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Synthesis of a Hexasaccharide Corresponding to a Porcine Zona Pellucida Fragment that Inhibits Porcine Sperm-Oocyte Interaction in Vitro.

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Abstract. The synthesis of hexasaccharide **1**, [Gal β (1-4)GlcNAc[6OSO₃] β (1-3)Gal β (1-4)GlcNAc β (1-3)Gal β (1-3)GalNAc α -O(CH₂)₃NH₂], which corresponds to a porcine zona pellucida fragment that inhibits porcine sperm-oocyte interaction, is described. Compound **1** was obtained from fully protected hexasaccharide **2**, which was in turn constructed from protected Gal β (1-3)GalNAc disaccharide **5**, containing an α -linked 3-azidopropyl spacer, and from lactosamine derivatives **3** and **4**. Disaccharide **3** and **4** were prepared by coupling of selenophenyl glycoside **6** with glycosyl acceptors containing anomeric thioethyl groups. NIS/TfOH promoted coupling of disaccharide **4** with **5** afforded **29**, which was transformed into the tetrasaccharide acceptor **30** by selective removal of the levulinoyl group. Glycosylation of **30** with **3** afforded protected hexasaccharide **2**. Removal of the phthalimido groups, acetylation, followed by selective removal of the allyl group and sulphation, and finally complete deprotection afforded hexasaccharide **1**. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

The zona pellucida (ZP) is the glycoprotein matrix that surrounds all mammalian oocytes. It regulates critical steps in the fertilisation process, including species-specific recognition, induction of the acrosome reaction, prevention of polyspermy, and physical protection of the growing embryo until implantation in the uterus^{1,2}.

Experimental evidence suggests that the initial interaction between sperm and oocyte is mediated by a protein-carbohydrate recognition system, involving specific sperm proteins and carbohydrate chains of the ZP^{3,4,5}. In order to get a deeper insight into the precise role of the carbohydrate chains of the ZP in this initial process of fertilisation, a number of studies concerning the structure and biological activity of ZP derived carbohydrate chains have been executed⁶⁻¹³. Porcine ZP, sharing cross-reactive antigens with human ZP¹⁴, is frequently used for these studies since it is relatively easy to obtain in large amounts.

In a retrosynthetic analysis of the target compound **1** (Figure 2) we revealed the fully protected hexasaccharide **2** as key intermediate. Compound **2** contains a temporary allyl protective group for the 6-hydroxyl group to be sulphated and acetyl and benzyl groups to protect free hydroxyl groups. Participating N-phthalimido groups are chosen to mask the amino groups of the glucosamine units, since they can promote the

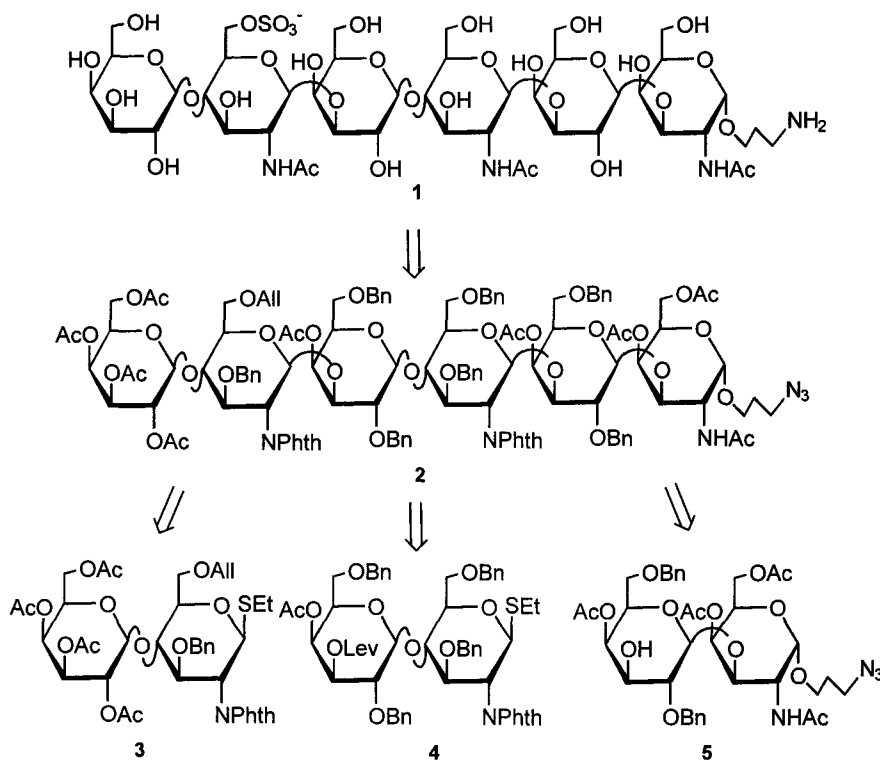


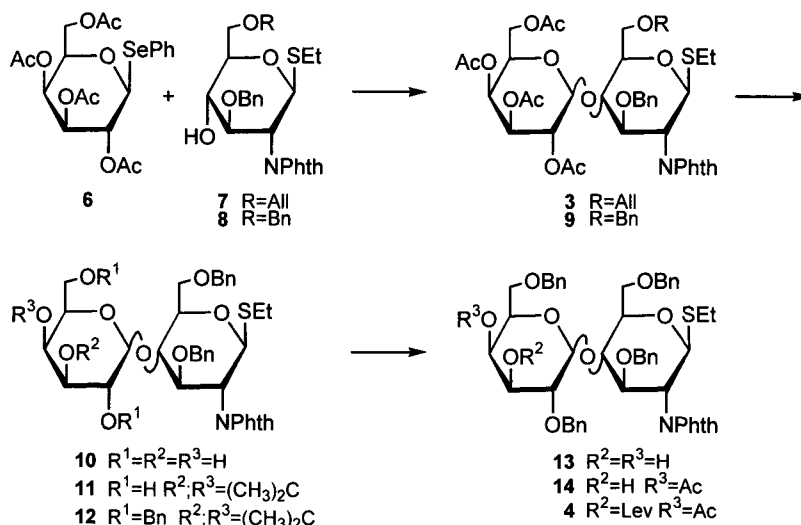
Figure 2. Retrosynthetic analysis of hexasaccharide 1.

formation of the required β -interglycosidic bonds. The amino group of the spacer is masked by an azido group, which can be converted to an amine by hydrogenolysis in the final step of the synthesis. An important property of the azido group is its stability during a variety of protecting group manipulations, such as the removal of phthalimido groups with hydrazine or ethylenediamine at elevated temperature. Other amine protecting groups, such as benzyloxycarbonyl groups, may be affected under these conditions¹⁷.

The protected hexasaccharide 2 has been obtained by a blockwise approach, using lactosamine derivatives 3 and 4, and the Gal β (1-3)GalNAc intermediate 5, that contains the α -oriented spacer. Compound 4 was coupled to 5, and the obtained tetrasaccharide was, after selective removal of the levulinoyl group, coupled to disaccharide 3.

Synthesis of disaccharides 3 and 4 (Scheme 1)

For the synthesis of disaccharides 3 and 4, compounds 7 and 8¹⁸ were selected as glycosyl acceptors since they contain an anomeric thioethyl group which can be readily activated in a later stage of the synthesis. Attempts to glycosylate 7 or 8 with 2,3,4,6-tetra-O-acetyl- α -D-galactopyranosyl bromide¹⁹ (in the presence of silver trifluoromethanesulfonate (AgOTf) at -40°C in CH_2Cl_2) or with 2,3,4,6-tetra-O-acetyl- α -D-galacto-



Scheme 1

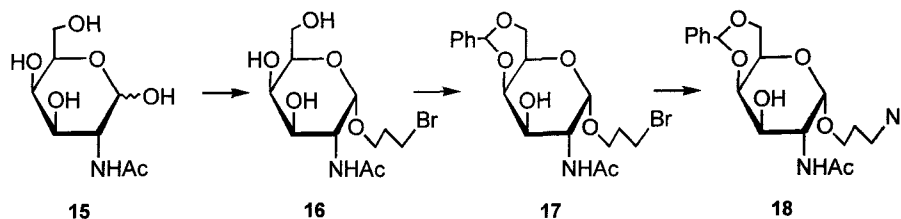
pyranosyl trichloroacetimidate²⁰ (catalysed by trimethylsilyl trifluoromethanesulfonate (TMSOTf) at -20°C in CH_2Cl_2) led to low yields of the desired disaccharides due to degradation of the acceptor. However, coupling of the selenophenyl glycoside **6**²⁴ with **7** and **8** using the conditions described by Pinto *et al.*^{21,22} (AgOTf, K_2CO_3 in CH_2Cl_2) gave good yields of the disaccharides **3** (71% yield) and **9** (80% yield), respectively, and the thioethyl groups of the acceptors were not affected under these conditions. On the other hand, a much lower yield of the disaccharides was obtained when **6** was activated by iodonium ions, generated in situ from *N*-iodosuccinimide (NIS) and trifluoromethanesulfonic acid (TfOH)^{23,24}.

