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Note

A facile synthesis of the GalNAcβ1→4Gal target sequence of respiratory pathogens

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Abstract—The carbohydrate sequence, GalNAcβ1→4Gal, is the target for the adhesion of several respiratory pathogens. The sequence was prepared in an optimized synthesis in forms that allow conjugation to scaffolds or surfaces.

Keywords: Bacterial adhesion; GalNAcβ1→4Gal synthesis

Pulmonary pathogens such as those infecting cystic fibrosis patients, *Pseudomonas aeruginosa*, *Haemophilus influenzae*, and *Staphylococcus aureus*, were previously shown to bind to the disaccharide sequence GalNAcβ1→4Gal present as part of glycolipids on the surface of lung tissue.[1] Conjugates of this sequence have potential as antibacterial agents by preventing adhesion and thus infection, as alternatives to conventional antibiotics. While we previously showed the synthesis and evaluation of multivalent versions of the sequence,[2] which resulted in a multivalent inhibition enhancement, the synthesis of the disaccharide in a form ready for conjugation had to be improved. The disaccharide synthesis has been achieved in 1984 by Lemieux et al.[3] but required many steps and consequently had a low overall yield. Since then, the synthesis of the GalNAcβ1→4Gal sequence was reported several times.[4] However, in many of these studies the sequence was part of a larger structure,[4c–h] necessitating the introduction of various protecting groups to allow attachment of additional sugar residues, which again resulted in a low overall yield. Furthermore, the obtained products were not always in a form allowing further conjugation[4a] and often the experimental data were incomplete or absent. Although over time procedures have improved since the first attempt, further improvement is needed for practical applications. However, the sequence is challenging, since it requires the attack of the relatively non-reactive axial galactose C-4 hydroxyl. We here describe a facile synthesis that has a 22% overall yield, requires few purification steps, and the products are compatible with conjugation to carboxylic acid or amine functionalities or via copper-catalyzed cycloaddition with acetylenes, the so-called 'click' chemistry.[5] Besides application to prevent microbial adhesion, for which the availability of significant amounts of material has been a limiting factor, further applications involving the association to the bacterial surface include the use of carbohydrate arrays,[5c,6] for example, for bacterial typing for diagnostic purposes.

In the synthesis, first peracetylated galactose **1** was brominated at C-1 (Scheme 1).[7] In order to make the thiophenyl derivative **2**, the bromide was exposed to a mixture of NBu_4HSO_4 and thiophenol in aq sodium carbonate and EtOAc. The acetate groups of **2** were subsequently removed using sodium methoxide, which was followed by treatment with α,α-dimethoxytoluene and a catalytic amount of *p*-TsOH in CH_3CN. This yielded the corresponding benzylidene compound, that could be purified by crystallization and resulted in **4** after acetylation of the remaining hydroxyl groups. The preparation thus far required no chromatography, gave a 70%

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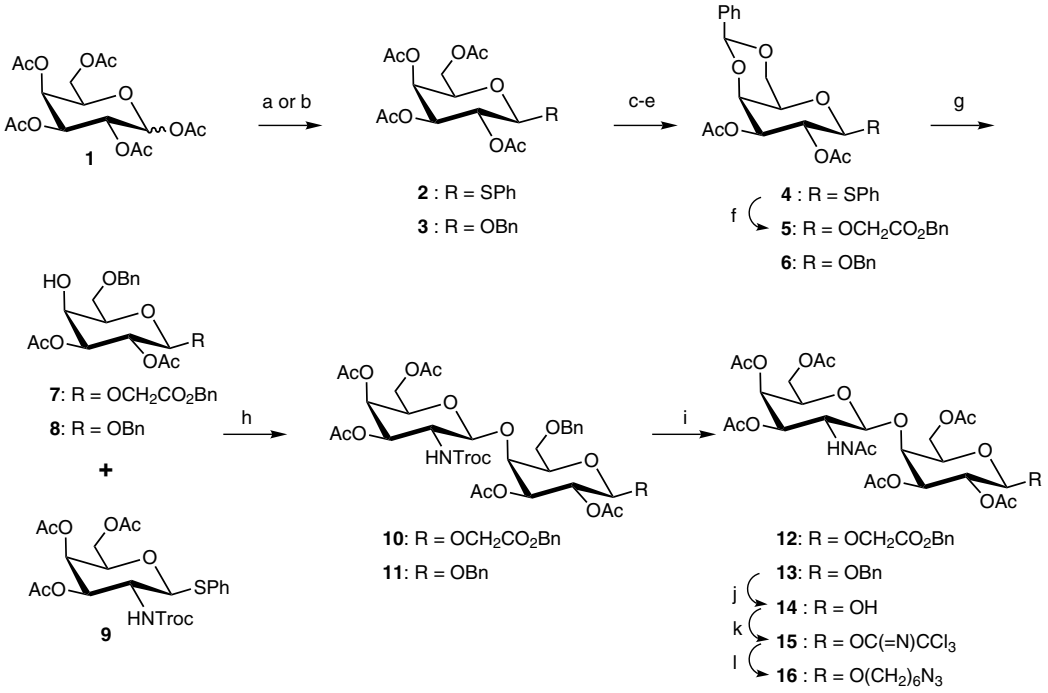

