/TESOTf (triethylsilyl triflate) (Scheme 3). If a larger excess of MeOH was used, the major reaction was addition of MeOH to the double bond.

Another known type of regioselective glycosylation, preferentially used in sialylation of galactose derivatives, was utilised to synthesise oligomers. In 2,3,4-triol galactose acceptors a high selectivity for the 3-hydroxyl group is usually observed in glycosylations, sialylation on 2,4-protected derivatives being often impossible.[22] Already anticipating this route when chosing galactosyl donor **3**, the acetyl groups of compound **13** were now removed chemoselectively by treatment with HCl in MeOH[23] to give the 2′,3′,4′-triol **14** (82%). In the coupling reaction to give the dimer it was most important to match the reactivity of the reacting species, acceptor, donor and promoter, to get a high yield of product (Table 1, Scheme 4). However, all the glycosylations were regioselective for the



Scheme 2. *i*: $(Bu_3SnO)_2$, MeOH; *ii*: BnBr (5 eq), TEABr, toluene, 90 °C; *iii*: AgOTf, CH_2Cl_2, 3Å MS, −40°C; *iv*: BzCl, pyridine.



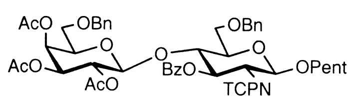
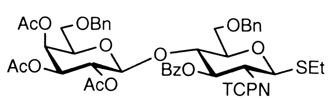
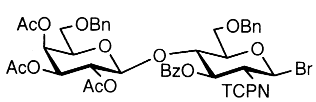
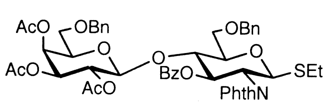
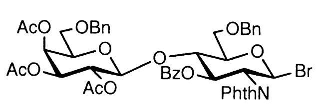
13 14

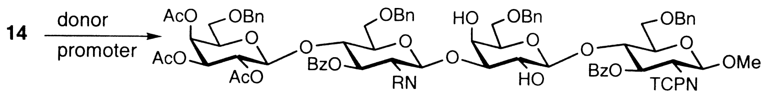
Scheme 3. *i*: MeOH, NIS, TESOTf, CH_2Cl_2, 3Å MS; ii: 5% HCl/MeOH.

3-position. The use of an effective promoter combined with especially tetrachlorophthalimido donors gave mainly decomposition of the donor. If the promoter was changed into a less active one or if a phthalimido donor was employed, good yields of the desired β-(1→3)-linked products **15** and **16** were obtained.

Choosing the most high-yielding conditions (donor **10**, promoter MeOTf) from this dimer glycosylation study, syntheses of a trimer and a tetramer were attempted (Scheme 5). Chemoselective removal of the three acetates from derivative **15**, again proceeded smoothly to give an 88% yield of compound **18**. Reasoning

Table 1. Glycosylation of Acceptor **14**. General Conditions: 1.7 Equiv of the Donor, 3Å MS, CH_2Cl_2, Ar. Bromide Donors were Prepared by Br_2 Treatment of the Corresponding Thioglycoside Just Prior to Coupling. [a]The Donor Decompose

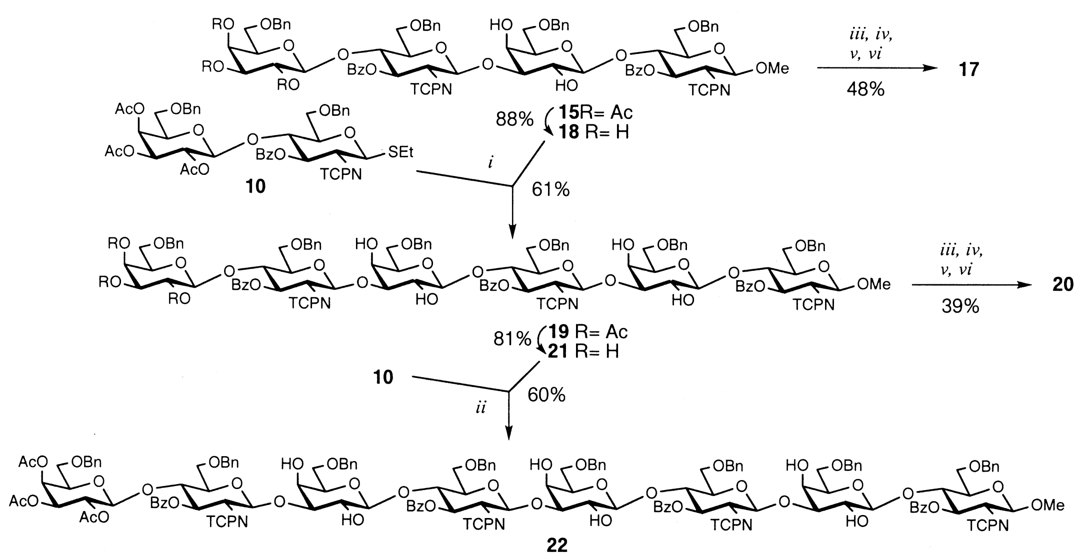
Donor	Promoter	temp	yield (%)/product
 **5**	NIS/TESOTf	rt	17%/**15**
	IDCT	rt	44%/**15**
 **10**	NIS/TMSOTf	rt	a
	MeOTf	rt	69%/ **15**
	DMTST	rt	61%/**15**
	AgOTf	-40 °C	a
 **12**	NIS/TESOTf	rt	50%/**16**
	AgOTf	-40 °C	62%/**16**



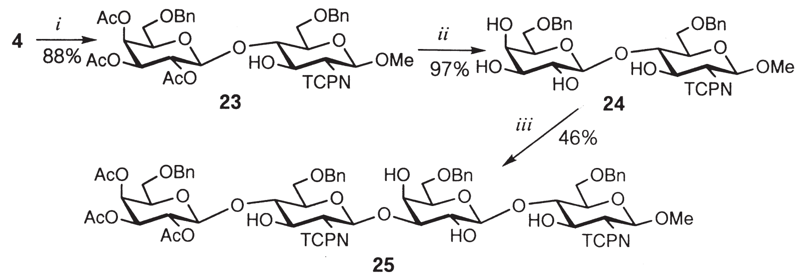
Scheme 4.

that glycosylation at the 3′-position should even more decrease the already low nucleophilicity of the 2′- and 4′-hydroxyl groups, pentaol **18** was tried as an acceptor in a glycosylation employing the conditions used above, and was found to give the trimer **19** in a most satisfactory yield (61%). Continued use of this methodology then effectively gave first the heptaol acceptor **21** (81%) and then the tetramer **22** (60%, 69% calculated on consumed acceptor).