Compound **9** was then saponified using K_2CO_3 in a mixture of methanol and dioxane, followed by selective 3',4'-*O*-isopropylideneation of **10** with 2,2-dimethoxypropane according to Catelani *et al.*²⁵ to give **11**. Benzoylation of **11** was accomplished without affecting the phthalimido group, using benzyl bromide, sodium hydride and a catalytic amount of tetrabutyl ammonium iodide in THF, giving **12** in 82% yield. Acid-catalysed hydrolysis of the isopropylidene group of **12** (to give **13**) was followed by conversion of the product into the 3',4'-cyclic methyl orthoester which was treated under acid conditions²⁶ to afford the 4'-*O*-acetylated derivative **14** regioselectively in almost quantitative yield. Finally 3'-*O*-levulinoylation afforded disaccharide **4**.

Synthesis of disaccharide **5** (Schemes 2 and 3)

For the synthesis of disaccharide **5**, a galactosamine unit was required which contains an α -linked 3-azido-propyl spacer. The most employed procedure for the synthesis of α -linked galactosamine derivatives takes advantage of a non-participating azido group at position 2 of the galactosamine unit. However, since the azido group had to be used to mask the amino group of the spacer, we envisaged an alternative method for the

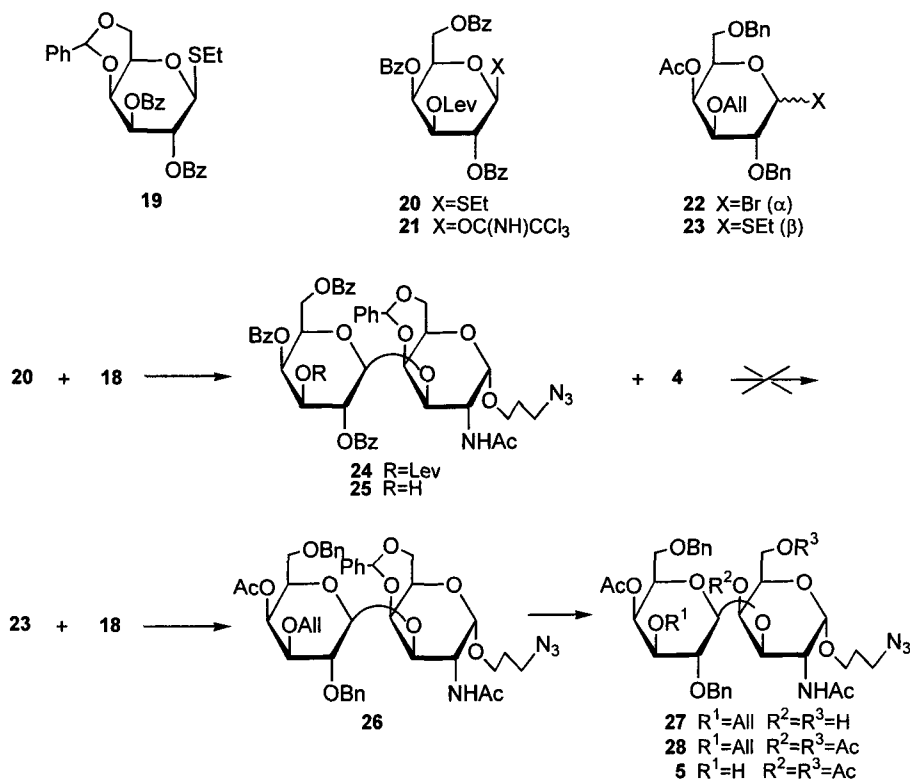
introduction of the α -linked spacer: the well-known Fischer method²⁷. This method is particularly effective when the glycosyl acceptor (the alcohol) is the solvent as well. However, since 3-azido-1-propanol cannot serve as a solvent in the Fischer reaction, the α -coupled 3-bromo-propyl derivative **16** was prepared first by heating N-acetyl-D-galactosamine (**15**) in 3-bromo-1-propanol in the presence of HCl (yield 84%). Subsequently, after introduction of the benzylidene group to give **17** (79%), the bromo atom of the spacer was substituted by an azido group with NaN_3 and Bu_4NOTf in DMF, to give the required N-acetyl- α -D-galactosamine derivative **18** in 54% yield.



Scheme 2

For the synthesis of the β -interglycosidic bond in the required $\text{Gal}\beta(1-3)\text{Gal}\alpha\text{-O}(\text{CH}_2)_3\text{N}_3$ disaccharide, several galactosyl donors were coupled with glycosyl acceptor **18**. In a first approach to the synthesis of the disaccharide, galactosyl donors containing participating 2-O-benzoyl groups were used in order to direct the formation of the β -interglycosidic bond. For instance, galactosyl donor **19**²⁸ was coupled to **18** in the presence of NIS and TfOH in CH_2Cl_2 at 0 °C. Unexpectedly, the reaction proceeded very slowly and the disaccharide was isolated in a β/α ratio of 1/1 (yield 70%). This low regioselectivity may be due to the presence of a conformationally rigid 4,6-O-benzylidene protected glycosyl donor and acceptor, which may inactivate the donor or may cause unfavourable steric interactions in the transition state leading to the required β -interglycosidic bond²⁹.

In a next attempt thiogalactoside **20**, containing benzoyl groups instead of the benzylidene group of **19**, was coupled with **18** in the presence of NIS and TfOH at 0 °C. Although only β -coupled product (**24**) was formed, the reaction proceeded in a low yield (24%). Coupling of the corresponding trichloroacetimidate **21** (promoted by TMSOTf) afforded solely the β -coupled product in an even lower yield of 14%. In this stage of the synthesis we did not try to optimise this glycosylation since we first liked to experience if the obtained disaccharide is a suitable acceptor for the glycosylation with disaccharide **4**. Thus, after removing the 3'-O-levulinoyl group of **24**, compound **25** was condensed with **4** in the presence of NIS and TfOH at 0 °C. Unfortunately, no glycosylation product could be isolated.