Scheme 1. Reagents and conditions: (a) (i) HBr, Ref. 7, quant; (ii) NBu_4HSO_4, PhSH Na_2CO_3, EtOAc, 15 min prod. **2**; (b) Ref. 11, 81% of **3**; (c) NaOMe, MeOH, 2 h; (d) $PhCH(OMe)_2$, p-TsOH, CH_3CN, 1.5 h; (e) Ac_2O, py 18 h, 70% for **6** from **3**; 70% for **4** from **1**; (f) $HOCH_2CO_2Bn$, NIS (1.3 equiv), TfOH, CH_2Cl_2, −70 °C, 1 h, 76%; (g) $NaCNBH_3$, HCl–Et_2O, THF, 3 h, 75–80%; (h) NIS, TfOH, CH_2Cl_2, MS 4 Å, −70 °C to rt, 2 h, 73% (**10**), 90% (**11**); (i) (i) Zn powder, Ac_2O, 6 h; (ii) $ZnCl_2$, Ac_2O–AcOH, 48 h. 70% (**12**), 81% (**13**); (j) Pd–C, THF, 18 h; (k) CCl_3CN, DBU, CH_2Cl_2, 1 h; (l) $HOC_6H_{12}N_3$, TMSOTf, CH_2Cl_2, −70 °C to 0 °C, 2 h. 71% from **13**.

yield from the starting galactoside **1** and was performed on a multigram scale. Glycosylation with benzyl glycolate and NIS–TfOH[8] in dichloromethane, furnished **5** in 76% yield (Table 1). Liberation of the 4-OH by reductive opening of the benzylidene ring by $NaCNBH_3$ was followed by NIS promoted glycosylation with donor **9**, which was prepared in three steps from D-(+)-galactosamine hydrochloride in 66% overall yield.[2] This donor, which was also readily made in multigram amounts, contained a Troc protecting group reported to significantly increase donor reactivity in coupling reactions while effecting good β-selectivity as well.[9] The coupling resulted solely in the β-linked-disaccharide **10**. The Troc group was replaced by an acetyl group by the action of Zn in Ac_2O. In order to facilitate deprotection at the multivalent or otherwise conjugated stage, it was deemed preferable to have only a single type of protecting group present on the sugar, that is, acetyl groups. While this was partially achieved under the Troc converting conditions, a report of Yang et al.[10] proved useful for the selective conversion of the 6-OBn by $ZnCl_2$ in AcOH–Ac_2O to an acetate, yielding **12**. This represents a protected GalNAcβ1→4Gal derivative, ready for conjugation to amine functionalized scaffolds or surfaces.

Similarly, a derivative was prepared that could be conjugated to carboxylic acid functionalized scaffolds or surfaces. While the previous synthesis necessitated the early incorporation of the aglycon part, in this method the use of an anomeric benzyl group allows for conjugation at the very end. As before, the synthesis started with acetylated galactose which was now converted to its C-1 benzyl functionalized derivative **3** by $BF_3{\cdot}OEt_2$.[11] The subsequent steps leading to the benzylidene derivative **6** and its ring opened counterpart **8** proceeded as before, setting the stage for glycosylation with donor **9**. The NIS-promoted glycosylation gave the desired β-linked-product **11** in a high yield of 90%. Again the two-step protecting group conversion by first Zn and then $ZnCl_2$ yielded **13**. The anomeric benzyl group was unaffected by the latter step, which selectively removed the benzyl group at the sixth position, to our knowledge a first observation of this type of selectivity. After hydrogenation of **13**, any desired spacer can be linked to C-1 using trichloroimidate chemistry. To this end, the hydrogenation product **14** was first converted to the trichloroacetamidate **15** and subsequently coupled in good yield to 6-azido-1-hexanol using TMSOTf as a catalyst. This protected GalNAcβ1→4Gal derivative can be conjugated to carboxylic acid bearing scaffolds or surfaces after hydrogenation or via copper-catalyzed cycloaddition with acetylenes, the so-called 'click' chemistry.[5]

