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Synthesis of the trisaccharide, 2-(*p*-trifluoroacetamidophenyl)ethyl *O*-α-D-galactopyranosyl-(1-4)-*O*-β-D-galactopyranosyl-(1-4)-2-acetamido-2-deoxy-β-D-glucopyranoside, corresponding to the blood group P_1 determinant

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The 2-(*p*-trifluoroacetamidophenyl)ethyl β-glycoside of the P_1 antigen trisaccharide, *O*-α-D-galactopyranosyl-(1-4)-*O*-β-D-galactopyranosyl-(1-4)-2-acetamido-2-deoxy-D-glucopyranose, was synthesized. Thioglycoside intermediates were used as building blocks.

Keywords: synthesis, thioglycoside, trichloroacetimidate, P_1 trisaccharide

The P_1 antigen belongs immunologically to the human blood group P system. The structure of the P_1 glycosphingolipid of human erythrocytes was established [1] as: α-D-Gal*p*-(1-4)-β-D-Gal*p*-(1-4)-β-D-GlcNAc*p*-(1-3)-β-D-Gal*p*-(1-4)-D-Glc*p*-ceramide. The trisaccharide located at the non-reducing end has been identified as the P_1 antigenic determinant [2].

Synthesis of the P_1 trisaccharide with a free reducing end, and its glycosides, has been described previously [3–5]. We now report a new synthetic route to the P_1 trisaccharide glycoside **19**, using thioglycoside intermediates. The 2-(*p*-trifluoroacetamidophenyl)ethyl moiety of **19** enables attachment to proteins to form artificial P_1 antigens.

Results and discussion

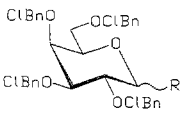
The strategy chosen for synthesis of the P_1 antigen trisaccharide was based on the galactosyl thioglycosides **1** and **8** and the glucosamine glycoside **9** [6] as monosaccharide building blocks. The two galactosyl blocks were condensed to give a digalactosyl block **10**, which was then condensed with the glucosamine block to give the protected trisaccharide **15**. Subsequent deprotection gave the target trisaccharide glycoside **19**.

The following synthetic steps were performed:

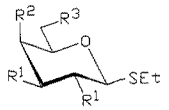
The terminal galactosyl block, ethyl tetra-*O*-*p*-chlorobenzyl-1-thio-β-D-galactopyranoside **1**, was prepared from 1,2,3,4,6-penta-*O*-acetyl-β-D-galactopyranose by successive treatment with ethanethiol/boron trifluoride etherate in chloroform [7], sodium methoxide in methanol, and *p*-chlorobenzyl chloride/sodium hydride in *N*,*N*-dimethylformamide. The *p*-chlorobenzyl protective group was chosen because of its better crystallization properties [8]. **1** was obtained in 69% yield by crystallization from the crude mixture.

The chain galactosyl block **8** was prepared from ethyl 1-thio-β-D-galactopyranoside [7, 9]. Treatment of this compound with benzaldehyde dimethylacetal/*p*-toluenesulfonic acid in tetrahydrofuran gave the 4,6-acetal **6** in 82% yield. Other reagents, such as benzaldehyde/zinc chloride or benzaldehyde/formic acid, which have been employed before for analogous conversions [10, 11], have given lower yields of benzylidenated products. Benzoylation of **6** with benzoyl chloride/pyridine gave **7** (93% yield). Reductive opening of the acetal ring in **7** with sodium cyanoborohydride/hydrogen chloride in tetrahydrofuran [12, 13] gave the desired 4-OH compound **8** in 79% yield.

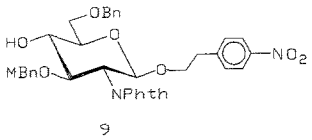
With the two blocks **1** and **8** at hand, construction of the digalactosyl block **10** was attempted. Condensation of the glycosyl bromide, obtained by bromine treatment of **1**, and **8** in the presence of silver triflate (method A) in dichloromethane/toluene at −20°C gave a multicomponent product mixture from which the disaccharide **10** could be purified in 17% yield. "Transglycosidation" products **1** and **2** were isolated in approximately 3% and 7% of the amount of glycosyl donor used, respectively, and the trisaccharide **13** and the tetrasaccharide **14** were isolated in 22% and 8% yields, respectively, based on the amount of aglycone **8** used.