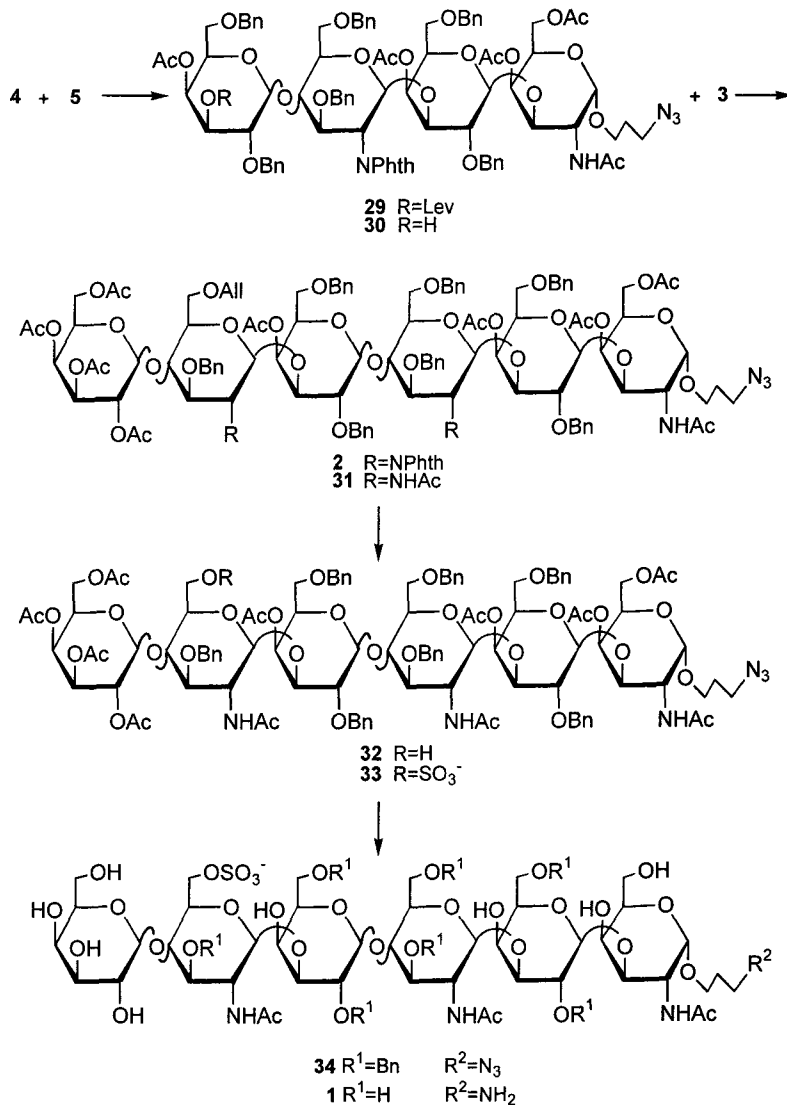
Scheme 3

The reason for this outcome might be the fact that bulky, electron-withdrawing benzoyl groups are present on the galactose unit of the glycosyl acceptor, which may strongly decrease the reactivity of the hydroxyl group of this acceptor. Consequently, it was reasoned that a Galβ(1-3)Gal- α -O(CH₂)₃N₃ disaccharide was required which contains electron-donating ether-protective groups instead of the deactivating benzoyl esters on the galactose unit. To this end, galactosyl donors **22** and **23**, which contain ether protective groups at position 2, 3 and 6, were coupled with **18**. Coupling of **22** with **18** was promoted by silver silicate-aluminate³⁰ to give the disaccharide **26** in a β/α ratio of 1/1 and in a very poor yield of 5%. On the other hand, coupling of thioglycoside **23** with **18** promoted by NIS/TfOH in acetonitrile gave a higher yield of **26** (β/α 2/1, yield (α + β): 60%).

The benzylidene group of **26** was then hydrolysed with 80% acetic acid, followed by acetylation of the 4- and 6-hydroxyl groups of **27** to give **28**. Isomerisation of the allyl group with 1,5-cyclooctadiene-bis[methyldiphenylphosphine]-iridium hexafluorophosphate³¹ and H₂, and subsequent removal of the propenyl group with NIS in a mixture of THF and water afforded disaccharide **5**.

Synthesis of protected hexasaccharide 2 and its deprotection to give 1 (Scheme 4)

Glycosylation of acceptor **5** with donor **4** in the presence of NIS and TfOH at 0 °C afforded the β -coupled tetrasaccharide **29** in a yield of 50%. Selective removal of the levulinoyl group with hydrazine acetate in pyridine afforded acceptor **30** (89%), which was in turn coupled with disaccharide **3** under the same glycosylation conditions as described for the synthesis of **29**, to give the fully protected hexasaccharide **2** in 58% yield.

**Scheme 4**

Deblocking and sulphation of the fully protected hexasaccharide **2** was accomplished using the following strategy. It is important to note that the final deprotection steps should be executed under mild conditions since sulphate groups may be unstable under strong basic (3,6-anhydro formation³²) or acid conditions (hydrolysis³³). Thus, the deblocking starts with the simultaneous cleavage of the phthalimido groups and the acetyl esters, by treatment of **2** with ethylenediamine in 1-butanol at 90 °C. Using this recently reported method³⁴, the phthalimido groups were removed in a high yield and the allyl group was not reduced, as was observed when model compounds were treated with hydrazine in refluxing ethanol. Acetylation of the amine and hydroxyl groups gave then compound **31**. In the next step the allyl group was removed and the resultant free hydroxyl group of **32** was sulphated with triethylamine sulphur trioxide complex in DMF at 40 °C to give **33**. In this respect it should be mentioned that when the sulphation reaction is performed at a higher temperature, (partial) N-sulphation may occur on the N-acetyl groups³⁵.

Mild saponification of **33** with potassium carbonate in methanol (to give **34**) was followed by simultaneously reduction of the benzyl ethers and the azido group with hydrogen in the presence of palladium on charcoal. However, the crude product after the reduction consisted of a mixture of the desired hexasaccharide **1**, and a compound that eluted faster than **1** from a Sephadex G-25 column, indicating that this by-product has a higher molecular weight than **1**. Model studies on a monosaccharide containing a β -linked 3-azido-propyl spacer showed that dimerization (to give [monosaccharide-O(CH₂)₃]₂NH) of compounds containing 3-azido-propyl spacers can occur during the hydrogenolysis with Pd/C in a mixture of DMF, water and acetic acid. It was found that the formation of the dimer could be suppressed by executing the hydrogenolysis with Pd/C in two steps: first, the azido group was reduced in a mixture of DMF and water containing ammonia. In the next step the benzyl ethers were reduced in a mixture of DMF, water and acetic acid. When this two-step reduction was applied on **34**, compound was obtained **1** in a high purity. Desalting of the product on Sephadex G-25 afforded the target hexasaccharide in a yield of 46% (overall yield from **32**). The structure and identity of **1** were confirmed by FAB mass analysis and ¹H NMR spectroscopy (600 MHz). The ¹H NMR data of **1** were in full accordance with the ¹H NMR data obtained from the corresponding fragment from porcine zonae pellucidae.

EXPERIMENTAL PART

General Procedures

Dioxane, pyridine and acetonitrile were stored over molecular sieves 4Å, toluene and ether over sodium wire and dichloromethane over basic alumina. Tetrahydrofuran was distilled from LiAlH₄. Reactions were performed under strict anhydrous conditions and at ambient temperature unless noted otherwise. TLC analysis was performed on Merck-Fertigplatten (kieselgel 60 F254, 5x10 cm) or on HPTLC Merck-Fertigplatten (kieselgel 60 F254, 5x5 cm). Compounds were visualized by spraying with sulphuric acid/ethanol (1/4, v/v). Normal phase column chromatography was performed on Kieselgel 60, 230-400 Mesh (Merck). Reversed phase column chromatography was performed on LiChroprep RP-18 (40-63 µm) (Merck). ¹H NMR spectra were recorded on a Bruker AC 200 or AM 360 spectrometer equipped with an ASPECT 3000 computer or a Bruker DRX 400 or DRX 600 spectrometer equipped with a silicon graphics indy; chemical shift are given in ppm (δ) relative to TMS as internal reference, or relative to D₂O. Fast Atom Bombardment (FAB) mass

spectra were recorded on a Finnigan MAT 90 mass spectrometer equipped with a WATV Cs ion gut. Glycerol or thioglycerol was used as the matrix.

Ethyl 6-O-allyl-3-O-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (7) - To a mixture of ethyl 2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (19.7 g, 55.8 mmol) in N,N-dimethylformamide (200 ml) was added acrolein dimethyl acetal (20.6 ml) and p-toluenesulfonic acid monohydrate (400 mg). After stirring for 1½ h, the mixture was poured out in a mixture of aqueous NaHCO₃ and ethyl acetate. The organic layer was washed with water, dried and concentrated. Purification of the residue on silicagel (toluene/ethyl acetate 9/1 → 6/4) afforded ethyl 4,6-O-allylidene-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (18.7 g, 86%). A solution of this compound in tetrahydrofuran (244 ml), containing benzyl bromide (8.5 ml), was added dropwise to NaH (3.87 g). After refluxing for 5 h, the mixture was filtered over Celite. The filtrate was diluted with ethyl acetate and successively washed with 0.01 N HCl, aqueous NaHCO₃ and water, dried and concentrated. Column chromatography (toluene → toluene/ethyl acetate 9/1) of the residue afforded ethyl 4,6-O-allylidene-3-O-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (16.5 g, 72%). R_f 0.30 (toluene/ethyl acetate 9/1). To a mixture of ethyl 4,6-O-allylidene-3-O-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (13.3 g, 27.6 mmol) and sodium cyanoborohydride (10 g) in tetrahydrofuran (455 ml) was added at 0 °C a saturated solution of hydrogen chloride in ether until the evolution of gas ceased. After stirring for 5 h at room temperature, a saturated solution of NaHCO₃ in water (500 ml) was added and the mixture was filtered over Celite. The filtrate was diluted with ethyl acetate and the organic layer was washed with water, dried and concentrated. Purification of the residue on silicagel (toluene → toluene/ethyl acetate 6/4) afforded **7** (14.0 g, 90%). R_f 0.52 (toluene/ethanol 8/2). ¹H NMR (200 MHz)(CDCl₃): δ 1.16 (m, 3H, SCH₂CH₃); 2.63 (m, 2H, SCH₂CH₃); 3.08 (bd, 1H, OH); 3.66 (m, 1H, H-5); 3.74 (dd, 1H, H-6a, J_{5,6a} 5.2 Hz, J_{6a,6b} 10.0 Hz); 3.78-3.85 (m, 2H, H-4, H-6b); 4.05-4.10 (m, 2H, OCH₂CH=); 4.23 (t, 1H, H-2, J_{1,2}=J_{2,3} 10.0 Hz); 4.28 (t, 1H, H-3, J_{2,3}=J_{3,4} 10.0 Hz); 4.55 (d, 1H, CH(H)Ph); 4.76 (d, 1H, CH(H)Ph); 5.20-5.34 (m, 2H, =CH₂); 5.27 (d, 1H, H-1 J_{1,2} 10.0 Hz); 5.91 (m, 1H, -CH=); 6.93-7.84 (m, 9H, H-arom).