In conclusion, we report a facile and efficient synthesis of two linkable versions of the GalNAcβ1→4Gal sequence, an important sequence to which various bacterial pathogens bind.[1,12] Of the two compounds, one

Table 1. ^{1}H NMR chemical shifts and coupling constants of the prepared compounds ($CDCl_3$)

	5	7	10	6	8	11	13	16
H-1	4.68	4.60	4.62	4.55	4.50	4.50	4.49	4.43
H-2	5.42	5.33	5.22	5.47	5.35	5.26	5.32	5.25
H-3	4.98	4.94	5.02	4.93	4.90	4.98	4.81	4.94
H-4	4.38	4.14	3.35	4.36	4.11	4.16	5.39	4.13
H-5	2.85	3.71	3.92	3.49	3.74	3.75	3.73	3.73
H-6a	4.03	3.71	3.92	4.07	3.74	3.75	4.32	4.29
H-6b	4.28	3.71	3.92	4.36	3.74	3.75	4.32	4.29
H-1′			4.75			4.81	5.13	5.12
H-2′			3.70			3.75	3.38	3.36
H-3′			4.75			5.26	5.92	5.93
H-4′			5.34			5.39	5.39	5.38
H-5′			3.92			3.85	3.93	3.90
H-6a′			3.92			3.98	4.09	4.05
H-6b′			3.92			4.10	4.09	4.05
$J_{1,2}$	8.0	8.0	7.7	8.0	8.0	8.0	8.0	8.0
$J_{2,3}$	10.4	10.1	10.2	10.2	10.2	10.2	10.4	10.4
$J_{3,4}$	3.6	3.0	3.0			2.7		2.5
$J_{4,5}$								
$J_{1',2'}$							8.2	8.2
$J_{2',3'}$							11.5	11.3
$J_{3',4'}$							3.3	3.3
C*H*Ph	5.49			5.51				
$CH_2C(O)$ (Linker)	4.38	4.34	4.35					
$CH_2C_6H_5$ (Linker)	5.17	5.16	5.17					
$CH_2C_6H_5$ (6-OBn)		4.54	4.53		4.57	4.59		
$CH_2C_6H_5$ (1-OBn)				4.65	4.62	4.63	4.64	
				4.93	4.90	4.81	4.81	
CH_2CCl_3			4.75			4.81		
$OC_6H_{12}N_3$								1.37
								1.59
								3.27
								3.48
								3.90

contained a protected carboxylic acid while the other featured a masked amino group, that is, an azide. They were both synthesized in 22% overall yield or 15% when considering the donor synthesis.

1. Experimental

1.1. General remarks

Chemicals were obtained from commercial sources and used without further purification unless stated otherwise. The solvents CH_2Cl_2, MeOH and dioxane were purchased from Biosolve, the Netherlands. The solvents CH_2Cl_2 and MeOH were stored on molecular sieves, 4 Å and 3 Å, respectively. Activated molecular sieves were prepared by flame-drying under diminished pressure followed by a nitrogen flush. Column chromatography was performed on E. Merck Kieselgel 60 (40–63 μm). TLC analysis was performed using E. Merck pre-coated silica gel 60 F-254 plates; spots were visualized by UV light (254 nm) and/or by charring after treatment with 10% H_2SO_4–MeOH. For neutralization, Dowex 50 × 8 (H^+-form; 20–50 mesh) purchased from Fluka was used. ^{1}H NMR spectra were recorded on a Varian G-300 spectrometer (300 MHz) in $CDCl_3$ and chemical shifts are given in ppm relative to Me_4Si (0 ppm). ^{13}C NMR spectra were recorded on a Varian G-300 spectrometer at 75.4 MHz in $CDCl_3$ (referenced to $CDCl_3$ at 77.0 ppm) using the attached proton test (APT) sequence. Electrospray ionization (ESI) mass spectrometry was carried out using a Shimadzu LCMS QP-8000 single quadrupole benchtop mass spectrometer (m/z range < 2000), coupled with a QP-8000 data system.