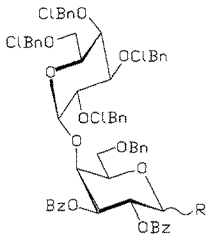
1: R = β-SEt
2: R = α-SEt
3: R = β-OCNHCCl$_3$
4: R = β-NHCOCCl$_3$
5: R = α-NHCOCCl$_3$



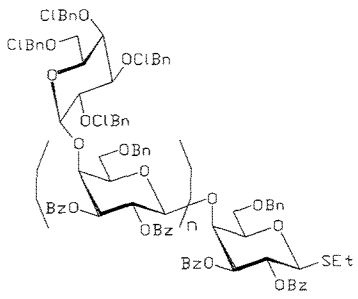
6: R^1 = OH, R^2,R^3 = OCH(Ph)O
7: R^1 = OBz, R^2,R^3 = OCH(Ph)O
8: R^1 = OBz, R^2 = OH, R^3 = OBn



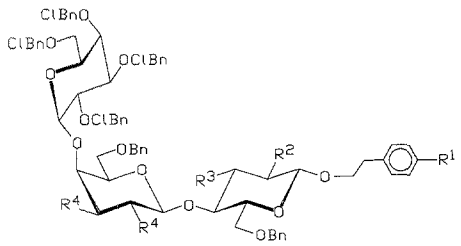
9



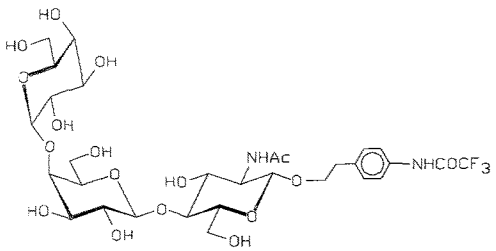
10: R = β-SEt
11: R = α-SEt
12: R = β-NHCOCCl$_3$



13: n = 1
14: n = 2



15: R^1 = NO$_2$, R^2 = NPhth, R^3 = OMBn, R^4 = OBz
16: R^1 = NO$_2$, R^2 = NPhth, R^3 = OH, R^4 = OBz
17: R^1 = NO$_2$, R^2 = NHAc, R^3 = OMBn, R^4 = OH
18: R^1 = NHCOCF$_3$, R^2 = NHAc, R^3 = OMBn, R^4 = OH



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An explanation of these results is that the 4-OH of **8** is slow to react and the thioethyl group of **8**, **10** or **13** can compete in the reaction with the glycosyl donor [14]. This gives rise to sulfonium intermediates, whose further reactions can explain the formation of **1**, **2**, **13** and **14**. If the reaction temperature was lowered to −75°C, the amount of transglycosidation products diminished, but the disaccharide **10** was still only obtained in 32% yield. Condensation in the presence of 1.5 equivalents of 2,6-di-t-butyl-4-methylpyridine at −20°C and −75°C gave **10** in 30% and 37% yield, respectively.

To test if trichloroacetimidate glycosylation [15] would give a higher yield of **10**, **1** was reacted with dimethyl(methylthio)sulfonium tetrafluoroborate [16] in acetonitrile/water followed by trichloroacetonitrile/potassium carbonate in dichloromethane [17] to give a 76% yield of the crystalline β-trichloroacetimidate **3**. The condensation of **3** and **8** in the presence of t-butyldimethylsilyl trifluoromethanesulfonate (method B) was performed in different solvents at room temperature [17]. Diethyl ether, according to TLC, gave the best result, but still the yield of **10** was only 35%. **1** and **2** were isolated in 16% and approx. 20% yields, respectively, based on the amount of aglycone **8** used. The β- and α-trichloroacetylated glycosylamines **4** and **5** were obtained in 11% and 22% yields based on the amount of trichloroacetimidate **3** used. That trichloroacetylated amines such as **4** and **5** can be formed in the trichloroacetimidate method has been reported [18]. A few percent of **12** were also isolated. If the condensation was performed at −10°C the α-thioethyl disaccharide **11** became an important by-product.

In conclusion, the yield of the disaccharide block **10** did not exceed 37%, irrespective of coupling method used. The main reason for the low yield was competing reactions, primarily transglycosidation at the thioglycoside function of the acceptor **8**. That the presence of this thioglycoside function was indeed the main reason for the low yield in the glycosidation reaction was shown by performing analogous glycosidations using the bromide derived from **1** and an analog of **8**, where the thioglycoside function had been replaced by a β-*O*-2-(*p*-trifluoroacetamidophenyl)ethyl group. Silver triflate promoted glycosidations in this case consistently gave yields better than 80% [Norbert T; unpublished results].