Ethyl 4-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-6-O-allyl-3-O-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (3) - A mixture of phenyl 2,3,4,6-tetra-O-acetyl-1-seleno-β-D-galactopyranoside (**6**) (23.8 g, 48.7 mmol), compound **7** (11.8 g, 24.4 mmol), K₂CO₃ (6.73 g) and molecular sieves 4Å (26 g) in dichloromethane (660 ml) was stirred for 30 minutes at room temperature. Silver triflate (25.2 g) was added over a period of 3 h. After stirring for another 3 h, the reaction mixture was filtered through Celite and the filtrate was washed with water, dried and concentrated. Silicagel column chromatography (toluene/ethyl acetate 95/5 → 9/1) afforded compound **3** (14.1 g, 71%). R_f 0.25 (dichloromethane/acetone 97/3). ¹H NMR (200 MHz)(CDCl₃): δ 1.16 (m, 3H, SCH₂CH₃); 1.98, 2.02, 2.09, 2.11 (4xs, 12H, 4xOC(O)CH₃); 2.63 (m, 2H, SCH₂CH₃); 3.55 (m, 1H, H-5); 3.75 (m, 2H, H-6a, H-6b); 3.85 (m, 1H, H-5'); 4.01 (m, 2H, H-6a', H-6b'); 4.00-4.21 (m, 2H, OCH₂CH=); 4.06 (m, 1H, H-4); 4.21 (t, 1H, H-2, J_{1,2}=J_{2,3} 10.0 Hz); 4.30 (dd, 1H, H-3, J_{3,4} 8.0 Hz); 4.46 (d, 1H, CH(H)Ph); 4.78 (d, 1H, H-1', J_{1',2'} 8.0 Hz); 4.82 (d, 1H, CH(H)Ph); 5.00 (dd, 1H, H-3', J_{2',3'} 10.0 Hz, J_{3',4'} 3.4 Hz); 5.20 (d, 1H, H-1, J_{1,2} 10.0 Hz); 5.23 (dd, 1H, H-2'); 5.22-5.37 (m, 2H, =CH₂); 5.34 (c, 1H, H-4'); 5.96 (m, 1H, -CH=); 6.85-7.80 (m, 9H, H-arom).

Ethyl 4-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-3,6-di-O-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (9) - Glycosyl donor **6** and glycosyl acceptor **8** were coupled using the procedure described for the synthesis of compound **3**, to give disaccharide **9** (yield 80%). R_f 0.22 (toluene/ethyl acetate 8/2). ¹H NMR (200 MHz)(CDCl₃): δ 1.18 (m, 3H, SCH₂CH₃); 1.88, 2.02, 2.02, 2.07 (4xs, 12H, 4xOC(O)CH₃); 2.63 (m, 2H, SCH₂CH₃); 3.57 (m, 1H, H-5); 3.65 (m, 1H, H-5'); 3.79 (m, 2H, H-6a, H-6b); 3.88 (m, 2H, H-6a', H-6b'); 4.02-4.18 (m, 2H, H-3, H-4); 4.21-4.82 (m, 4H, 2xCH₂Ph); 4.27 (m, 1H, H-2); 4.61 (d, 1H, H-1', J_{1',2'} 8.1 Hz); 4.85 (dd, 1H, H-3', J_{2',3'} 10.4 Hz, J_{3',4'} 3.4 Hz); 5.16 (dd, 1H, H-2'); 5.23 (c, 1H, H-1); 5.28 (dd, 1H, H-4', J_{4',5'} 1.2 Hz); 6.83-7.81 (m, 14H, H-arom).

Ethyl 4-O-(β-D-galactopyranosyl)-3,6-di-O-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (10) - To a solution of **9** (4.4 g, 5.1 mmol) in a mixture of dioxane and methanol (220 ml, 1/1) was added K₂CO₃ (2.7 g) at 0 °C. After stirring for 4 h at 0 °C, the mixture was neutralized with Dowex 50 (H⁺) resin. The resin

was filtered off, washed and the filtrate was evaporated to give **10** (3.5 g, 100%). R_f 0.32 (toluene/ethyl acetate 8/2).

Ethyl 4-O-(3,4-O-isopropylidene-β-D-galactopyranosyl)-3,6-di-O-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (11) - To a mixture of **10** (2.5 g, 3.6 mmol) in 2,2-dimethoxypropane (73 ml) was added camphorsulfonic acid (50 mg). After stirring for 16 h, triethylamine (0.8 ml) was added and stirring was continued for 15 minutes. The solution was concentrated, coevaporated with toluene (3x), and a mixture of methanol and water (120 ml, 10/1) was added. After refluxing for 2 h, the mixture was concentrated. Purification of the residue on silicagel (toluene/ethyl acetate 9/1 → 7/3) gave **11** (2.1 g, 79%). R_f 0.28 (heptane/ethyl acetate 4/6).

Ethyl 4-O-(2,6-di-O-benzyl-3,4-O-isopropylidene-β-D-galactopyranosyl)-3,6-di-O-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (12) - A solution of **11** (1.54 g, 2.09 mmol) and benzyl bromide (1.25 ml) in tetrahydrofuran (67 ml) was added dropwise to a mixture of NaH (376 mg) and tetrabutylammonium iodide (82 mg). After stirring for 6 h, the mixture was diluted with dichloromethane, successively washed with 0.01 N HCl, aqueous NaHCO₃ and water, dried and concentrated. Column chromatography (heptane/ethyl acetate 8/2 → 7/3) of the residue gave **12** (1.57 g, 82%). R_f 0.50 (heptane/ethyl acetate 6/4).

Ethyl 4-O-(2,6-di-O-benzyl-β-D-galactopyranosyl)-3,6-di-O-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (13) - Compound **12** (5.33 g, 5.82 mmol) was dissolved in 70% acetic acid (130 ml). After stirring for 3 h at 60 °C, toluene was added and the mixture was concentrated to give **13** (5.10 g, 100%). R_f 0.33 (heptane/ethyl acetate 1/1).

Ethyl 4-O-(4-O-acetyl-2,6-di-O-benzyl-β-D-galactopyranosyl)-3,6-di-O-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (14) - To a stirred mixture of **13** (5.10 g, 5.82 mmol) in acetonitrile (50 ml) was added trimethyl orthoacetate (1.8 ml) and a catalytic amount of p-toluenesulfonic acid monohydrate. After stirring for 10 minutes, 80% acetic acid (90 ml) was added and stirring was continued for 15 minutes. Dichloromethane was added and the organic layer was successively washed with aqueous NaHCO₃ and water, dried and concentrated to give **14** (5.29 g, 99%). R_f 0.44 (heptane/ethyl acetate 1/1). ¹H NMR (200 MHz)(CDCl₃): δ 1.18 (m, 3H, SCH₂CH₃); 1.99 (s, 3H, OC(O)CH₃); 2.65 (m, 2H, SCH₂CH₃); 5.25 (d, 1H, H-1, $J_{1,2}$ 10.1 Hz); 5.33 (dd, 1H, H-4', $J_{3',4'}$ 3.4 Hz, $J_{4',5'}$ 1.0 Hz).

Ethyl 4-O-(4-O-acetyl-2,6-di-O-benzyl-3-O-levulinoyl-β-D-galactopyranosyl)-3,6-di-O-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (4) - To a stirred solution of **14** (5.29 g, 5.76 mmol) and levulinic acid (1.0 g) in dichloromethane (100 ml) was added at 0 °C 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (1.69 g) and a catalytic amount of 4-dimethylaminopyridine. After stirring for 1½ h at 0 °C, 0.2 N HCl was added and the organic layer was washed with aqueous NaHCO₃ and water, dried and concentrated. Column chromatography of the residue (toluene/ethyl acetate 9/1) afforded **4** (5.79 g, 99%). R_f 0.68 (toluene/ethyl acetate 8/2). ¹H NMR (200 MHz)(CDCl₃): δ 1.19 (m, 3H, SCH₂CH₃); 1.96, 2.18 (2xs, 6H, OC(O)CH₃, C(O)(CH₂)₂C(O)CH₃); 2.30-2.90 (m, 6H, C(O)(CH₂)₂C(O)CH₃, SCH₂CH₃); 3.48-3.60 (m, 3H, H-2', H-5, H-5'); 4.11 (m, 1H, H-4); 4.20-4.87 (m, 8H, 4xCH₂Ph); 4.26-4.38 (m, 2H, H-2, H-3); 4.52 (d, 1H, H-1', $J_{1',2'}$ 10.0 Hz); 4.87 (dd, 1H, H-3', $J_{2',3'}$ 8.0 Hz, $J_{3',4'}$ 3.4 Hz); 5.23 (c, 1H, H-1); 5.34 (dd, 1H, H-4', $J_{4',5'}$ 1.0 Hz); 6.82-7.85 (m, 24H, H-arom).