1.2. Synthesis

1.2.1. Multi-step protocol for synthesis of phenyl 2,3-di-*O*-acetyl-4,6-*O*-benzylidene-1-thio-β-D-galactopyranoside (4)—optimized conditions. Acetylated galactose **1** was obtained from galactose (2.5 g, 13.9 mmol) and converted to the corresponding bromide.[7] It was dissolved in EtOAc (100 mL) and added to a mixture of Bu_4NHSO_4 (4.7 g, 13.9 mmol), thiophenol (2 mL, 19.5 mmol) in 1 M Na_2CO_3 (100 mL) and vigorously stirred at rt for 15 min. After the addition of EtOAc (5 mL), the organic phase was separated and washed with 1 M NaOH (50 mL), water (2 × 50 mL) and brine (50 mL), dried (Na_2SO_4), concentrated, and dissolved in MeOH (100 mL). In order to deprotect the hydroxyl groups,

NaOMe (33% v/v in MeOH, 2 mL) was added and the mixture was stirred for 2 h, after which it was neutralized with Dowex/H^+ ion-exchange resin, filtered, and concentrated under diminished pressure. Prior to benzylidene formation the residue was subjected to high vacuum, thereby removing all traces of MeOH and facilitating the attachment of α,α-dimethoxytoluene. The product was suspended in CH_3CN (10 mL), and after the addition of a catalytic amount of *p*-TsOH (80 mg, 0.4 mmol), 3 Å molecular sieves and α,α-dimethoxytoluene (3.11 mL, 20.9 mmol), the mixture was stirred for 90 min at rt, yielding phenyl 4,6-*O*-benzylidene-1-thio-β-D-galactopyranoside. After crystallization from EtOH, this compound was obtained pure in 50% yield. More material was harvested (up to 20%) after filtration over a silica plug to remove excess α,α-dimethoxytoluene, followed by another crystallization from EtOH. The collected crystals were acetylated using Ac_2O (40 mL) and pyridine (60 mL) and after completion the mixture was concentrated and coevaporated with toluene, EtOH and CH_2Cl_2, giving **4** in 70% overall yield (4.32 g, white solid). Spectroscopic data were consistent with those in Ref. 13.

1.2.2. Benzyloxycarbonylmethyl 2,3-di-*O*-acetyl-4,6-*O*-benzylidene-β-D-galactopyranoside (5). To a soln of **4** (444 mg, 1 mmol) in dry CH_2Cl_2 (10 mL) were added 4 Å molecular sieves (crushed and activated) and benzyl glycolate (117 μL, 1.2 mmol). The mixture was first stirred at rt for 20 min under N_2, after which it was cooled to −70 °C. Then, NIS (318 mg, 1.3 mmol) and a cat. amount of TfOH (17 μL) was added and stirring was continued for approx. 1 h (monitored by TLC, 1:1 EtOAc–hexane). When complete conversion was reached, the mixture was filtered over Celite, taken up in CH_2Cl_2 (75 mL) and subsequently washed with aq $NaHCO_3$ (sat., 75 mL), water (75 mL) and brine (75 mL). The organic layer was dried using $NaSO_4$, concentrated under diminished pressure and subjected to silica gel column chromatography with 1:2 EtOAc–hexane as eluent. Yield: 380 mg (76%, white solid): ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 2.05$, 2.07 ($2\times$s, 6H, $2\times$C(O)CH_3), 7.34–7.51 (m, 10H, $10\times H_{arom}$); ^{13}C NMR (75.4 MHz, $CDCl_3$): $\delta = 20.7$, 20.9 ($2\times$OC(O)*C*H$_3$), 64.0, 66.5, 68.6 (C-6, O*C*H$_2$CO, *C*H$_2$C$_6$H$_5$), 66.3, 67.9, 71.6, 73.0 (C-2, C-3, C-4, C-5), 100.0, 100.9 (C-1, PhCH), 126.2–129.0 ($10\times CH_{arom}$), 135.2, 137.3 ($2\times C_{arom}$), 169.4, 169.7, 170.6 ($3\times$C=O); HRMS *m*/*z* calcd for $C_{26}H_{28}O_{10}$ $[M+Na]^+$ 523.1580, found 523.1535.