Since the 3-position of the glucosamine residue, as discussed and shown, is quite unreactive also, perhaps its protection was not necessary, i.e., the regioselective glycosylation could be performed on a 3,2′,3′,4′-tetraol acceptor using a 3-OH donor. This approach would have the advantage of fewer reaction steps (no benzoylation) and more easily performed deacetylation steps (nonchemoselective). To test this hypothesis the pentenyl glycoside **4** was transformed into the methyl glycoside **23** using the same conditions as for the benzoylated analogue **5** above, and in comparable yield (88%) (Scheme 6). Removal of the acetyl protecting groups then gave the tetraol **24** in almost quantitative yield (97%). Coupling between acceptor **24** and the non-benzoylated donor **9** using methyl triflate as promoter pro-



Scheme 5. *i*: HCl/MeOH-CH_2Cl_2 1:1; *ii*: MeOTf, CH_2Cl_2, 3Å MS; *iii*: H_2NNH_2-H_2O, MeCN-THF-EtOH 2:1:1, 70 °C; *iv*: Ac_2O, pyridine; v: H_2, Pd/C; *vi*: 1M NaOMe, MeOH.



Scheme 6. *i*: MeOH, NIS, TESOTf, CH_2Cl_2, 3Å MS; *ii*: 5% HCl/MeOH; *iii*: 9, MeOTf, CH_2Cl_2, 3Å MS.

ceeded to give one major product, which by NMR was shown to be the expected β-(1→3)-linked tetrasaccharide **25** (46%), proving this to be an attractive alternative pathway to lactosamine oligomers.

Deprotection of the oligomers **15** and **19** was accomplished using standard conditions, hydrazine hydrate at elevated temperature followed by acetylation, hydrogenolysis and deacetylation with sodium methoxide (Scheme 5). Although rather straightforward, the yield in the deprotection is acceptable but not high, as is also the case with deprotections of similar compounds found in the literature.[3–8] The yields are normally in the range of 40–50% corresponding to an 80–84% yield in each step in a four-step deprotection scheme.

EXPERIMENTAL

General Methods. Organic solutions were dried over $MgSO_4$ before concentrations, which were performed under reduced pressure at ≤40°C (water bath). TLC was performed on silica gel 60 F_{254} (Merck) with detection by UV light and/or charring with 8% sulfuric acid, ninhydrin or AMC (ammonium molybdate 10g, cerium(IV) sulfate 2 g, dissolved in aq 10% H_2SO_4 2 L). Silica gel (0.0404–0.063 mm, Amicon) was used for column chromatography. NMR spectra were recorded in $CDCl_3$ at 25 °C (internal standard $Me_4Si, \delta = 0.00$) unless otherwise stated, using a Varian Mercury 300 at 300 MHz (^{1}H) or 75 MHz (^{13}C) or a Varian Inova 400 at 400 MHz (^{1}H) or 100 MHz (^{13}C).

Pent-4-enyl 6-*O*-Benzyl-2-deoxy-2-tetrachlorophthalimido-β-D-glucopyranoside (2). Bis(tributyltin) oxide (14.5 mL, 28.54 mmol) was added to a solution of **1**[19] (10.5 g, 20.39 mmol) in dry MeOH (120 mL) stirred under argon. After stirring at 75°C for 2 h the reaction mixture was concentrated and dried *in vacuo*. The oily tin ether complex was dissolved in benzyl bromide (40 mL) and tetrabutylammonium bromide (7.22 g, 22.4 mmol) was added. After stirring at 90°C for 20 h benzyl bromide was distilled from the reaction vessel. Flash chromatography (two columns: toluene-EtOAc 20:1→5:1 and toluene-EtOAc 5:1) of the oily residue furnished **2** (8.51 g, 14.07 mmol, 69%) having NMR data in agreement with those reported elsewhere.[20]