3-Bromopropyl 2-acetyl-amino-2-deoxy-α-D-galactopyranoside (16) - To a solution of N-acetyl-D-galactosamine (2.0 g, 9.0 mmol) in 3-bromo-1-propanol (30 ml) was added dropwise at 0 °C acetyl chloride (1.68 ml). After stirring for 5½ h at 70 °C, the reaction mixture was neutralized with Dowex OH⁻. The mixture was filtered and the filtrate was chromatographed on silicagel (dichloromethane → dichloromethane/methanol 3/2) to give compound **16** (2.6 g, 84%) R_f 0.84 (dichloromethane/methanol 3/2). ¹H NMR (200 MHz)(MeOD): δ 2.00 (s, 3H, NC(O)CH₃); 2.12 (m, 2H, OCH₂CH₂CH₂Br); 4.25 (dd, 1H, H-2, $J_{1,2}$ 3.8 Hz, $J_{2,3}$ 11.0 Hz); 4.83 (d, 1H, H-1).

3-Bromopropyl 2-acetyl-amino-4,6-O-benzylidene-2-deoxy- α -D-galactopyranoside (17) - To a mixture of compound **16** (2.5 g, 7.3 mmol) in N,N-dimethylformamide (40 ml) and benzaldehyde dimethyl acetal (4 ml) was added a catalytic amount of p-toluenesulfonic acid monohydrate. After stirring for 4 h at room temperature, the mixture was poured out in a mixture of aqueous NaHCO₃ and dichloromethane. The organic layer was washed with water, dried and concentrated to give **17** (2.47 g, 79%). R_f 0.5 (dichloromethane/acetone 3/1).

3-Azidopropyl 2-acetyl-amino-4,6-O-benzylidene-2-deoxy- α -D-galactopyranoside (18) - To a solution of compound **17** (2.47 g, 5.7 mmol) in N,N-dimethylformamide (40 ml) was added NaN₃ (1.5 g) and Bu₄NOTf (670 mg). After stirring for 2 h at 50 °C, the mixture was diluted with ethyl acetate and the organic layer was washed with water, dried and concentrated. The crude mixture was eluted from a silicagel column (dichloromethane/acetone 86/14 → 8/2) to give **18** (1.19 g, 54%). R_f 0.17 (dichloromethane/acetone 85/15). ¹H NMR (200 MHz)(CDCl₃): δ 1.91 (m, 2H, OCH₂CH₂CH₂N₃); 2.03 (s, 3H, NHC(O)CH₃); 3.41 (m, 2H, OCH₂CH₂CH₂N₃); 3.56 (m, 1H, OCH(H)CH₂CH₂N₃); 3.68 (m, 1H, H-5); 3.79-3.92 (m, 2H, OCH(H)CH₂CH₂N₃, H-3); 4.08 (dd, 1H, H-6a, J_{5,6a} 1.9 Hz, J_{6a,6b} 12.3 Hz); 4.24 (dd, 1H, H-4); 4.29 (dd, 1H, H-6b, J_{5,6b} 1.9 Hz); 4.48 (ddd, 1H, H-2, J_{1,2} 3.8 Hz, J_{2,3} 11.0 Hz, J_{2,NH} 8.4 Hz); 4.98 (d, 1H, H-1, J_{1,2} 3.8 Hz); 5.59 (s, 1H, Ph(H)C); 5.88 (d, 1H, NHC(O)CH₃); 7.34-7.58 (m, 5H, H-arom).

Ethyl 2,4,6-tri-O-benzoyl-3-O-levulinoyl-1-thio- β -D-galactopyranoside (20) - Compound **20** was prepared from ethyl 1-thio- β -D-galactopyranoside²³ by successive 3,4-O-isopropylidation, benzylation of 2-OH and 6-OH, removal of the isopropylidene, selective 4-O-benzylation via the 3,4-cyclic phenyl orthoester, and levulinoylation of the 3-OH. R_f 0.55 (toluene/ethyl acetate 3/1). ¹H NMR (200 MHz)(CDCl₃): δ 1.30 (m, 3H, SCH₂CH₃); 1.93 (s, 3H, C(O)(CH₂)₂C(O)CH₃); 2.34-2.59 (m, 4H, C(O)(CH₂)₂C(O)CH₃); 2.75-2.85 (m, 2H, SCH₂CH₃); 4.27 (m, 1H, H-5); 4.37 (dd, 1H, H-6a); 4.62 (dd, 1H, H-6b); 4.78 (d, 1H, H-1, J_{1,2} 10.0 Hz); 5.40 (dd, 1H, H-3, J_{2,3} 10.0 Hz, J_{3,4} 3.4 Hz); 5.65 (t, 1H, H-2, J_{1,2}=J_{2,3} 10.0 Hz); 5.88 (dd, 1H, H-4, J_{4,5} 1.4 Hz); 7.39-8.18 (m, 15H, H-arom).

2,4,6-tri-O-benzoyl-3-O-levulinoyl- β -D-galactopyranosyl trichloroacetimidate (21) - Compound **21** was prepared from **20** by successive hydrolysis of the anomeric thioethyl group with NOBF₄ in a mixture of acetonitrile and water³⁶, and introduction of the trichloroacetimidate group using CCl₃CN and Cs₂CO₃. R_f 0.49 (dichloromethane/acetone 95/5). ¹H NMR (200 MHz)(CDCl₃): δ 1.94 (s, 3H, C(O)(CH₂)₂C(O)CH₃); 2.38-2.58 (m, 4H, C(O)(CH₂)₂C(O)CH₃); 4.38-4.18 (m, 2H, H-5, H-6a); 4.67 (m, 1H, H-6b); 5.48 (dd, 1H, H-3, J_{2,3} 10.0 Hz, J_{3,4} 3.4 Hz); 5.88 (dd, 1H, H-2, J_{1,2} 8.0 Hz); 5.91 (bd, 1H, H-4); 6.11 (d, 1H, H-1, J_{1,2} 8.0 Hz); 7.20-8.20 (m, 15H, H-arom); 8.70 (s, 1H, OC(NH)CCl₃).

4-O-Acetyl-3-O-allyl-2,6-di-O-benzyl- α -D-galactopyranosyl bromide (22) - Compound **22** was prepared from compound **23** by successive hydrolysis of the anomeric thioethyl group and reaction with oxalylbromide/DMF. ¹H NMR (200 MHz)(CDCl₃): δ 1.97 (s, 3H, OC(O)CH₃); 3.42 (m, 2H, H-6a, H-6b); 3.60 (dd, 1H, H-2, J_{1,2} 3.8 Hz, J_{2,3} 9.6 Hz); 3.80 (dd, 1H, H-3, J_{3,4} 3.4 Hz); 3.94-4.18 (m, 2H, OCH₂CH=); 4.32-4.76 (m, 4H, 2xCH₂Ph); 4.28 (m, 1H, H-5); 5.07-5.29 (m, 2H, =CH₂); 5.48 (dd, 1H, H-4, J_{4,5} 1.4 Hz); 5.71-5.91 (m, 1H, -CH=); 6.36 (d, 1H, H-1, J_{1,2} 3.8 Hz); 7.11-7.33 (m, 10H, H-arom).

Ethyl 4-O-acetyl-3-O-allyl-2,6-di-O-benzyl-1-thio- β -D-galactopyranoside (23) - Compound **23** was prepared from ethyl 1-thio- β -D-galactopyranoside²³ by successive 3,4-O-isopropylidation, benzylation of 2-OH and 6-OH, removal of the isopropylidene, selective 3-O-allylation via a 3,4-O-dibutylstannylene intermediate, and acetylation of the 4-OH. R_f 0.58 (heptane/ethyl acetate 4/1). ¹H NMR (200 MHz)(CDCl₃): δ 1.30 (m, 3H, SCH₂CH₃); 2.06 (s, 3H, OC(O)CH₃); 2.66-2.84 (m, 2H, SCH₂CH₃); 3.43-3.61 (m, 4H, H-2, H-3, H-6a, H-6b); 3.72 (m, 1H, H-5); 3.96-4.27 (m, 2H, OCH₂CH=); 4.40-4.58 (m, 4H, 2xCH₂Ph); 4.48 (d, 1H, H-1, J_{1,2} 9.0 Hz); 5.13-5.34 (m, 2H, =CH₂); 5.72 (dd, 1H, H-4, J_{3,4} 2.0 Hz); 5.78-5.98 (m, 1H, -CH=); 7.25-7.45 (m, 10H, H-arom).