Condensation of disaccharide **10** and glucosamine glycoside **9** in the presence of methyl triflate [19] and 2,6-di-t-butyl-4-methylpyridine in diethyl ether at room temperature gave a 95% yield of trisaccharide derivative **15**. If the condensation was performed in the absence of base, **15** was obtained in 48% yield and the trisaccharide that had lost the *p*-methoxybenzyl protective group, compound **16**, was obtained in 29% yield.

The phthalimido group in **15** was removed by treatment with hydrazine acetate in toluene/95% ethanol. Acetylation with acetic anhydride/pyridine and de-*O*-acylation with

sodium methoxide in methanol gave **17** in 73% yield. Compound **18** was obtained in 79% yield from **17** through reduction of the nitro group with hydrogen over platinum oxide and *N*-trifluoroacetylation with trifluoroacetic anhydride/pyridine. The *O*-benzyl protective groups were finally removed by hydrogenolysis over Pd/C and the trisaccharide **19** could be isolated after chromatographic purification in 88% yield. The ^{13}C and ^{1}H NMR parameters of **19** agreed excellently with those reported [3, 5] for analogous P_1 trisaccharide derivatives.

Experimental

General methods

Melting points are corrected. Concentrations were performed under reduced pressure at $<40°C$ bath temperature. Optical rotations were measured at 23°C ($c = 0.5$, chloroform) unless otherwise stated, using a Perkin-Elmer 241 Polarimeter. NMR spectra were recorded at 300 K with a Bruker AM 500 instrument. The following reference signals were used: Me_4Si, δ 0.0 (^{1}H in C^2HCl_3); $CHCl_3$, δ 77.0 (^{13}C in C^2HCl_3); Me_2CO, δ 2.225 (^{1}H in ^2H_2O); and external dioxan, δ 67.4 (^{13}C in ^2H_2O). Only selected NMR data are reported. Assignments were based on 2D COSY, *J* resolved, decoupling, DEPT and proton–carbon correlation (CHORTLE) [20] experiments. The FAB-MS spectrum was recorded with a VG ZAB-SE mass spectrometer. TLC was performed on Silica Gel F_{254} (Merck, Darmstadt, Germany) with detection by u.v. light and/or by charring with sulfuric acid. Column chromatography was performed on silica gel (Matrex, 60 Å, 20–45 μm or 35–70 μm; Grace, Worms, Germany). Organic solutions were dried over sodium sulfate. Powdered molecular sieves (3 Å or 4 Å; Fluka, Buchs, Switzerland) and potassium carbonate were heated to 300°C under vacuum overnight. DMF and dichloromethane were distilled from P_2O_5 and THF from $LiAlH_4$. Toluene and diethyl ether were dried over sodium wire.

Ethyl 2,3,4,6-tetra-O-p-chlorobenzyl-1-thio-β-D-galactopyranoside (**1**)

To a solution of 1,2,3,4,6-penta-*O*-acetyl-β-D-galactopyranose (39.0 g, 0.10 mol) and ethanethiol (11.1 ml, 0.15 mol) in chloroform (100 ml) was added boron trifluoride etherate (5.0 ml, 40 mmol). The solution was stirred at room temperature for 24 h, diluted with dichloromethane, washed with saturated sodium hydrogen carbonate and water, dried and concentrated. The residue was treated with sodium methoxide in methanol (100 ml, 0.05 M) overnight, neutralized with Dowex 50 (H^+) resin, filtered, concentrated and coevaporated with toluene. The residue was dissolved together with *p*-chlorobenzyl chloride (70.9 g, 0.44 mol) in *N*,*N*-dimethylformamide (400 ml) and added while stirring to sodium hydride (19.2 g, 0.80 mol) at 0°C under a nitrogen atmosphere. The mixture was allowed to attain room temperature overnight. Excess methanol was added and the mixture was partitioned between toluene and water. The organic layer was washed with water, dried and concentrated. Crystallization from ethyl acetate/light petroleum gave **1** (49.9 g, 69%).