1.2.3. Benzyloxycarbonylmethyl 2,3-di-*O*-acetyl-6-*O*-benzyl-β-D-galactopyranoside (7). Ethereal HCl was added dropwise to a stirred mixture of **5** (1.19 g, 2.38 mmol), $NaCNBH_3$ (1.38 g, 21.9 mmol) and molecular sieves (4 Å) in dry THF (20 mL) until gas evolution ceased. The reaction was monitored with TLC analysis (2% MeOH–CH_2Cl_2). When completed, solid $NaHCO_3$, (≈2 g), CH_2Cl_2 (40 mL) and satd aq $NaHCO_3$ (25 mL) were added, and the mixture was filtered over hyflo. After the addition of additional CH_2Cl_2 (40 mL), the organic phase was washed with aq $NaHCO_3$ (satd 2×50 mL), water (50 mL) and dried with Na_2SO_4. After concentration, product **7** was purified with silica gel column chromatography with 2% MeOH–CH_2Cl_2 as an eluent. Yield: 0.96 g (80%, clear oil): ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 2.03$, 2.08 ($2\times$s, 6H, $2\times$C(O)CH_3), 7.30 (m, 10H, $10\times H_{arom}$); ^{13}C NMR (75.4 MHz, $CDCl_3$): $\delta = 20.7$, 20.8 ($2\times$OC(O)*C*H$_3$), 64.5, 66.6, 69.0, 73.6 (C-6, O*C*H$_2$CO, $2\times$*C*H$_2$C$_6$H$_5$), 67.6, 68.7, 73.0, 73.1 (C-2, C-3, C-4, C-5), 100.4 (C-1), 127.7–128.5 ($10\times CH_{arom}$), 135.1, 137.4 ($2\times C_{arom}$), 169.2, 169.9, 170.2 ($3\times$C=O); HRMS *m*/*z* calcd for $C_{26}H_{30}O_{10}$ $[M+Na]^+$ 525.1737, found 525.1418.

1.2.4. Benzyloxycarbonylmethyl *O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-(2′,2′,2′-trichloroethoxycarbonyl-amino))-(β-D-galactopyranosyl)-(1→4)-2,3-di-*O*-acetyl-6-*O*-benzyl-β-D-galactopyranoside (10). Compound **7** (0.96 g, 1.9 mmol) and **9** (0.87 g, 1.82 mmol) were dissolved in dry CH_2Cl_2 (2.5 mL) and stirred under argon in the presence of activated crushed 4 Å molecular sieves. After 30 min, the mixture was cooled to −70 °C and NIS (580 mg, 2.4 mmol) and a cat. amount of TfOH were added. The reaction mixture was stirred while the temperature was allowed to rise to 20 °C. Progression of the reaction was checked with TLC analysis 1:1 EtOAc–hexane. After 2 h, the reaction mixture was filtered over hyflo, CH_2Cl_2 (to a volume of 150 mL) was added after which the mixture was washed with aq $NaHCO_3$ (satd, 100 mL), Na_2SO_3 (satd, 100 mL) and water (100 mL). The organic phase was dried with Na_2SO_4 and concentrated under diminished pressure. Purified product was obtained after silica gel column chromatography with 2:3 EtOAc–hexane as eluent. Yield: 1.28 g (73%, white solid): $[\alpha]_D^{23}$ −23.8 (*c* 1.0 $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 1.99$, 2.00, 2.01, 2.12, 2.13 ($5\times$s, 15H, $5\times$C(O)CH_3), 5.51 (d, $J_{NH,2'} = 7.1$ Hz, 1H, NH), 7.27–7.43 (m, 10H, $10\times H_{arom}$); ^{13}C NMR (75.4 MHz, $CDCl_3$): $\delta = 20.6$ ($5\times$C(O)*C*H$_3$), 52.8 (C-2′), 61.3, 64.4, 66.6, 69.1, 73.4, 74.2 ($6\times CH_2$), 66.5, 68.7, 68.9, 70.4, 72.9, 73.6, 73.7 (C-2, C-3, C-3′, C-4, C-4′, C-5, C-5′), 100.2, 101.0 (C-1, C-1′), 127.5–128.6 ($10\times CH_{arom}$), 135.2, 137.9 ($2\times C_{arom}$), 153.9 (C=O Troc), 169.3–170.3 ($6\times$C=O); HRMS *m*/*z* calcd for $C_{41}H_{48}Cl_3NO_{19}$ $[M+Na]^+$ 986.1784, found 986.1278.