Pent-4-enyl (2,3,4-Tri-*O*-acetyl-6-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-3-*O*-benzoyl-6-*O*-benzyl-2-deoxy-2-tetrachlorophthalimido-β-D-glucopyranoside (5). A solution of ethyl 2,3,4-tri-*O*-acetyl-6-*O*-benzyl-1-thio-β-D-galactopyranoside[21] (6.59 g, 14.96 mmol) and bromine (2.3 mL, 44.89 mmol) in CH_2Cl_2 (100 mL) was stirred under argon for 30 min at room temperature. The mixture was then concentrated and co-concentrated twice with toluene (Na-dried). A solution of the residue and **2** (6.87 g, 11.26 mmol) in CH_2Cl_2 (150 mL) was stirred with 3Å MS for 30 min at −60°C before AgOTf (7.30 g, 28.40 mmol) was added. The reaction was quenched with triethylamine (6 mL) after 1 h and the mixture was stirred for another 15 min, filtered through Celite and concentrated. Flash chromatography of the residue (toluene-EtOAc 6:1) gave pent-4-enyl (2,3,4-tri-*O*-acetyl-6-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-6-*O*-benzyl-2-deoxy-2-tetrachlorophthalimido-β-D-glucopyranoside (**4**) (9.51 g, 9.79 mmol, 87%). NMR ($CDCl_3$): ^{13}C, δ 21.1 (2C), 21.2 (CH_3 acetyl), 29.1, 30.4 (CH_2 pentenyl), 56.7, 67.4, 68.0, 68.2, 69.0, 69.1, 69.6, 71.0, 72.5, 73.6, 73.8, 74.4, 81.74 (C-2–6, 2′-6′, OCH_2 pentenyl, CH_2 benzyl), 98.0 (C-1), 101.3 (C-1′), 115.0 (CH_2 = pentenyl), 125.6–140.1 (aromatic C, CH = pentenyl), 162.4, 162.5 (C═O TCP), 169.4, 170.1, 170.3 (C═O acetyl); ^1H, δ 1.53–1.64 (m, 2H, CH_2 pentenyl), 1.89–1.94 (m, 2H, CH_2 pentenyl), 1.97, 1.98, 2.06 (s, 9H, CH_3 acetyl), 3.41–3.87 (m, 8H, OCH_2 pentenyl, H-4, 5, 5′, 6, and 6′), 4.17 (dd, 1H, H-2), 4.25–4.55 (5H, H-1′, 3, CH_2 benzyl), 4.71 (d, 1H, CH_2 benzyl), 4.82–4.87 (m, 2H, CH_2 = pentenyl), 4.94 (dd, 1H, H-3′), 5.09 (d, 1H, $J_{1,2}$ 8.42 Hz, H-1), 5.17 (dd, 1H, H-2′), 5.37 (d, 1H, H-4′), 5.58–5.74 (m, 1H, CH= pentenyl), 7.15–7.36 (m, 10H, aromatic H). Benzoyl chloride (1.63 mL, 14.0 mmol) and pyridine (2.3 mL) were added to a stirred solution of **4** (1.38 g, 1.40 mmol) in dry CH_2Cl_2 (20 mL) and the reaction mixture was stirred at ambient temperature overnight and then concentrated. The residue was dissolved in CH_2Cl_2 and the organic layer was washed with aq 1M HCl, satd aq $NaHCO_3$, and H_2O, dried and concentrated. Flash chromatography (toluene-EtOAc 8:1) of the residue gave **5** (1.35 g, 1.24 mmol, 88%). NMR ($CDCl_3$): ^{13}C, δ 21.1 (2C), 21.3 (CH_3 acetyl), 29.1, 30.4 (CH_2 pentenyl), 56.1, 66.3, 67.2, 67.8, 69.5, 70.0, 71.6, 72.3, 73.5, 74.2, 75.1, 75.9 (C-2–6, 2′-6′, OCH_2 pentenyl, CH_2 benzyl), 98.2 (C-1), 100.7 (C-1′), 115.2 (CH_2= pentenyl), 127.8–140.4 (aromatic C, CH = pentenyl), 162.6, 162.8 (C═O TCP) 165.6 (C═O benzoyl), 169.2, 170.2 (2C) (C═O acetyl); ^1H, δ 1.56–1.64 (m, 2H, CH_2 pentenyl), 1.85, 1.92, 1.94 (s, 9H, CH_3 acetyl), 1.89–1.98 (m, 2H, CH_2 pentenyl), 3.47–3.51 (m, 1H, OCH_2 pentenyl), 2.70 (dd, 1H), 2.91 (dd, 1H) 3.65–3.69 (m, 1H), 3.74–3.87 (m, 3H, OCH_2 pentenyl, H-6), 4.07 (d, 1H, CH_2 benzyl), 4.16 (dd, 1H, H-4), 4.25 (d, 1H, CH_2 benzyl), 4.38 (dd, 1H, H-2), 4.45 (d, 1H, $J_{1,2}$ 8.06 Hz, H-1′), 4.54 (d, 1H, CH_2 benzyl), 4.74–4.89 (m, 4H, H-3′, CH_2 benzyl, CH_2= pentenyl), 4.94 (dd, 1H, H-2′), 5.22 (d, 1H, H-4′), 5.35 (d, 1H, $J_{1,2}$ 8.42 Hz, H-1), 5.64–4.74 (m, 1H, CH= pentenyl), 5.88 (dd, 1H, H-3) 7.14–8.17 (m, 15H, aromatic H). MS (MALDI-TOF): $(M+Na)^+$ 1108.1.

Ethyl (2,3,4-Tri-*O*-acetyl-6-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-3-*O*-benzoyl-6-*O*-benzyl-2-deoxy-2-tetrachlorophthalimido-1-thio-β-D-glucopyranoside (10). Bis(tributyltin) oxide (3.53 mL, 6.93 mmol) was added to a solution of **6**[24] (3.09 g, 6.3 mmol) in dry MeOH (60 mL) stirred under argon. The reaction was stirred at 75°C for 2 h, concentrated and dried in vacuo. The yellow oil was dissolved in toluene (Na-dried) (70 mL) and benzyl bromide (3.75 mL, 31.5 mmol) and tetrabutylammonium bromide (2.13 g, 6.62 mmol) were added. The reaction was kept at 90°C for 21 h and then concentrated. Purification by flash chromatography (toluene-EtOAc 4:1) furnished ethyl 6-*O*-benzyl-2-deoxy-2-tetrachlorophthalimido-1-thio-β-D-glucopyranoside (**7**) (2.45 g, 4.22 mmol, 67%). NMR ($CDCl_3$): ^{13}C, δ 15.3 (CH_3CH_2S), 24.5 (CH_2S), 56.2, 70.6, 72.4, 73.7, 74.0, 78.3 (C-2–6, CH_2 benzyl), 81.1 (C-1), 125.5–140.4 (aromatic C). Ethyl 2,3,4-tri-*O*-acetyl-6-*O*-benzyl-1-thio-β-D-galactopyranoside[21] (1.9 g, 4.34 mmol) was converted into the corresponding bromosugar **3**, by treatment with bromine (0.45 mL, 8.68 mmol). A mixture of **3**, acceptor **7** (1.8 g, 3.1 mmol) and 3Å MS in CH_2Cl_2 (40 mL) was stirred at −60°C for 30 min, whereafter AgOTf (2.23 g, 8.68 mmol) was added. After 45 min the reaction was quenched by addition of triethylamine (1 mL) and the mixture was diluted with CH_2Cl_2 and filtered through Celite and concentrated. Flash chromatography (toluene-EtOAc 6:1) gave ethyl (2,3,4-tri-*O*-acetyl-6-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-6-*O*-benzyl-2-deoxy-2-tetrachlorophthalimido-1-thio-β-D-glucopyranoside (**9**) (2.61 g, 2.73 mmol, 88%). NMR ($CDCl_3$): ^{13}C, δ 15.3 (CH_3CH_2S), 20.9, 21.0, 21.1 (CH_3 acetyl), 24.3 (CH_2S), 56.0, 67.6, 68.2, 68.5, 69.3, 70.6, 71.2, 72.7, 73.8, 74.0, 78.6, 81.0 (C-2–6, 2′-6′, CH_2 benzyl), 81.6 (C-1), 101.5 (C-1′), 125.9–140.3 (aromatic C), 162.9, 163.4 (C═O TCP), 169.3, 170.0, 170.2 (C═O acetyl). Benzoyl chloride (1.40 mL, 12.0 mmol) was added to a stirred solution of **9** (2.3 g, 2.40 mmol) in pyridine (35 mL). After stirring at room temperature overnight the reaction mixture was concentrated and worked up as described for **5**. Flash chromatography (toluene-EtOAc 4:1) of the crude product afforded **10** (2.17 g, 2.04 mmol, 85%). $[\alpha]_D$ +69°(*c* 1.1, $CHCl_3$) NMR ($CDCl_3$): ^{13}C, α15.0 (CH_3CH_2S), 20.4, 20.5, 20.7 (CH_3 acetyl), 24.2 (CH_2S), 54.5, 65.6, 66.6, 67.4, 69.4, 71.0 (2C), 72.3, 72.9, 73.6, 74.9, 78.7 (C-2–6, 2′-6′, CH_2 benzyl), 80.5 (C-1), 100.0 (C-1′), 125.0–140.1 (aromatic C), 162.1, 162.6 (C═O TCP), 165.0 (C═O benzoyl), 168.6, 169.6 (C═O acetyl); ^1H, δ 1.24 (t, 3H, CH_3CH_2S), 1.83, 1.91, 1.94 (s, 9H, CH_3 acetyl), 2.64–2.75 (m, 3H, CH_2S), 2.89–2.92 (m, 1H), 3.30–3.34 (m, 1H), 3.70–3.79 (3H, H-5, H-6), 4.09 (d, 1H, CH_2 benzyl), 4.19 (dd, 1H, H-4), 4.26 (d, 1H, CH_2 benzyl), 4.46–4.55 (m, 3H, H-2, H-1′, CH_2 benzyl), 4.76–4.79 (m, 2H, H-3′, CH_2 benzyl), 4.95 (dd, 1H, H-4′), 5.47 (d, 1H, $J_{1,2}$ 10.62 Hz, H-1), 5.95 (dd, 1H, H-3), 7.12–8.10 (m, 15H, aromatic H).