M.p. 95–96°C, $[\alpha]_D$ +18°. NMR data (C^2HCl_3): ^{13}C, δ 15.1, 24.9 (SEt), 68.5, 71.9, 72.7, 73.7, 74.8 (C-6, OCH_2Ph), 73.8, 76.9, 78.4, 83.8, 85.3 (C-1,2,3,4,5), 128.3–137.0 (aromatic C); ^{1}H, δ 3.51 (dd, $J_{2,3}$ 9.4 Hz, $J_{3,4}$ 2.9 Hz, H-3), 3.57 (m, H-5, H-6a, H-6b), 3.75 (t, $J_{1,2}$ 9.4 Hz, H-2), 3.90 (d, H-4), 4.40 (d, H-1). Analytical data calculated for $C_{36}H_{36}Cl_4O_5S$: C, 59.8; H, 5.0; S, 4.4. Found: C, 59.5; H, 4.9; S, 4.4.

O-(2,3,4,6-Tetra-O-p-chlorobenzyl-β-D-galactopyranosyl)trichloroacetimidate (**3**)

Dimethyl(methylthio)sulfonium tetrafluoroborate [16] (2.03 g, 10.3 mmol) was added to a stirred mixture of **1** (4.98 g, 6.89 mmol) in 70 ml acetonitrile/water, 9/1 by vol. A clear solution was obtained. After 5 min the solution was concentrated to about one fourth its volume and partitioned between ethyl acetate and saturated sodium hydrogen carbonate. The organic layer was washed with water, dried, concentrated and coevaporated with toluene. A syrupy crude mixture of the α and β reducing sugars was obtained (5.1 g). NMR data (C^2HCl_3): ^{13}C, δ 91.6, 97.7 (C-1α,1β).

Potassium carbonate (3.5 g) and trichloroacetonitrile (3.5 ml, 35 mmol) were added to a solution of this material (5.1 g) in dichloromethane (35 ml). The mixture was vigorously stirred for 16 h at room temperature under a nitrogen atmosphere. The reaction was monitored by TLC (toluene/ethyl acetate/triethylamine, 7/1/0.24 by vol. R_F of **3** is 0.29). The mixture was filtered through a layer of Celite, concentrated and the residue was crystallized from diethyl ether/light petroleum to give **3** (4.28 g, 76%).

M.p. 117–119°C, $[\alpha]_D$ +36°. NMR data (C^2HCl_3): ^{13}C, δ 67.9, 72.3, 72.7, 74.0, 74.3 (C-6, OCH_2Ph), 73.7, 74.2, 78.0, 81.9 (C-2,3,4,5), 98.6 (C-1), 128.5–136.8 (aromatic C), 161.4 (C=NH); ^{1}H, δ 3.59 (dd, $J_{2,3}$ 9.8 Hz, $J_{3,4}$ 2.8 Hz, H-3), 3.92 (dd, $J_{4,5}$ 0.9 Hz, H-4), 4.01 (dd, $J_{1,2}$ 8.0 Hz, H-2), 5.73 (d, H-1), 8.65 (s, NH). Analytical data calculated for $C_{36}H_{32}Cl_7NO_6$: C, 52.6; H, 3.9; N, 1.7. Found: C, 52.5; H, 3.9; N, 1.6.

Ethyl 4,6-O-benzylidene-1-thio-β-D-galactopyranoside (**6**)

Ethyl 1-thio-β-D-galactopyranoside [7, 9] (18.0 g, 80 mmol) and *p*-toluenesulfonic acid monohydrate (2.7 g, 14 mmol) were suspended in tetrahydrofuran (160 ml). Benzaldehyde dimethylacetal (24.4 g, 160 mmol) was added and the mixture was stirred for 1 h. The mixture was partitioned between ethyl acetate and saturated sodium hydrogen carbonate. The organic layer was washed with water, dried and concentrated. Crystallization from dichloromethane/light petroleum gave **3** (20.6 g, 82%).

M.p. 155–157°C, $[\alpha]_D$ −67°; lit. m.p. 154–156°C, $[\alpha]_D$ −55° (c = 3.0, chloroform) [10]. NMR data (C^2HCl_3): ^{13}C, δ 15.2, 23.4 (SEt), 69.2 (C-6), 69.6, 67.0, 73.8, 75.6 (C-2,3,4,5), 85.2 (C-1), 101.3 (CHPh), 126.3, 128.2, 129.2, 137.5 (aromatic C); ^1H, δ 3.48 (broad q, H-5), 3.66 (m, $J_{2,3} = J_{3,OH}$ 9.3 Hz, $J_{3,4}$ 3.7 Hz, H-3), 3.79 (m, $J_{1,2}$ 9.3 Hz, $J_{2,OH}$ 1.5 Hz, H-2), 4.00 (dd, $J_{5,6a}$ 1.6 Hz, $J_{6a,6b}$ 12.5 Hz, H-6a), 4.22 (dd, $J_{4,5}$ 0.9 Hz, H-4), 4.32 (dd, $J_{5,6b}$ 1.6 Hz, H-6b), 4.33 (d, H-1).