1.2.5. Benzyloxycarbonylmethyl *O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-acetamido-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-acetyl-β-D-galactopyranoside (12). Compound **10** (0.52 g, 0.54 mmol) was dissolved in Ac_2O (50 mL),

excess Zn (2.5 g) was added and the mixture was vigorously stirred for 6 h. When TLC analysis (2% MeOH in CH_2Cl_2) showed that complete conversion had taken place, the mixture was filtered over hyflo, the filter cake was washed with CH_2Cl_2 (20 mL) and the filtrates were concentrated under diminished pressure at 50 °C, followed by coevaporation with toluene (2 × 50 mL). The crude Troc-deprotected residue was dissolved in a mixture of Ac_2O (8 mL) and AcOH (4 mL), $ZnCl_2$ (0.55 g, 4.1 mmol) was added and the mixture was stirred for 48 h. After filtration over hyflo, the reaction mixture was concentrated. The residue was taken up in CH_2Cl_2 (150 mL), washed with water (100 mL), $NaHCO_3$ (satd, 100 mL), water (100 mL) and brine (100 mL). Finally, compound **12** was obtained in pure form after column chromatography with 4:1 EtOAc–hexane as eluent. Overall yield: 0.34 g (70%, white solid): $[\alpha]_D^{23}$ −27.2 (*c* 1.0 $CHCl_3$); spectroscopic data were consistent with those reported in Ref. 2; HRMS *m*/*z* calcd for $C_{35}H_{45}NO_{19}$ $[M+Na]^+$ 806.2484, found 806.2555.

1.2.6. Benzyl 2,3-di-*O*-acetyl-4,6-*O*-benzylidene-β-D-galactopyranoside (6). Compound **3**, obtained from galactose penta-acetate according to Ref. 11, (0.74 g, 81%, 1.62 mmol) was deacetylated after stirring for 2 h in a solution of NaOMe (33% v/v in MeOH, 2 mL) in MeOH (100 mL). After neutralization with Dowex/H^+ ion-exchange resin, the resin was removed by filtration and the filtrate was concentrated under diminished pressure and coevaporated several times with CH_2Cl_2. Thus obtained deacetylated intermediate was then suspended in CH_3CN (6 mL). Next, α,α-dimethoxytoluene (363 μL, 2.43 mmol) and a cat. amount of *p*-TsOH (9 mg) were added and the mixture was stirred. After a few minutes, the product precipitated as a white solid. More CH_3CN was added (20 mL), and stirring was continued for 50 min. The solvent was evaporated under diminished pressure and the benzylidene sugar was crystallized from EtOH. Acetylation of the intermediate was accomplished by stirring for 18 h in a mixture of Ac_2O (10 mL) and pyridine (15 mL). After completion of the reaction, the mixture was concentrated and coevaporated with toluene, EtOH and CH_2Cl_2, giving compound **6**.[14] Overall yield: 0.50 g (70%, white solid): mp 188 °C, lit.[14] 188–189 °C; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 2.02$, 2.06 (2 × s, 6H, 2 × C(O)CH_3), 7.28–7.54 (m, 10H, 10 × H_{arom}); ^{13}C NMR (75.4 MHz, $CDCl_3$): $\delta = 20.6$, 20.7 (2 × C(O)CH_3), 66.2, 68.4, 71.8, 73.2 (C-2, C-3, C-4, C-5), 68.7, 69.9 (C-6, $CH_2C_6H_5$), 99.5, 100.8 (CHC_6H_5, C-1), 126.2–128.9 (10 × CH_{arom}), 137.1, 137.4 (2 × C_{arom}), 169.2, 170.6 (2 × C=O); ESI-MS: $m/z = 465.4$ $[M+Na]^+$.