Anal. Calcd for $C_{49}H_{47}O_{15}NCl_4$: C, 55.33; H, 4.45%. Found: C, 55.19; H, 4.48%.

Ethyl (2,3,4-Tri-*O*-acetyl-6-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-3-*O*-benzoyl-6-*O*-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (12). A solution of ethyl 2,3,4-tri-*O*-acetyl-6-*O*-benzyl-1-thio-β-D-galactopyranoside[21] (3.45 g, 7.83 mmol) and bromine (0.8 mL, 15.65 mmol) in CH_2Cl_2 (60 mL) was stirred for 30 min at room temperature and under argon. The mixture was then concentrated and co-evaporated twice from toluene (Na-dried). A solution of the residue and ethyl 6-*O*-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside[17] (**8**, 2.89 g, 6.53 mmol) in CH_2Cl_2 (75 mL) was stirred with 3Å MS for 30 min at −60°C before AgOTf (4.0 g, 15.67 mmol) was added. After 1 h, triethylamine (3 mL) was added and the mixture was allowed to attain room temperature, whereafter it was filtered through Celite and concentrated. Flash chromatography of the residue (toluene-EtOAc 6:1) gave ethyl (2,3,4-tri-*O*-acetyl-6-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-6-*O*-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (**11**) (3.70 g, 4.51 mmol, 69%). NMR ($CDCl_3$): ^{13}C, δ 15.4 (CH_3CH_2S), 20.9 (2C), 21.1 (CH_3 acetyl), 24.2 (CH_2S), 55.5, 67.6, 67.7, 68.5, 69.3, 71.0, 71.2, 72.6, 73.8, 73.9, 78.6, 81.3 (C-2–6, 2′-6′, CH_2 benzyl), 81.7 (C-1), 101.5 (C-1′), 123.4–138.3 (aromatic C), 167.8, 168.2 (C═O NPhth), 169.4, 170.0, 170.2 (C═O acetyl). Benzoyl chloride (2.09 mL, 18.04 mmol) was added to a stirred solution of **11** (3.70 g, 4.51 mmol) in pyridine (100 mL). After stirring in room temperature for 15 h the reaction was concentrated and then worked up as described for compound **5**. Flash chromatography (toluene-EtOAc 6:1) gave **12** (3.63 g, 3.92 mmol, 87%). NMR ($CDCl_3$): ^{13}C, δ 15.6 (CH_3CH_2S), 20.9, 20.9, 21.1 (CH_3 acetyl), 24.5 (CH_2S), 54.2, 66.1, 67.1, 68.0, 69.9, 71.5, 72.8 (2C), 73.3, 74.0, 75.8, 79.2 (C-2–6, 2′-6′, CH_2 benzyl), 81.3 (C-1), 100.57 (C-1′), 125.5–138.0 (aromatic C), 165.2 (C═O benzoyl), 167.5, 167.8 (C═O NPhth), 169.1, 170.1 (2C) (C═O acetyl); ^1H, δ 1.23 (t, 3H, CH_3CH_2S), 1.84, 1.91, 1.94 (s, 9H, CH_3 acetyl), 2.61–2.72 (m, 3H, CH_2S), 2.90 (dd, 1H), 3.30 (dd, 1H), 3.74–3.80 (m, 3H, H-5, 6), 4.07 (d, 1H, CH_2 benzyl), 4.18 (dd, 1H, H-4), 4.25 (d, 1H, CH_2 benzyl), 4.47–4.55 (m, 3H, H-1 ($J_{1,2}$ 10.2 Hz), H-2, CH_2 benzyl), 4.75–4.79 (m, 2H, H-3′, CH_2 benzyl), 4.96 (dd, 1H, H-2′), 5.22 (d, 1H, H-4′), 5.50 (d, 1H, $J_{1,2}$ 10.6 Hz, H-1′), 6.03 (dd, 1H, H-3), 7.14–8.12 (m, 19H, aromatic H). MS (MALDI-TOF): $(M+Na)^+$ 948.3; $(M+K)^+$ 964.3.