3-Azidopropyl 3-O-(2,4,6-tri-O-benzoyl-3-O-levulinoyl- β -D-galactopyranosyl)-2-acetyl-amino-4,6-O-benzylidene-2-deoxy- α -D-galactopyranoside (24) - A mixture of glycosyl donor **20** (121 mg, 0.191 mmol) and glycosyl acceptor **18** (50 mg, 0.127 mmol) and molecular sieves 4 Å (75 mg) in dichloromethane was stirred for 30 minutes at room temperature. A freshly prepared solution containing N-iodosuccinimide (47 mg) and trifluoromethanesulfonic acid (4 μ l) in a mixture of dichloromethane and dioxane (2.1 ml, 1/1) was added dropwise to the mixture at 0 °C. After stirring for 30 minutes at 0 °C, the reaction mixture was filtered and poured out in a mixture of dichloromethane, aqueous Na₂S₂O₃ and aqueous NaHCO₃. The organic layer was washed with water, dried and concentrated. Purification of the residue was effected by chromatography on successively Sephadex LH-20 (dichloromethane/methanol 1/1) and on silicagel (toluene/ethyl acetate 1/1 $\rightarrow$ 2/3) to give **24** (29 mg, 24%). *R*_f 0.20 (toluene/ethyl acetate 1/1). ¹H NMR (200 MHz)(CDCl₃): δ 1.40, 1.90 (2xs, 6H, NHC(O)CH₃, C(O)(CH₂)₂C(O)CH₃); 1.82 (m, 2H, OCH₂CH₂CH₂N₃); 2.30-2.58 (m, 4H, C(O)(CH₂)₂C(O)CH₃); 3.30 (m, 2H, OCH₂CH₂CH₂N₃); 3.42-3.53 (m, 2H, OCH₂(H)CH₂CH₂N₃, H-5); 3.59-3.81 (m, 2H, OCH(H)CH₂CH₂N₃, H-6a); 3.99 (dd, 1H, H-3, J_{2,3} 11.4 Hz, J_{3,4} 3.4 Hz); 4.15 (dd, 1H, H-6b, J_{5,6b} 1.8 Hz J_{6a,6b} 12.3 Hz); 4.40 (c, 1H, H-4); 4.58 (m, 1H, H-2); 5.06 (d, 1H, H-1', J_{1',2'} 7.9 Hz); 5.09 (d, 1H, H-1, J_{1,2} 3.4 Hz); 5.38 (dd, 1H, H-3', J_{2',3'} 10 Hz, J_{3',4'} 3.5 Hz); 5.39 (s, 1H, Ph(H)C); 5.47 (d, 1H, NHC(O)CH₃); 5.61 (dd, 1H, H-2'); 5.83 (dd, 1H, H-4', J_{4',5'} 1.2 Hz); 7.24-8.17 (m, 20H, H-arom).

3-Azidopropyl 3-O-(2,4,6-tri-O-benzoyl- β -D-galactopyranosyl)-2-acetyl-amino-4,6-O-benzylidene-2-deoxy- α -D-galactopyranoside (25) - Compound **24** (28 mg, 0.030 mmol) was dissolved in a mixture of hydrazine monohydrate (0.044 mmol), acetic acid (0.376 ml) and pyridine (0.60 ml) and was subsequently stirred for 7 minutes. The reaction mixture was then diluted with dichloromethane and successively washed with water, 1N HCl, aqueous NaHCO₃ and water, dried and concentrated. The crude mixture was purified on silicagel (toluene/ethyl acetate 1/1 $\rightarrow$ 2/3) to give **25** (18 mg, 72%). *R*_f 0.20 (toluene/ethyl acetate 2/3). ¹H NMR (200 MHz)(CDCl₃): δ 1.33 (s, 3H, NHC(O)CH₃); 1.75 (m, 2H, OCH₂CH₂CH₂N₃); 3.22 (m, 2H, OCH₂CH₂CH₂N₃); 4.94 (d, 1H, H-1', J_{1',2'} 7.9 Hz); 5.02 (d, 1H, H-1, J_{1,2} 3.4 Hz); 5.27 (s, 1H, Ph(H)C); 5.34 (dd, 1H, H-2', J_{2',3'} 10.0 Hz); 5.43 (d, 1H, NHC(O)CH₃, J_{NH,2} 8.0 Hz); 5.68 (dd, 1H, H-4', J_{3',4'} 3.6 Hz, J_{4',5'} 1.2 Hz); 7.04-8.10 (m, 20H, H-arom). ¹³C NMR (CDCl₃): δ 97.974 (C-1); 100.853, 101.734 (C-1', Ph(H)C).

3-Azidopropyl 3-O-(4-O-acetyl-3-O-allyl-2,6-di-O-benzyl- β -D-galactopyranosyl)-2-acetyl-amino-4,6-O-benzylidene-2-deoxy- α -D-galactopyranoside (26) - A mixture of glycosyl donor **23** (4.17 g, 8.57 mmol), glycosyl acceptor **18** (1.68 g, 4.29 mmol) and molecular sieves 4 Å (1.8 g) in acetonitrile (50 ml) was stirred for 30 minutes at room temperature. A freshly prepared solution of N-iodosuccinimide (1.93 g) and trifluoromethanesulfonic acid (155 μ l) in acetonitrile (30 ml) was added dropwise to the mixture. After stirring for 30 minutes at 0 °C, the mixture was filtered and poured out in a mixture of ethyl acetate, aqueous Na₂S₂O₃ and aqueous NaHCO₃. The organic layer was washed with water, dried and concentrated. Purification of the residue on silicagel (dichloromethane/acetone 95/5 $\rightarrow$ 9/1) afforded β -coupled **26** together with the α -coupled isomer (2.07 g, 60%, α/β 1/2). *R*_f (**26 β**) 0.28; *R*_f (**26 α**) 0.25 (dichloromethane/acetone 9/1). ¹H NMR (200 MHz)(CDCl₃): δ 4.51 (d, 1H, H-1'(**26 β**), J_{1',2'} 7.8 Hz); 5.00 (d, 1H, H-1(**26 α**), J_{1,2} 3.4 Hz); 5.05 (d, 1H, H-1'(**26 α**), J_{1',2'} 3.4 Hz); δ 5.15 (d, 1H, H-1(**26 β**), J_{1,2} 3.4 Hz).

3-Azidopropyl 3-O-(4-O-acetyl-3-O-allyl-2,6-di-O-benzyl- β -D-galactopyranosyl)-4,6-di-O-acetyl-2-acetyl-amino-2-deoxy- α -D-galactopyranoside (28) - A mixture of compound **26 α** and **26 β** (3.2 g, 3.9 mmol) was dissolved in 80% acetic acid (300 ml). After stirring for 1 h at 60 °C, toluene was added and the mixture was concentrated. The residual oil (compound **27**) was dissolved in a mixture of pyridine and acetic anhydride (250 ml, 3/2) and a catalytic amount of 4-dimethylaminopyridine was added. After stirring for 16 h, toluene was added and the mixture was concentrated. The crude compound was chromatographed on silicagel (heptane/ethyl acetate 1/1 $\rightarrow$ 2/3) to give compound **28 β** together with the α -coupled isomer (2.5 g, 80%). *R*_f 0.30 (dichloromethane/acetone 9/1).

3-Azidopropyl 3-O-(4-O-acetyl-2,6-di-O-benzyl- β -D-galactopyranosyl)-4,6-di-O-acetyl-2-acetyl-amino-2-deoxy- α -D-galactopyranoside (5) - A mixture of compound **28 β** and **28 α** (380 mg, 0.47 mmol) was dissolved in freshly distilled tetrahydrofuran (30 ml). In order to remove impurities that may interfere with the

isomerisation, the solution was treated with 10% Pd/C (150 mg). After stirring for 30 minutes the mixture was filtered and a catalytic amount of 1,5-cyclooctadiene-bis[methyldiphenylphosphine]-iridium hexafluorophosphate was added to the filtrate. The stirred solution was degassed, placed under hydrogen for 2 minutes, degassed and placed under nitrogen. After stirring for 2 h, the mixture was diluted with water (3 ml) and N-iodosuccinimide (230 mg) was added. The reaction mixture was stirred for 1 h, then diluted with dichloromethane, washed with aqueous Na₂S₂O₃ and water, dried and concentrated. The α/β -mixture was now separated on silicagel (toluene/acetone 8/2) to give β -coupled **5** (144 mg, 40%). *R*_f 0.19 (toluene/acetone 7/3). ¹H NMR (200 MHz)(CDCl₃): δ 1.87 (m, 2H, OCH₂CH₂CH₂N₃); 1.77, 2.04, 2.05, 2.12 (4xs, 12H, NHC(O)CH₃, 3xOC(O)CH₃); 2.29 (d, 1H, OH); 3.35 (m, 2H, OCH₂CH₂CH₂N₃); 3.40 (dd, 1H, H-2', J_{1,2'} 8.0 Hz); 3.48-3.55 (m, 2H, OCH(H)CH₂CH₂N₃); 3.78 (m, 1H, OCH(H)CH₂CH₂N₃); 4.43-4.89 (m, 4H, 2xCH₂Ph); 4.51 (d, 1H, H-1', J_{1,2'} 8.0 Hz); 4.90 (d, 1H, H-1, J_{1,2} 3.0 Hz); 5.33 (dd, 1H, H-4', J_{3,4'} 3.0 Hz, J_{4',5'} 1.0 Hz); 5.50 (bd, 1H, H-4); 5.76 (d, 1H, NHC(O)CH₃) 7.15-7.37 (m, 10H, H-arom).