1.2.7. Benzyl 2,3-di-*O*-acetyl-6-*O*-benzyl-β-D-galactopyranoside (8). Compound **6** (250 mg, 0.48 mmol) was dissolved in THF (10 mL). $NaCNBH_3$ (277 mg, 4.41 mmol) and 4 Å molecular sieves were added to this mixture, after which ethereal HCl was added dropwise until TLC analysis (2% MeOH in CH_2Cl_2) revealed complete conversion. The reaction mixture was quenched with solid $NaHCO_3$, (≈1 g), CH_2Cl_2 (20 mL) and satd $NaHCO_3$ (5 mL) were added and the mixture was filtered over hyflo. The organic phase was washed with satd $NaHCO_3$ (2 × 50 mL), water (50 mL) and dried with Na_2SO_4. After concentration, the product was purified by silica gel column chromatography with 2% MeOH–CH_2Cl_2 as an eluent. Yield: 160 mg (75%, clear oil): ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 1.99$, 2.06 (2 × s, 6H, 2 × C(O)CH_3), 7.25–7.37 (m, 10H, 10 × H_{arom}); ^{13}C NMR (75.4 MHz, $CDCl_3$): $\delta = 20.6$, 20.7 (2 × C(O)CH_3), 67.7, 69.2, 73.1, 73.3 (C-2, C-3, C-4, C-5), 69.0, 70.3, 73.6 (C-6, 2 × $CH_2C_6H_5$), 99.7 (C-1), 127.5–128.4 (10 × CH_{arom}), 136.9, 137.5 (2 × C_{arom}), 169.5, 170.3 (2 × C=O); HRMS *m*/*z* calcd for $C_{24}H_{28}O_8$ $[M+Na]^+$ 467.1682, found 467.1620.

1.2.8. Benzyl (3,4,6-tri-*O*-acetyl-2-deoxy-2-(2′,2′,2′-trichloroethoxycarbonyl-amino))-(β-D-galactopyranosyl)-(1→4)-2,3-di-*O*-acetyl-6-*O*-benzyl-β-D-galactopyranoside (11). Donor **9** (213 mg, 0.44 mmol) and acceptor **8** (220 mg, 0.42 mmol) were dissolved in dry CH_2Cl_2 (10 mL), activated ground molecular sieves (4 Å) were added and the mixture was stirred under argon for 30 min. Then, the mixture was cooled to −70 °C and NIS (134 mg, 0.55 mmol) and a cat. amount of TfOH were added. Stirring was continued for 2 h, while the mixture was allowed to warm up to rt. Successful product formation was observed by TLC analysis 1:1 EtOAc–hexane. The mixture was filtered over hyflo, CH_2Cl_2 (40 mL) was added and the mixture was washed with aq $NaHCO_3$ (satd, 30 mL), Na_2SO_3 (satd, 30 mL) and water (30 mL). The organic phase was then dried with $NaSO_4$ and concentrated under diminished pressure. The product was obtained in pure form after silica gel column chromatography with 1:2 EtOAc–hexane as eluent. Yield: 343 mg (90%, white solid): mp 58 °C; $[\alpha]_D^{23}$ −33.3 (*c* 1.0 $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 1.97$, 1.99, 2.00, 2.11, 2.14 (5 × s, 15H, 5 × C(O)CH_3), 5.39 (m, 2H, 4′-H, NH), 7.33 (m, 10H, 10 × H_{arom}); ^{13}C NMR (75.4 MHz, $CDCl_3$): $\delta = 20.5$, 20.6 (5 × C(O)CH_3), 52.7 (C-2′), 61.2 (C-6′), 66.4, 68.9, 69.4, 70.3, 73.1, 73.5, 73.9 (C-2, C-3, C-3′, C-4, C-4′, C-5, C-5′), 69.2, 70.1, 73.3, 74.1 (2 × $CH_2C_6H_5$, C-6, CH_2CCl_3), 95.7 (CCl_3), 99.4, 100.8 (C-1, C-1′), 127.3–128.3 (10 × CH_{arom}), 136.9, 138.0 (2 × C_{arom}), 153.8, 168.9, 170.0, 170.2 (6 × C=O); HRMS *m*/*z* calcd for $C_{39}H_{46}Cl_3NO_{17}$ $[M+Na]^+$ 928.1729, found 928.0938.