Methyl (6-*O*-Benzyl-β-D-galactopyranosyl)-(1→4)-3-*O*-benzoyl-6-*O*-benzyl-2-deoxy-2-tetrachlorophthalimido-β-D-glucopyranoside (14). A solution of **5** (2.23 g, 2.05 mmol) and dry MeOH (0.412 mL, 10.25 mmol) in CH_2Cl_2 (100 mL) was stirred under argon for 30 minutes, whereafter NIS (0.922 g, 4.10 mmol) and TESOTf (0.927 mL, 4.10 mmol) were added. After 15 min TLC indicated complete reaction, and the mixture was diluted with CH_2Cl_2, filtered through Celite, washed with satd aq $Na_2S_2O_3$ and satd aq $NaHCO_3$, dried and concentrated. Flash chromatography (toluene-EtOAc 5:1) yielded methyl (2,3,4-tri-*O*-acetyl-6-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-3-*O*-benzoyl-6-*O*-benzyl-2-deoxy-2-tetrachlorophthalimido-β-D-glucopyranoside (**13**) (1.97 g, 1.91 mmol, 93%). NMR ($CDCl_3$): ^{13}C, δ 20.8 (2C), 21.0 (CH3 acetyl), 55.7, 57.1, 67.9, 66.9, 67.4, 69.7, 71.2, 71.3, 71.9, 73.2, 73.9, 74.8, 75.5 (C-2–6, 2′-6′, CH_2 benzyl, CH_3O), 98.8 (C-1), 100.3 (C-1′), 125.3–140.1 (aromatic C), 162.3, 162.4 (C═O TCP), 165.3 (C═O benzoyl), 168.9, 169.9 (2C) (C═O acetyl); 1H, δ 1.85, 1.91, 1.94 (s, 9H, CH_3 acetyl), 2.66 (dd, 1H), 2.89 (dd, 1H), 3.26–3.41 (m, 1H), 3.47 (s, 3H, CH_3O), 3.68 (m, 1H, H-5), 3.80 (m, 2H, H-6), 4.08 (d, 1H, CH_2 benzyl), 4.17 (dd, 1H, H-2), 4.25 (d, 1H, CH_2 benzyl), 4.38 (dd, 1H, H-4), 4.44 (d, 1H, $J_{1,2}$ 7.87 Hz, H-1′), 4.55 (dd, 1H, H-3′), 4.80 (d, 1H, CH_2 benzyl), 4.94 (dd, 1H, H-2′), 5.22 (d, 1H, H-4′), 5.28 (d, 1H, $J_{1,2}$ 8.42 Hz, H-1), 5.89 (dd, 1H, H-3). Disaccharide **13** (1.74 g, 1.68 mmol) was dissolved in 5% HCl/MeOH (70 mL) and the mixture was stirred for 15 h and then concentrated. Flash chromatography (toluene-EtOAc 1:1–1:3) of the residue afforded **14** (1.25 g, 1.38 mmol, 82%). $[\alpha]_D$ +35° (c 1.0, $CHCl_3$) NMR: ^{13}C ($CDCl_3$), δ 55.7, 57.3, 67.6, 68.3, 68.5, 72.3, 72.4, 72.9, 73.3 (2C), 73.7, 74.8, 76.9 (C-2–6, 2′-6′, CH_2 benzyl, CH_3O), 98.9 (C-1), 103.7 (C-1′), 127.3–140.0 (aromatic C), 162.4, 162.5 (C═O TCP), 166.3 (C═O benzoyl); ^1H (CD_3OD + 2 drops of $CDCl_3$), δ 2.90 (dd, 1H), 3.09 (dd, 1H), 3.22–3.34 (m, 5H, H-3′, CH_3O), 3.39 (dd, 1H, H-2′), 3.65 (d, 1H, H-4′), 3.82 (m, 1H, H-5), 3.95 (dd, 1H, H-6), 4.12 (dd, 1H, H-6), 4.17–4.34 (m, 4H, CH_2 benzyl, H-1′, 2), 4.65 (dd, 2H, CH_2 benzyl), 5.30 (d, 1H, H-1), 5.85 (dd, 1H, H-3).

Anal. Calcd for $C_{42}H_{38}O_{12}NCl_4$: C, 56.64; H, 4.30%. Found: C, 56.44; H, 4.50%.

Methyl (2,3,4-Tri-*O*-acetyl-6-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-(3-*O*-benzoyl-6-*O*-benzyl-2-deoxy-2-tetrachlorophthalimido-β-D-glucopyranosyl)-(1→3)-(6-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-(3-*O*-benzoyl-6-*O*-benzyl-2-deoxy-2-tetrachlorophthalimidoβ-D-glucopyranoside) (15). Methyl triflate (0.28 mL, 1.5 mmol) was added to a stirred mixture of **14** (0.417 g, 0.46 mmol), **10** (0.685 g, 0.644 mmol) and 3Å MS in CH_2Cl_2. The reaction mixture was stirred under argon for 18 h, quenched by addition of triethylamine (1 mL), filtered through Celite and concentrated. Flash chromatography of the residue gave **15** (0.615 g, 0.322 mmol, 69%). NMR ($CDCl_3$): ^{13}C, δ 20.9 (2C), 21.1 (CH_3 acetyl), 55.8, 57.2, 66.1, 67.0, 67.5, 67.6, 68.3, 69.9, 71.1, 71.4, 71.5, 71.8, 72.4, 72.8, 73.4, 73.4, 73.8, 74.0, 74.4, 74.9, 75.6, 76.7, 77.5 (C-2–6, 2′-6′, 2″-6″, 2—6‴, CH_3O, CH_2 benzyl), 99.1, 99.2, 100.5, 103.3 (C-1–1‴), 125.4–140.2 (aromatic C), 162.3, 163.7 (C═O TCP), 165.3, 165.7 (C═O benzoyl), 169.0, 170.0 (2C) (C═O acetyl). An aliquot of **15** was acetylated (pyridine-acetic anhydride 3:2) to give material having NMR ($CDCl_3$): ^1H, δ 1.86, 1.90, 1.91, 1.97 (CH_3 acetyl), 2.54–2.68 (m, 2H), 2.86–2.92 (m, 2H), 2.23–3.30 (m, 2H), 3.44 (s, 3H, CH_3O), 3.55–3.61 (m, 2H, H-3′), 3.68–3.77 (m, 5H), 4.05–4,24 (m, 8H, H-4, 4″, 2″, 1′, CH_2 benzyl), 4.35 (dd, 1H, H-2), 4.46–4.62 (m, 4H, H-1‴, 2′, CH_2 benzyl), 4.75–4.82 (m, 3H, H-3‴, CH_2 benzyl), 4.97 (dd, 1H, H-2‴), 5.13 (d, 1H, $J_{1,2}$ 8.42 Hz, H-1″), 5.20–5.22 (m, 2H, H-4‴, 1) 5.34 (d, 1H, H-4′), 5.74 (dd, 1H, H-3), 5.94 (dd, 1H, H-3″), 7.12–7.83 (m, 30H, aromatic H). The signals for H-2′ (4.61 ppm) and H-4′ (5.34 ppm) in the acetylated tetrasaccharide was shifted downfield, proving selective coupling at 3′-OH of the acceptor.