Ethyl 2,3-di-O-benzoyl-4,6-O-benzylidene-1-thio-β-D-galactopyranoside (**7**)

Benzoyl chloride (28 ml, 240 mmol) was added dropwise to a stirred solution of **6** (25.0 g, 80 mmol) in pyridine (300 ml) at 0°C. The mixture was allowed to attain room temperature. Ice-water was added, and after 15 min stirring the mixture was diluted with toluene. The organic layer was washed with cold 1 M sulfuric acid, saturated sodium hydrogen carbonate and water, dried and concentrated. Crystallization from dichloromethane/light petroleum gave **7** (38.8 g, 93%).

M.p. 184–185°C (sinters at 153–154°C), $[\alpha]_D$ +110°; lit. m.p. 148–150°C, $[\alpha]_D$ +99.5° (c = 4.0, chloroform) [10]. NMR data (C^2HCl_3): ^{13}C, δ 14.8, 22.9 (SEt), 67.2, 69.9, 73.8, 73.9 (C-2,3,4,5), 69.2 (C-6), 82.9 (C-1), 101.0 (CHPh), 126.3–137.6 (aromatic C), 165.3, 166.1 (C=O); ^1H, δ 3.72 (broad q, H-5), 4.09 (dd, $J_{5,6a}$ 1.7 Hz, $J_{6a,6b}$ 12.5 Hz, H-6a), 4.41 (dd, $J_{5,6b}$ 1.7 Hz, H-6b), 4.62 (broad d, $J_{3,4}$ 3.7 Hz, H-4), 4.73 (d, $J_{1,2}$ 9.9 Hz, H-1), 5.39 (dd, $J_{2,3}$ 9.9 Hz, H-3), 5.95 (t, H-2).

Ethyl 2,3-di-O-benzoyl-6-O-benzyl-1-thio-β-D-galactopyranoside (**8**)

Diethyl ether saturated with hydrogen chloride was added at 0°C to a stirred mixture of **7** (20.0 g, 38.4 mmol), sodium cyanoborohydride (21.7 g, 345 mmol), 3 Å molecular sieves (38 g) and tetrahydrofuran (500 ml) until the mixture was strongly acidic (as shown by pH paper). The mixture was stirred at room temperature for 24 h and hydrogen chloride in diethyl ether was added in portions to keep the mixture acidic. The reaction mixture was diluted with toluene, filtered through a layer of Celite, washed with saturated sodium hydrogen carbonate and water, dried and concentrated. Column chromatography (toluene/ethyl acetate, 5/1 by vol) and crystallization from diethyl ether/light petroleum gave **8** (15.9 g, 79%).

M.p. 98–99°C, $[\alpha]_D$ +69°. NMR data (C^2HCl_3): ^{13}C, δ 14.9, 23.9 (SEt), 67.9, 68.3, 75.4, 77.0 (C-2,3,4,5), 69.5, 73.8 (C-6, OCH_2Ph), 83.8 (C-1), 127.7–137.5 (aromatic C), 165.4, 165.8 (C=O); ^1H, δ 4.44 (broad t, H-4), 4.69 (d, $J_{1,2}$ 9.9 Hz, H-1), 5.33 (dd, $J_{2,3}$ 9.9 Hz, $J_{3,4}$ 3.0 Hz, H-3), 5.85 (t, H-2). Analytical data calculated for $C_{29}H_{30}O_7S$: C, 66.6; H, 5.8; S, 6.1. Found: C, 66.4; H, 5.8; S, 6.2.