3-Azidopropyl 3-O-{3-O-[4-O-(4-O-acetyl-2,6-di-O-benzyl-3-O-levulinoyl- β -D-galactopyranosyl)-3,6-di-O-benzyl-2-deoxy-2-phthalimido- β -D-glucopyranosyl]-4-O-acetyl-2,6-di-O-benzyl- β -D-galactopyranosyl]-4,6-di-O-acetyl-2-acetyl-amino-2-deoxy- α -D-galactopyranoside (29)} - Compound **29** was prepared by reaction of thioglycoside **4** and glycosyl acceptor **5** in the same way as described for the synthesis of **24** starting from **18** and **20**. Yield of compound **29**: 50%. *R*_f 0.33 (toluene/acetone 7/3). ¹H NMR (400 MHz)(CDCl₃): δ 1.76 (m, 2H, OCH₂CH₂CH₂N₃); 1.64, 1.86, 1.91, 2.01, 2.07, 2.16 (6xs, 18H, NHC(O)CH₃, 4xOC(O)CH₃, C(O)(CH₂)₂C(O)CH₃); 2.37-2.82 (m, 4H, C(O)(CH₂)₂C(O)CH₃); 3.26 (m, 2H, OCH₂CH₂CH₂N₃); 3.38 (m, 1H, OCH(H)CH₂CH₂N₃); 3.65 (m, 1H, OCH(H)CH₂CH₂N₃); 5.57 (d, 1H, NHC(O)CH₃, J_{NH,2a} 8.0 Hz); 6.79-7.46 (m, 34H, H-arom).

unit a*: 4.84 (d, 1H, H-1, J_{1,2} 3.0 Hz); 4.42 (c, 1H, H-2); 3.97 (c, 1H, H-3, J_{3,4} 3.0 Hz); 5.42 (bd, 1H, H-4).

unit b: 4.44 (d, 1H, H-1, J_{1,2} 9.0 Hz); 3.38 (c, 1H, H-2); 3.66 (c, 1H, H-3); 5.38 (bd, 1H, H-4, J_{3,4} 3.6 Hz).

unit c: 5.27 (d, 1H, H-1, J_{1,2} 8.2 Hz); 4.15 (c, 1H, H-2).

unit d: 4.54 (c, 1H, H-1); 3.50 (c, 1H, H-2); 4.86 (dd, 1H, H-3, J_{2,3} 8.2 Hz, J_{3,4} 3.6 Hz); 5.33 (bd, 1H, H-4).

*The monosaccharide units are denoted from the reducing end (unit a) to the non-reducing end (unit d).

3-Azidopropyl 3-O-{3-O-[4-O-(4-O-acetyl-2,6-di-O-benzyl- β -D-galactopyranosyl)-3,6-di-O-benzyl-2-deoxy-2-phthalimido- β -D-glucopyranosyl]-4-O-acetyl-2,6-di-O-benzyl- β -D-galactopyranosyl]-4,6-di-O-acetyl-2-acetyl-amino-2-deoxy- α -D-galactopyranoside (30)} - compound **29** was delevulinoylated using the procedure described for the synthesis of **25**. Purification of the crude compound on silicagel (toluene/ethyl acetate 8/2) afforded compound **30** in 89% yield. *R*_f 0.18 (toluene/ethyl acetate 8/2). ¹H NMR (360 MHz)(CDCl₃): δ 1.77 (m, 2H, OCH₂CH₂CH₂N₃); 1.34, 1.86, 1.97, 2.01, 2.07, (5xs, 15H, NHC(O)CH₃, 4xOC(O)CH₃); 3.26 (m, 2H, OCH₂CH₂CH₂N₃); 3.42 (m, 1H, OCH(H)CH₂CH₂N₃); 3.64 (m, 1H, OCH(H)CH₂CH₂N₃); 5.55 (d, 1H, NHC(O)CH₃, J_{NH,2a} 8.0 Hz); 6.75-7.47 (m, 34H, H-arom).

unit a*: 4.85 (d, 1H, H-1, J_{1,2} 3.4 Hz); 4.43 (c, 1H, H-2); 3.97 (c, 1H, H-3); 5.42 (bd, 1H, H-4).

unit b: 4.43 (c, 1H, H-1); 3.37 (c, 1H, H-2); 3.64 (c, 1H, H-3); 5.40 (bd, 1H, H-4).

unit c: 5.28 (d, 1H, H-1, J_{1,2} 8.0 Hz); 4.15 (c, 1H, H-2); 4.26 (c, 1H, H-3); 4.07 (c, 1H, H-4).

unit d: 4.49 (d, 1H, H-1, J_{1,2} 8.0 Hz); 3.97 (c, 1H, H-2); 3.58 (c, 1H, H-3); 5.32 (bd, 1H, H-4).

*The monosaccharide units are denoted from the reducing end (unit a) to the non-reducing end (unit d).

Synthesis of the protected hexasaccharide (2) - Glycosyl donor **3** and glycosyl acceptor **30** were coupled using the same conditions as described for the synthesis of **24**. The crude product was successively chromatographed on Sephadex LH-20 (dichloromethane/methanol 1/1) and on silicagel (ethyl acetate/heptane 7/3) to give **2** in 58% yield. *R*_f 0.29 (dichloromethane/methanol 98/2). ¹H NMR (600 MHz)(CDCl₃): δ 1.75 (m, 2H, OCH₂CH₂CH₂N₃); 1.33, 1.83, 1.97, 1.98, 2.00, 2.01, 2.02, 2.07, 2.11 (9xs, 27H, NHC(O)CH₃, 8xOC(O)CH₃); 3.25 (m, 2H, OCH₂CH₂CH₂N₃); 3.38 (m, 1H, OCH(H)CH₂CH₂N₃); 3.64 (m, 1H, OCH(H)CH₂CH₂N₃); 4.02 (m, 1H, OCH(H)CH=); 4.05-4.81 (m, 14H, 7xCH₂Ph); 4.22 (m, 1H, OCH(H)CH=); 5.18-5.32 (m, 2H, =CH₂); 5.54 (d, 1H, NHC(O)CH₃, J_{NH,2a} 8.2 Hz); 5.94 (m, 1H, -CH=); 6.76-7.48 (m, 43H, H-arom).

unit a*: 4.84 (d, 1H, H-1, $J_{1,2}$ 3.6 Hz); 4.38 (c, 1H, H-2); 3.95 (c, 1H, H-3); 5.40 (d, 1H, H-4, $J_{3,4}$ 3.5 Hz); 3.90 (c, 1H, H-5).

unit b: 4.31 (d, 1H, H-1, $J_{1,2}$ 8.0 Hz); 3.28 (c, 1H, H-2); 3.52 (c, 1H, H-3); 5.32 (c, 1H, H-4).

unit c: 5.13 (d, 1H, H-1, $J_{1,2}$ 8.0 Hz); 4.06 (c, 1H, H-2); 3.90 (c, 1H, H-3); 3.91 (c, 1H, H-4); 3.16 (c, 1H, H-5).

unit d: 4.28 (d, 1H, H-1, $J_{1,2}$ 8.0 Hz); 3.32 (dd, 1H, H-2, $J_{2,3}$ 10.0 Hz); 3.56 (dd, 1H, H-3, $J_{3,4}$ 3.4 Hz); 5.28 (d, 1H, H-4).

unit e: 5.24 (d, 1H, H-1, $J_{1,2}$ 8.0 Hz); 4.05 (c, 1H, H-2); 3.94 (c, 1H, H-3).

unit f: 4.78 (d, 1H, H-1, $J_{1,2}$ 8.0 Hz); 5.21 (c, 1H, H-2, $J_{2,3}$ 10.0 Hz); 4.99 (dd, 1H, H-3, $J_{3,4}$ 3.4 Hz); 5.34 (c, 1H, H-4).