Methyl (2,3,4-Tri-*O*-acetyl-6-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-(3-*O*-benzoyl-6-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→3)-(6-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-(3-*O*-benzoyl-6-*O*-benzyl-2-deoxy-2-tetrachlorophthalimido-β-D-glucopyranoside) (16). A solution of **12** (0.90 g, 0.97 mmol) and bromine (0.1 mL, 1.95 mmol) in CH_2Cl_2 (10 mL) was stirred under argon for 30 min. The mixture was then concentrated and co-concentrated twice with toluene (Na-dried). A solution of the obtained glycosyl bromide and **14** (0.50 g, 0.55 mmol) in CH_2Cl_2-MeCN 3:1 (20 mL) was stirred with 3Å MS for 30 min at −40 °C before AgOTf (0.51 g, 1.98 mmol) was added. After stirring at −40°C for 1 h the reaction was allowed to warm to −10 °C, and after an additional 4 h Et_3N (1 mL) was added. The mixture was stirred for another 15 min then filtered through Celite and concentrated. Flash chromatography (toluene-EtOAc 3:1) of the residue yielded 594 mg of **16** (0.34 mmol, 62%). NMR ($CDCl_3$): ^{13}C, δ 20.8, 20.9, 21.1 (CH_3 acetyl), 55.1, 55.8, 57.2, 66.1, 67.0, 67.5, 68.2, 69.9, 71.0, 71.4, 71.5, 71.8, 72.5 (2C), 72.7 (2C), 73.3, 73.4, 73.6, 74.0, 74.6, 75.0, 75.9, 76.6, 82.3 (C-2–6, 2′-6′, 2″-6″, 2—6‴, CH_3O), 99.0, 99.2, 100.6, 103.4 (C-1–1‴), 123.7–138.2 (aromatic C), 162.4, 162.6 (C═O TCP), 165.1, 165.7 (C═O benzoyl), 167.6, 167.8 (C═O NPhth) 169.1, 170.1 (2C) (C═O acetyl). An aliquot of **16** was treated with benzoyl chloride (10 equiv) in pyridine and then acetylated (pyridine-Ac_2O 3:2) to give the corresponding 2′-*O*-acetylated-4′-*O*-benzoylated tetrasaccharide with NMR ($CDCl_3$): ^1H, δ 1.70, 1.84, 1.91, 1.95 (s, 12H, CH_3 acetyl), 2.52–2-62 (m, 2H), 2.79–2.90 (m, 2H), 3.20–3.25, 3.35–3.43 (m, 5H, H-5′, 5‴, CH_3O), 3.57 (m, 1H, H-5), 3.65–3.78 (m, 7H, H-3′), 3.99–4.25 (m, 8H, H-4, 4″, 2, 1′), 4.37 (dd, 1H, H-2″), 4.46–4.52 (m, 4H, H-1‴, CH_2 benzyl), 4.72–4.86 (m, 4H, H-3‴, 2′, CH_2 benzyl), 4.96 (dd, 1H, H-2‴), 5.20–5.24 (m, 2H, H-4‴, 1″ ($J_{1,2}$ 8.24 Hz)), 4.36 (d, 1H, $J_{1,2}$ 8.24 Hz), 5.61 (d, 1H, H-4′), 5.79 (dd, 1H, H-3″), 5.92 (dd, 1H, H-3), 6.86–8.14 (m, 34H, aromatic H). The downfield shift for H-2′ (4.72–4.85 ppm) and H-4′ (5.61 ppm) in the acylated tetrasaccharide confirmed the (1→3)-linkage.

Methyl (β-D-Galactopyranosyl)-(1→4)-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-(β-D-galactopyranosyl)-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranoside (17). Hydrazine hydrate (0.145 mL, 3 mmol) was added to a solution of tetrasaccharide **15** (0.130 g, 0.068 mmol) in MeCN-THF-EtOH (2:1:1, 4 mL). After stirring at 70°C for 18 h, the mixture was concentrated and co-concentrated with EtOH. The residue was purified on a short silica gel column (EtOAc-MeOH-H_2O 7:2:1). The obtained material was dissolved in pyridine (3 mL) and acetic anhydride (1 mL), and the mixture was stirred overnight and concentrated. Flash chromatography ($CHCl_3$-MeOH 30:1→10:1) followed by further purification by size exclusion chromatography (LH-20 Sephadex, eluted with MeOH) of the residue gave the fully acetylated tetrasaccharide (63 mg, 0.044 mmol, 65%), which was hydrogenolysed (120 psi) over Pd/C (10%) in a solution of EtOAc-MeOH-H_2O (7:2:1, 3 mL). After 12 h the mixture was filtered through Celite and concentrated. The residue was dissolved in MeOH and a catalytic amount of 1 M NaOMe in MeOH was added. The mixture was stirred for 5 h, then neutralised with Dowex H^+ ion exchange resin, filtered and concentrated. Size exclusion chromatography (Biogel P2 column, eluted with H_2O containing 1% *n*-BuOH) gave **17** (25 mg, 0.033 mmol, 49% from **15**). $[\alpha]_D$ −12° (*c* 1.1, H_2O), [Lit.[3] −10° (*c* 1.0, H_2O)]. NMR (D_2O): ^1H (selected data) δ 2.04, 2.05 (s, 6H, CH_3 acetyl), 3.51 (s, 3H, CH_3O), 4.48 (2d, 3H), 4.71 (2d, 1H). NMR data were in agreement with those reported elsewhere.[3,6,8]