Ethyl 2,3-di-O-benzoyl-6-O-benzyl-4-O-(2,3,4,6-tetra-O-p-chlorobenzyl-α-D-galactopyranosyl)-1-thio-β-D-galactopyranoside (**10**)

Method A. Bromine (282 µl, 6.31 mmol) in dichloromethane (30 ml) was added under nitrogen to a stirred mixture of **1** (4.00 g, 5.56 mmol) and 4 Å molecular sieves (15 g) in dichloromethane (30 ml) at 0°C. After 15 min cyclohexene (1.0 ml) was added, followed by **8** (2.64 g, 5.05 mmol) and, in some experiments, the base, 2,6-di-t-butyl-4-methylpyridine (1.56 g, 7.57 mmol). The mixture was cooled to −20°C or −75°C and silver triflate (2.14 g, 8.34 mmol) in toluene (30 ml) was added dropwise during 15 min. After 1 h the temperature was increased to −20°C and pyridine (5 ml) and then 0.5 M aqueous sodium thiosulfate (50 ml) were added. The mixture was filtered through a layer of Celite and the organic layer was washed with water, 1 M sulfuric acid, water and saturated sodium hydrogen carbonate, dried and concentrated. The residue was purified by column chromatography (toluene/ethyl acetate, 20/1 by vol). The fractions containing **10** (R_F 0.25) were concentrated and chromatographed once more (isooctane/acetone, 2/1 by vol) to give pure **10** as an amorphous material in the following yields: 17% (−20°C, no base), 32% (−75°C, no base), 30% (−20°C, base), 37% (−75°C, base).

$[\alpha]_D$ +84°. NMR data (C^2HCl_3): ^{13}C, δ 15.2, 23.6 (SEt), 67.3, 67.5, 71.9, 72.0, 72.7, 73.2, 74.0 (C-6,6', OCH_2Ph), 68.0, 69.2, 74.6, 75.0, 75.6, 76.4, 77.5, 78.6 (C-2,3,4,5,2',3',4',5'), 83.4 (C-1), 99.5 (C-1'), 127.6–137.7 (aromatic C), 165.5, 166.1 (C=O); ^1H, δ 2.85 (dd, $J_{5',6'a}$ 5.0 Hz, $J_{6'a,6'b}$ 8.0 Hz, H-6'a), 3.28 (broad t, $J_{5',6'b}$ 9.5 Hz, H-6'b), 3.95 (dd, $J_{1',2'}$ 3.4 Hz, $J_{2',3'}$ 10.3 Hz, H-2'), 3.97 (d, $J_{3',4'}$ 2.6 Hz, H-4'), 4.08 (dd, H-3'), 4.28 (broad dd, H-5'), 4.49 (d, $J_{3,4}$ 2.8 Hz, H-4), 4.69 (d, $J_{1,2}$ 10.0 Hz, H-1), 4.99 (d, H-1'), 5.26 (dd, $J_{2,3}$ 10.1 Hz, H-3), 5.84 (t, H-2).

Method B. A solution of t-butyldimethylsilyl trifluoromethanesulfonate (26 µl, 0.11 mmol) in diethyl ether (10 ml) was added during 40 min to a stirred mixture of **3** (400 mg, 0.486 mmol), **8** (196 mg, 0.374 mmol) and 4 Å molecular sieves (3.0 g) in diethyl ether (20 ml) at room temperature under a nitrogen atmosphere. After 1 h saturated sodium hydrogen carbonate and dichloromethane were added. The mixture was filtered and the organic layer was dried and concentrated. The residue was purified by column chromatography (toluene/ethyl acetate, 20/1 by vol, followed by isooctane/acetone, 3/1 by vol) to give pure **10** (156 mg, 35%). R_F 0.25 (toluene/ethyl acetate, 20/1 by vol), R_F 0.14 (isooctane/acetone, 3/1 by vol).

The following compounds were also isolated from the glycosidation reactions described above:

Ethyl 2,3,4,6-tetra-O-p-chlorobenzyl-1-thio-α-D-galactopyranoside (**2**). R_F 0.36 (toluene/ethyl acetate, 20/1 by vol). NMR data (C^2HCl_3): ^{13}C, δ 14.6, 23.4 (SEt), 68.7, 71.4, 72.6,

72.6, 74.0 (C-6, OCH_2Ph), 69.3, 75.3, 76.1, 79.2, 82.9 (C-1,2,3,4,5), 128.3–137.0 (aromatic C); ^1H, δ 3.50 (dd, $J_{5,6a}$ 6.7 Hz, $J_{6a,6b}$ 9.5 Hz, H-6a), 3.56 (dd, $J_{5,6b}$ 6.2 Hz, H-6b), 3.75 (dd, $J_{2,3}$ 9.9 Hz, $J_{3,4}$ 2.9 Hz, H-3), 3.88 (d, H-4), 4.22 (dd, $J_{1,2}$ 5.5 Hz, H-2), 4.29 (broad t, H-5), 5.51 (d, H-1).