1.2.9. Benzyl (3,4,6-tri-*O*-acetyl-2-deoxy-2-acetamido-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-acetyl-β-D-galactopyranoside (13). Compound **11** (395 mg, 0.436 mmol) was dissolved in Ac_2O (10 mL) and Zn powder (4 g)

was added. In the second step, the intermediate still bearing the benzyl group at C-6 was dissolved in a mixture of Ac_2O (10 mL) and AcOH (5 mL), to which $ZnCl_2$ (446, 3.27 mmol) was added. The product **13** was successfully obtained in 81% yield (256 mg, white solid). Work-up as described above for **12**, mp 70 °C, lit.[4b] 82–92 °C; $[\alpha]_D^{23}$ −33.6 (*c* 1.0 $CHCl_3$), lit.[4b] $[\alpha]_D^{25}$ −37.5 (*c* 0.64 $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$): $\delta = 1.99$–2.14 ($7\times$ s, 21H, $7\times C(O)CH_3$), 6.01 (d, $J_{NH,2'} = 6.9$ Hz, 1H, NH), 7.28–7.38 (m, 5H, $5\times H_{arom}$); ^{13}C NMR (75.4 MHz, $CDCl_3$): $\delta = 20.5$, 20.7, 23.2 ($7\times C(O)CH_3$), 53.2 (C-2′), 61.4, 63.0 (C-6, C-6′), 66.8, 67.8, 69.0, 70.1, 72.0, 72.6, 73.1 (C-2, C-3, C-3′, C-4, C-4′, C-5, C-5′), 70.4 ($CH_2C_6H_5$), 98.7, 99.7 (C-1, C-1′), 127.6, 127.8, 128.2 ($5\times CH_{arom}$), 169.4, 169.6, 170.0, 170.3, 170.4, 170.7, 171.2 ($7\times C{=}O$); HRMS *m/z* calcd for $C_{33}H_{43}NO_{17}$ $[M+Na]^+$ 748.2429, found 748.1372.

1.2.10. 6-Azidohexyl (3,4,6-tri-*O*-acetyl-2-deoxy-2-acetamido-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-acetyl-β-D-galactopyranoside (16). Compound **13** (0.50 g, 0.689 mmol) was dissolved in THF (15 mL) and hydrogenated overnight in a Parr-apparatus, using $Pd(OH)_2$ on carbon (75 mg) as a catalyst. After complete conversion to **14**, the mixture was filtered over hyflo and concentrated under diminished pressure. Crude **14** was dissolved in dry CH_2Cl_2 (10 mL) and stirred under N_2. DBU (82 μL, 0.55 mmol) and trichloroacetonitrile (2.2 mL, 21.7 mmol) were added and the mixture was stirred at rt for 1 h to yield **15**. The reaction mixture was coevaporated twice with toluene, after which the concentrate was dissolved in dry CH_2Cl_2 (6 mL). To this soln, 6-azido-1-hexanol (296 mg, 2.07 mmol) and activated ground molecular sieves (4 Å) were added, after which the mixture was stirred under an argon atmosphere. After 30 min, the mixture was cooled to −70 °C and TMSOTf (50 μL) was added. The reaction was monitored with TLC analysis (3:1 EtOAc–hexane) and after completion, the mixture was filtered over hyflo, CH_2Cl_2 (up to a total volume of 50 mL) was added and the mixture was washed with aq $NaHCO_3$ (satd, 30 mL), Na_2SO_3 (satd, 30 mL) and water (30 mL). Subsequently, the organic phase was dried ($NaSO_4$) and evaporated under diminished pressure. Finally, **16** was obtained pure after silica gel column chromatography with 3:1 EtOAc–hexane as eluent. Overall yield: 71% (351 mg, white solid): $[\alpha]_D^{23}$ −17.0 (*c* 1.0 $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$): $\delta = 1.99$–2.14 ($7\times$ s, 21H, $7\times C(O)CH_3$), 5.82 (d, $J_{NH,2'} = 7.1$ Hz, 1H, NH); ^{13}C NMR (75.4 MHz, $CDCl_3$): $\delta = 20.6$, 20.8, 23.4 ($7\times C(O)CH_3$), 25.3, 26.3, 28.6, 29.1 ($CH_2CH_2CH_2CH_2CH_2CH_2$), 51.2 (CH_2N_3), 53.4 (C-2′), 61.4, 63.0 (C-6, C-6′), 66.8, 67.9, 69.1, 70.2, 71.9, 72.7, 73.1 (C-2, C-3, C-3′, C-4, C-4′, C-5, C-5′), 69.9 (OCH_2CH_2), 98.8, 101.0 (C-1, C-1′), 169.5, 169.7, 170.1, 170.4, 170.5, 170.8, 171.3 ($7\times C{=}O$); HRMS *m/z* calcd for $C_{32}H_{48}N_4O_{17}$ $[M+Na]^+$ 783.2912, found 783.3254.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.carres.2005.08.001.

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