Methyl (β-D-galactopyranosyl)-(1→4)-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-(β-D-galactopyranosyl)-(1→4)-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-(β-D-galactopyranosyl)-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranoside (20). Tetrasaccharide **15** (71 mg, 0.037 mmol) was dissolved in 5% HCl/MeOH-CH_2Cl_2 (1:1, 4 mL), and the solution stirred under argon for 37 h, then concentrated and purified by flash chromatography (toluene-EtOAc 2:3) to afford methyl (6-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-(3-*O*-benzoyl-6-*O*-benzyl-2-deoxy-2-tetrachlorophthalimido-β-D-glucopyranosyl)-(1→3)-(6-*O*-benzyl—D-galactopyranosyl)-(1→4)-(3-*O*-benzoyl-6-*O*-benzyl-2-deoxy-2-t etrachlorophthalimido-β-D-glucopyranoside) (**18**) (58 mg, 0.033 mmol, 88%). NMR ($CDCl_3$): ^{13}C, δ 55.7 (2C), 57.3, 67.5, 67.7, 67.8, 68.2, 68.3, 68.5, 70.9, 72.2, 72.5, 72.8, 72.9, 73.4, 73.4, 73.7, 73.8, 74.4, 74.6, 76.6, 77.5, 77.7, 82.4 5 (C-2–6, 2′-6′, 2″-6″, 2—6‴, CH_3O), 99.0, 99.2, 103.3, 103.8 (C-1–1‴), 127.2–140.3 (aromatic C), 162.7, 163.4 (C═O TCP), 165.7, 165.7 (C═O benzoyl). A mixture of **18** (52 mg, 0.029 mmol), **10** (53 mg, 0.05 mmol), and 3Å MS in CH_2Cl_2 (2 mL) was treated with methyl triflate (13 μL, 0.116 mmol) under argon. After stirring for 48 h, Et_3N (0.250 mL) was added and stirring was continued for 15 min. The mixture was then diluted with CH_2Cl_2, filtered through Celite, and the solvent removed under reduced pressure. Flash chromatography (toluene-EtOAc 3:1) of the residue gave methyl (2,3,4-tri-*O*-acetyl-6-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-(3-*O*-benzoyl-6-*O*-benzyl-2-deoxy-2-tetrachlorophthalimido-β-D-glucopyranosyl)-(1→3)-(6-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-(3-*O*-benzoyl-6-*O*-benzyl-2-deoxy-2-tetrachlorophthalimido-β-D-glucopyranosyl)-(1→3)-(6-*O*-benzyl-β-D-ga lactopyranosyl)-(1→4)-(3-*O*-benzoyl-6-*O*-benzyl-2-deoxy-2-tetrachlorophthalimido-β-D-glucopyranoside) (**19**) (49 mg, 0.018 mmol, 61%). NMR ($CDCl_3$): ^{13}C, 20.8, 21.1, 21.0 (CH_3 acetyl), 55.7 (3C), 57.3, 66.0, 67.0, 67.4, 67.6, 68.1, 68.2, 69.8, 71.0, 71.4, 71.5, 71.8, 72.1, 72.3, 72.7, 73.3, 73.4, 73.6, 73.7, 73.9, 74.0, 74.3, 74.4, 74.9, 75.6, 76.3, 77.6, 82.4 (C-2–6, 2′-6′, 2″-6″, 2—6‴, 2⁗-6⁗, 2‴‴-6‴‴, CH_3O, CH_2 benzyl), 99.0, 99.1, 99.3, 100.5, 103.3 (2C) (C-1–1‴‴), 125.5–140.3 (aromatic C), 162.7, 163.3 (C═O TCP), 165.3, 165.6, 165.7 (C═O benzoyl), 170.0, 170.1 (C═O acetyl). An aliquot of **19** was acetylated (pyridine-Ac_2O 3:2). ^1H and COSY NMR experiments showed downfield shifts for H-2′, 2‴ (4.50–4.66 ppm), H-4′, 4‴ (5.29–5.34 ppm), whereas H-3′, 3‴ (3.5–3.7 ppm) which proved the (1→3)-linkage. Hexasaccharide **19** (100 mg, 0.036 mmol) was deprotected as described for compound **15** to give 16 mg (0.013 mmol, 39%) of **20**. $[\alpha]_D$ −8°(*c* 1.0, H_2O), [Lit.[3] −6° (*c* 1.0, H_2O)]. NMR (D_2O): ^1H, (selected data) δ 2.03 (3s, 9H, CH_3 acetyl), 3.50 (s, 3H, CH_3O), 3.93 (d, 1H), 4.16 (d, 2H), 4.48 (2d, 4H), 4.71 (2d, 2H). NMR data were in agreement with those reported elsewhere.[3,6,8]

Methyl (2,3,4-Tri-*O*-acetyl-6-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-(3-*O*-benzoyl-6-*O*-benzyl-2-deoxy-2-tetrachlorophthalimido-β-D-glucopyranosyl)-(1→3)-(6-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-(3-*O*-benzoyl-6-*O*-benzyl-2-deoxy-2-tetrachlorophthalimido-β-D-glucopyranosyl)-(1→3)-(6-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-(3-*O*-benzoyl-6-*O*-benzyl-2-deoxy-2-tetrachlorophthalimido-β-D-glucopyranosyl)-(1→3)-*O*-(6-*O*-benzyl-β-D-

galactopyranosyl)-(1→4)-*O*-(3-*O*-benzoyl-6-*O*-benzyl-2-deoxy-2-tetrachlorophthalimido-β-D-glucopyranoside) (22). 5% HCl/MeOH (1 mL) was added to a stirred solution of **19** (50 mg, 0.018 mmol) in CH_2Cl_2 (1 mL). After 42 h the mixture was concentrated and the residue subjected to flash chromatography (toluene-EtOAc 1:1) to give **21** (39 mg, 0.015 mmol, 81%). NMR (selected data) ($CDCl_3$): ^{1}H δ 3.41 (s, 3H, CH_3O), 5.21, 5.48, 5.58 (d, 3H, H-1, 1″, 1″″), 5.86, 5.99 (dd, 3H, H-3, 3″, 3″″). Compound **21** (39 mg) and **10** (27 mg, 0.026 mmol) was dissolved in CH_2Cl_2 (1 mL) and stirred with 3Å MS for 30 min when methyl triflate (9 μL, 0.075 mmol) was added. After 24 h the reaction was quenched by addition of Et_3N (0.1 mL), and the mixture diluted with CH_2Cl_2, filtered through Celite and concentrated. Flash chromatography (toluene-EtOAc 3:1→2:1) of the crude product gave **22** (31 mg, 0.009 mmol, 60%). Further elution (toluene-EtOAc 1:1) rendered 7 mg of unreacted **19** (0.002 mmol, 13%). **22**: NMR ($CDCl_3$): ^{13}C, 20.9, 21.0, 21.1 (CH_3 acetyl), 55.8 (4C), 57.2, 66.1, 67.0, 67.1, 67.5, 67.6, 67.7, 68.1, 68.3, 69.8, 71.0, 71.3, 71.5, 71.8, 72.0, 72.4, 72.8, 73.4 (2C), 73.6, 73.7, 74.0, 74.4, 74.9, 75.6, 76.3, 76.6, 76.7, 77.5, 82.4 (C-2–6, 2′-6′, 2″-6″, 2—6‴, 2″″-6″″, 2‴″-6‴″, 2″″″-6″″″, 2‴‴-6‴‴, CH_2 benzyl, CH_3O), 99.0 (2C), 99.2 (2C), 100.5, 103.3 (3C) (C-1–1 ′) 128.3–140.1 (aromatic C), 162.7, 163.4 (C═O TCP), 165.3, 165.6 (2C), 165.7 (C═O benzoyl), 169.0, 170.0, 170.1 (C═O acetyl).