N-(2,3,4,5-tetra-O-p-chlorobenzyl-β-D-galactopyranosyl)-trichloroacetamide (**4**). R_F 0.19 (toluene/ethyl acetate, 20/1 by vol). NMR data (C^2HCl_3): ^{13}C, δ 67.7, 72.0, 72.7, 74.2, 74.2 (C-6, OCH_2Ph), 73.7, 75.1, 77.7, 81.1, 83.1 (C-1,2,3,4,5), 128.2–136.5 (aromatic C), 161.6 (C=O); ^1H, δ 3.64 (dd, $J_{2,3}$ 9.2 Hz, $J_{3,4}$ 2.8 Hz, H-3), 3.77 (t, $J_{1,2}$ 9.2 Hz, H-2), 3.96 (d, H-4), 5.08 (t, $J_{1,NH}$ 9.2 Hz, H-1), 7.03 (d, NH).

N-(2,3,4,6-tetra-O-p-chlorobenzyl-α-D-galactopyranosyl)-trichloroacetamide (**5**). R_F 0.25 (toluene/ethyl acetate, 20/1 by vol), R_F 0.18 (isooctane/acetone, 3/1 by vol). NMR data (C^2HCl_3): ^{13}C, δ 67.3, 72.1, 72.3, 72.6, 73.4 (C-6, OCH_2Ph), 71.8, 73.5, 74.8, 76.2m 77.6 (C-1,2,3,4,5), 128.4–136.4 (aromatic C), 161.9 (C=O); ^1H, δ 3.61 (dd, $J_{2,3}$ 8.5 Hz, $J_{3,4}$ 2.4 Hz, H-3), 3.99 (broad t, $J_{4,5}$ 2.2 Hz, H-4), 4.07 (dd, $J_{1,2}$ 4.7 Hz, H-2), 5.65 (dd, $J_{1,NH}$ 6.7 Hz, H-1).

Ethyl 2,3-di-O-benzoyl-6-O-benzyl-4-O-(2,3,4,6-tetra-O-p-chlorobenzyl-α-D-galactopyranosyl)-1-thio-α-D-galactopyranoside (**11**). R_F 0.31 (toluene/ethyl acetate, 20/1 by vol). NMR data (C^2HCl_3): ^{13}C, δ 14.6, 23.9 (SEt), 67.5, 68.1, 71.8, 72.0, 73.1, 73.1, 74.1 (C-6,6', OCH_2Ph), 68.7, 69.4, 69.6, 72.1, 75.0, 76.1, 76.5, 78.7, 82.2 (C-1,2,3,4,5,2',3',4',5'), 99.8 (C-1'), 127.3–138.0 (aromatic C), 165.7, 166.3 (C=O); ^1H, δ 3.95 (dd, $J_{1',2'}$ 3.5 Hz, $J_{2',3'}$ 10.3 Hz, H-2'), 4.08 (dd, $J_{3',4'}$ 2.8 Hz, H-3'), 4.47 (d, $J_{3,4}$ 2.9 Hz, H-4), 4.97 (d, H-1'), 5.51 (m, H-3), 5.87 (m, H-1,2).

N-[2,3-Di-O-benzoyl-6-O-benzyl-4-O-(2,3,4,6-tetra-O-p-chlorobenzyl-α-D-galactopyranosyl)-β-D-galactopyranosyl]-trichloroacetamide (**12**). R_F 0.19 (toluene/ethyl acetate, 20/1 by vol), R_F 0.11 (isooctane/acetone, 3/1 by vol). NMR data (C^2HCl_3): ^{13}C, δ 66.9, 67.5, 71.6, 72.1, 73.2, 73.3, 74.1 (C-6,6', OCH_2Ph), 69.3, 69.4, 73.9, 74.4, 74.6, 75.8, 76.3, 78.5, 80.8 (C-1,2,3,4,5,2',3',4',5'), 99.5 (C-1'), 127.6–137.6 (aromatic C), 162.0 (C=O NHCOCCl$_3$), 166.1, 166.9 (C=O OBz); ^1H, δ 4.08 (dd, $J_{2',3'}$ 10.3 Hz, $J_{3',4'}$ 2.7 Hz, H-3'), 4.52 (d, $J_{3,4}$ 3.0 Hz, H-4), 5.04 (d, $J_{1',2'}$ 3.7 Hz, H-1'), 5.35 (t, $J_{1,2} = J_{1,NH}$ 9.0 Hz, H-1), 5.43 (dd, $J_{2,2}$ 10.6 Hz, H-3), 5.72 (dd, H-2), 7.70 (d, NH).