*The monosaccharide units are denoted from the reducing end (unit a) to the non-reducing end (unit f).

Deprotection and sulphation of hexasaccharide 2

a) Cleavage of the phthalimido groups and simultaneous saponification of the acetyl esters, acetylation and subsequent removal of the allyl group (32) - Compound **2** (460 mg, 0.193 mmol) was dissolved in a mixture of 1-butanol (15 ml) and ethylenediamine (3.4 ml). After stirring for 20 h at 90 °C, the mixture was concentrated. The residual oil (R_f 0.22 (toluene/ethanol 8/2)) was acetylated in a mixture of pyridine and acetic anhydride (72 ml, 3/1), containing a catalytic amount of 4-dimethylaminopyridine. After stirring for 3 h, toluene was added and the mixture was concentrated. The residue was purified by successively gel filtration on Sephadex LH-20 (dichloromethane/methanol 1/1) and by reversed phase chromatography (acetonitrile/water 6/4 → 7/3) to give **31** (350 mg, 83%). R_f 0.52 (toluene/ethanol 8/2). The allyl group was isomerised in the presence of 1,5-cyclooctadiene-bis[methyldiphenylphosphine]-iridium hexafluorophosphate using the procedure as described for the synthesis of compound **5**. Removal of propenyl group was effected using HgO (120 mg) and HgCl₂ (150 mg) in a mixture of dioxane (6 ml) and water (0.5 ml). After stirring for 2½ h the reaction mixture was diluted with dichloromethane, filtered through celite, washed with aqueous KI, aqueous Na₂S₂O₃ and water, dried and concentrated. Purification of the crude compound by reversed phase chromatography (acetonitrile/water 65/35 → 7/3) gave compound **32** in 67% yield. R_f 0.53 (dichloromethane/methanol 93/7). ¹H NMR (600 MHz)(CDCl₃): δ 1.85 (m, 2H, OCH₂CH₂CH₂N₃); 1.35, 1.38, 1.65, 2.00, 2.01, 2.04, 2.05, 2.06, 2.06, 2.11, 2.13 (11xs, 33H, 3xNHC(O)CH₃, 8xOC(O)CH₃); 3.35 (m, 2H, OCH₂CH₂CH₂N₃); 3.48 (m, 1H, OCH(H)CH₂CH₂N₃); 3.72 (m, 1H, OCH(H)CH₂CH₂N₃); 4.27-4.93 (m, 14H, 7xCH₂Ph); 5.04, 5.14, 5.81 (3xd, 3H, 3xNHC(O)CH₃); 7.23-7.37 (m, 35H, H-arom).

unit a: 4.91 (c, 1H, H-1); 4.61 (c, 1H, H-2); 4.08 (dd, 1H, H-3, $J_{2,3}$ 10.6 Hz, $J_{3,4}$ 3.4 Hz); 5.53 (bd, 1H, H-4); 4.06 (c, 1H, H-5); 3.98 (c, 1H, H-6); 4.15 (dd, 1H, H-6').

unit b: 4.51 (d, 1H, H-1, $J_{1,2}$ 8.0 Hz); 3.53 (c, 1H, H-2). 3.65 (dd, 1H, H-3, $J_{2,3}$ 10.0 Hz, $J_{3,4}$ 3.6 Hz); 5.32 (bd, 1H, H-4); 3.62 (c, 1H, H-5).

unit c: 4.92 (d, 1H, H-1, $J_{1,2}$ 8.0 Hz); 3.29 (c, 1H, H-2); 3.85 (c, 1H, H-3); 3.97 (c, 1H, H-4); 3.34 (c, 1H, H-5); 3.61 (c, 1H, H-6); 3.73 (c, 1H, H-6').

unit d: 4.48 (d, 1H, H-1, $J_{1,2}$ 7.6 Hz); 3.51 (c, 1H, H-2); 3.52 (c, 1H, H-3); 5.58 (bd, 1H, H-4, $J_{3,4}$ 3.6 Hz).

unit e: 4.90 (d, 1H, H-1, $J_{1,2}$ 8.0 Hz); 3.39 (c, 1H, H-2); 3.89 (c, 1H, H-3); 3.38 (c, 1H, H-4).

unit f: 4.73 (c, 1H, H-1, $J_{1,2}$ 8.0 Hz); 5.22 (dd, 1H, H-2, $J_{2,3}$ 8.0 Hz); 5.05 (dd, 1H, H-3, $J_{3,4}$ 3.4 Hz); 5.37 (bd, 1H, H-4); 3.86 (c, 1H, H-5); 3.99 (c, 1H, H-6); 4.06 (c, 1H, H-6').

b) Sulphation (33) - To a solution of compound **32** (155 mg, 0.072 mmol) in N,N-dimethylformamide (6.0 ml) was added Et₃N·SO₃ (139 mg, 0.72 mmol). After stirring for 2 h at 40 °C, the solution was cooled to 0 °C and NaHCO₃ (240 mg) was added. The mixture was stirred for another 15 minutes, after which time dichloromethane and water were added. The organic layer was washed with aqueous NaCl and dried. Methanol (15 ml) was added and dichloromethane was evaporated. The solution of compound **33** (R_f 0.48 (dichloromethane/methanol 92/8)) was directly used for the next reaction.

c) Deacetylation, reduction of the azide group and reduction of the benzyl ethers (1) - To a stirred solution of compound **33** in methanol (15 ml) was added K₂CO₃ (45 mg). After stirring for 20 h the mixture was neutralized with a 10% solution of acetic acid in methanol. N,N-Dimethylformamide (6 ml) was added and methanol was evaporated. To the solution was subsequently added water (6 ml), ammonia (0.02 ml) and 10%

Pd/C (120 mg). The mixture was stirred under hydrogen atmosphere for 2½ h after which time the ammonia was removed by a stream of nitrogen. Acetic acid (3 droplets) was added and the mixture was stirred under hydrogen for another 16 h. The catalyst was removed by filtration and the filtrate was evaporated to dryness. The crude compound was desalted on G-25 Sephadex column, to give compound **1** (40.5 mg, 46% overall yield from **32**). R_f 0.36 (pyridine/ethyl acetate/acetic acid/water 5/7/1.6/4); $[\alpha]_D^{20} +34.6^\circ$ (c 0.5, H₂O); FAB(+): 1251.7 (M+H)⁺; FAB(-): 1249.8 (M-H)⁻. ¹H NMR (600 MHz)(D₂O): δ 1.88 (m, 2H, OCH₂CH₂CH₂NH₂); 1.89, 1.90, 1.91 (3xs, 9H, 3xNHC(O)CH₃); 3.06 (m, 2H, OCH₂CH₂CH₂NH₂); 3.53 (m, 2H, OC(H)HCH₂CH₂NH₂); 3.75 (m, 2H, OC(H)HCH₂CH₂NH₂).
 unit a*: 4.84 (d, 1H, H-1, $J_{1,2}$ 3.8 Hz); 4.27 (dd, 1H, H-2, $J_{2,3}$ 11.0 Hz); 3.95 (dd, 1H, H-3, $J_{3,4}$ 3.0 Hz); 4.17 (d, 1H, H-4); 3.90 (c, 1H, H-5).
 unit b: 4.39 (d, 1H, H-1, $J_{1,2}$ 7.9 Hz); 3.51 (m, 1H, H-2); 3.61 (c, 1H, H-3); 4.17 (d, 1H, H-4, $J_{3,4}$ 3.2 Hz); 3.58 (c, 1H, H-5).
 unit c: 4.65 (d, 1H, H-1, $J_{1,2}$ 7.8 Hz); 3.50-3.73 (m, 6H, H-2, H-3, H-4, H-5, H-6, H-6')
 unit d: 4.40 (d, 1H, H-1, $J_{1,2}$ 8.0 Hz); 3.53 (c, 1H, H-2); 3.65 (c, 1H, H-3); 4.13 (d, 1H, H-4, $J_{3,4}$ 3.0 Hz); 3.61 (c, 1H, H-5).
 unit e: 4.65 (d, 1H, H-1, $J_{1,2}$ 7.8 Hz); 3.50-3.75 (m, 4H, H-2, H-3, H-4, H-5); 4.24 (m, 1H, H-6); 4.33 (m, 1H, H-6');
 unit f: 4.46 (d, 1H, H-1, $J_{1,2}$ 7.8 Hz); 3.46 (dd, 1H, H-2, $J_{2,3}$ 9.6 Hz); 3.61 (dd, 1H, H-3, $J_{3,4}$ 3.2 Hz); 3.86 (d, 1H, H-4).

*The monosaccharide units are denoted from the reducing end (unit a) to the non-reducing end (unit f).

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