An aliquot of **22** was acetylated and used for NMR analysis to prove the selectivity in the coupling. ^{1}H and COSY NMR experiments showed the characteristic downfield shifts for H-2′, 2‴, 2‴″, 4′, 4‴ and 4‴″ compared to H-3′, 3‴, and 3‴″, and it could be concluded that the coupling was selective for 3‴″-OH.

Methyl (6-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-6-*O*-benzyl-2-deoxy-2-tetrachlorophthalimido-β-D-glucopyranoside (24). 120 mg (0.122 mmol) of **4** was treated with dry MeOH (25 μL, 0.61 mmol), NIS (55 mg, 0.244 mmol) and TESOTf (55 μL, 0.244 mmol) in CH_2Cl_2 and then worked up as described for **13**. Flash chromatography (toluene-EtOAc 5:1) gave methyl (2,3,4-tri-*O*-acetyl-6-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-6-*O*-benzyl-2-deoxy-2-tetrachlorophthalimido-β-D-glucopyra-noside (**23**) (99 mg, 0.107 mmol, 88%). NMR ($CDCl_3$): ^{13}C δ 20.9, 21.0, 21.1 (CH_3 acetyl), 56.8, 57.0, 67.6, 68.2, 68.3, 69.3, 69.7, 71.2, 72.6, 73.7, 73.9, 74.6, 81.9 (C-2–6, C-2′-6′, CH_2 benzyl, CH_30), 99.1, 101.5 (C-1, C-1′), 125.5–138.2 (aromatic C), 169.3, 170.0, 170.1 (C═O acetyl). Disaccharide **23** (95 mg, 0.102 mmol) was dissolved in 5% HCl/MeOH and stirred at room temperature for 36 h. The mixture was then concentrated and the residue subjected to flash chromatography (toluene-EtOAc 1:2) to give tetraol **24** (79 mg, 0.099 mmol, 97%). NMR ($CDCl_3$): ^{13}C δ 56.8, 56.9, 69.0, 69.2, 69.5, 69.6, 71.5, 73.7, 73.8, 73.9 (2C), 74.5, 82.9 (C-2–6, C-2′-6′, CH_3O, CH_2 benzyl), 99.0, 103.9 (C-1, C-1′), 127.5–140.1 (aromatic C), 163.1 (C═O TCP).

Methyl (2,3,4-Tri-*O*-acetyl-6-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-(6-*O*-benzyl-2-deoxy-2-tetrachlorophthalimido-β-D-glucopyranosyl)-(1→3)-(6-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-(6-*O*-benzyl-2-deoxy-2-tetrachlorophthalimidoβ-D-glucopyranoside) (25). Methyl triflate (40 μL, 0.35 mmol), was added to a stirred solution of thioglucoside **9** (117 mg, 0.122 mmol),

24 (70 mg, 0.087 mmol) and 3Å MS in CH_2Cl_2 (3 mL). The reaction mixture was stirred under argon for 24 h, quenched by addition of triethylamine (0.25 mL), filtered through Celite and concentrated. Flash chromatography (toluene-EtOAc 4:1–3:1) gave **25** (68 mg, 40 mmol, 46%). NMR ($CDCl_3$): ^{13}C δ 20.9, 21.0, 21.1, 56.6, 56.7, 57.0, 67.6, 68.2, 68.3, 68.5, 68.8, 69.3, 69.6, 69.7, 69.9, 70.3, 71.2, 72.4, 73.5, 73.6, 73.8, 73.9, 74.0, 74.4, 77.5, 81.9, 83.1, 84.7 (C-2–6, 2′-6′, 2″-6″, 2—C-6‴, CH_3O, CH_2 benzyl), 99.0, 99.3, 101.4, 104.0 (C-1–1‴), 127.4–140.2 (aromatic C), 163.5, 163.9 (C═O TCP), 169.2, 170.0, 170.1 (C═O acetyl). An aliquot of **25** was acetylated (pyridine:Ac_2O 3:2) to give the corresponding 3,2′,4′,3″-*O*-acetylated tetrasaccharide with NMR ($CDCl_3$): ^1H δ 1.77, 1.82, 1.90, 1.96, 1.99, 2.01, 2.04 (CH_3 acetyl), 3.25 (m, 1H), 3.33–3.58 (m, 15H), 3.62–3.68 (m, 3H, H-2 GlcNTCP, H-4×2 GlcNTCP), 4.16 (dd, 1H, H-2 GlcNTCP), 4.20 (d, 1H, H-1′), 4.34–4.66 (m, 6H, H-1‴, 2′), 4.77 (m, 2H), 4.91 (dd, 1H, H-3‴), 5.02 (dd, 1H, H-2‴), 5.11 (d, 1H, H-1 GlcNTCP), 5.15 (d, 1H, H-1 GlcNTCP), 5.41–5-47 (m, 3H, H-3 GlcNTCP, H-4′, 4‴), 5.66 (dd, 1H, H-3 GlcNTCP). The downfield shift for H-3, 2′, 4′ and 3″ confirmed the regioselective coupling.

ACKNOWLEDGMENTS

This is a collaboration with Professor Fred Brewer at the Albert Einstein College of Medicine, New York. Financial support from the Swedish Natural Science Research Council is gratefully acknowledged.

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Received March 23, 2001
Accepted July 25, 2001