Ethyl-O-(2,3,4,5-tetra-O-p-chlorobenzyl-α-D-galactopyranosyl)-(1-4)-O-(2,3-di-O-benzoyl-6-O-benzyl-β-D-galactopyranosyl)-(1-4)-2,3-di-O-benzoyl-6-O-benzyl-1-thio-β-D-galactopyranoside (**13**). R_F 0.18 (toluene/ethyl acetate, 20/1 by vol). NMR data (C^2HCl_3): ^{13}C, δ 14.6, 23.1 (SEt), 67.0, 67.2, 69.1, 71.9, 71.9, 72.9, 73.1, 73.4, 74.1 (C-6,6',6'', OCH_2Ph), 68.0, 69.1, 70.2, 72.5, 73.4, 74.4, 74.6, 74.9, 75.4, 76.4, 77.6, 78.8, 83.5 (C-1,2,3,4,5,2',3',4',5',2'',3'',4'',5''), 99.8, 100.8 (C-1',1''), 127.4–138.2 (aromatic C), 164.6, 165.7, 165.9, 166.5 (C=O); ^1H, δ 3.94 (dd, $J_{1'',2''}$ 3.5 Hz, $J_{2'',3''}$ 10.3 Hz, H-2''), 4.13 (dd, $J_{3'',4''}$ 2.7 Hz, H-3''), 4.62 (d, $J_{1,2}$ 9.9 Hz, H-1), 4.95 (d, $J_{1',2'}$ 7.7 Hz, H-1'), 4.97 (d, H-1''), 5.09 (dd, $J_{2',3'}$ 10.8 Hz, $J_{3',4'}$ 2.9 Hz, H-3'), 5.43 (dd, $J_{2,3}$ 9.9 Hz, $J_{3,4}$ 2.7 Hz, H-3), 5.61 (t, H-2), 5.83 (dd, H-2').

Ethyl O-(2,3,4,6-tetra-O-p-chlorobenzyl-α-D-galactopyranosyl)-(1-4)-O-(2,3-di-O-benzoyl-6-O-benzyl-β-D-galactopyranosyl)-(1-4)-O-(2,3-di-O-benzoyl-6-O-benzyl-β-D-galactopyranoside (**14**). R_F 0.12 (toluene/ethyl acetate, 20/1 by vol). NMR data (C^2HCl_3). The designations ', '', and ''' are arbitrary: ^{13}C, δ 14.7, 22.2 (SEt), 66.9, 67.2, 68.5, 68.9, 71.3, 71.9, 73.0, 73.0, 73.3, 73.4, 74.0 (C-6,6',6'',6''', OCH_2Ph), 67.7, 69.1, 70.2, 70.3, 71.4, 71.4, 73.5, 73.5, 74.3, 74.5, 74.7, 74.9, 75.2, 76.3, 77.5, 78.6, 82.7 (C-1,2,3,4,5,2',3',4',5',2'',3'',4'',5'', 2''',3''',4''',5'''), 100.1, 100.3, 100.5 (C-1',1'',1'''), 127.4–138.6 (aromatic C), 164.5, 164.8, 165.5, 165.6, 166.2, 166.5 (C=O); ^1H, δ 3.92 (dd, $J_{1''',2'''}$ 3.7 Hz, $J_{2''',3'''}$ 10.4 Hz, H-2'''), 4.05 (dd, $J_{3''',4'''}$ 2.4 Hz, H-3'''), 4.50 (d, $J_{1,2}$ 9.8 Hz, H-1), 4.90 (d, $J_{1',2'}$ 7.9 Hz, H-1'), 4.96 (d, H-1'''), 5.07 (d, $J_{1'',2''}$ 7.9 Hz, H-1''), 5.08 (dd, $J_{2'',3''}$ 10.4 Hz, $J_{3'',4''}$ 3.0 Hz, H-3''), 5.28 (dd, $J_{2',3'}$ 10.4 Hz, $J_{3',4'}$ 3.0 Hz, H-3'), 5.36 (dd, $J_{2,3}$ 9.8 Hz, $J_{3,4}$ 3.0 Hz, H-3), 5.51 (t, H-2), 5.57 (dd, H-2'), 5.86 (dd, H-2'').

2-(p-Nitrophenyl)ethyl O-(2,3,4,6-tetra-O-p-chlorobenzyl-α-D-galactopyranosyl)-(1-4)-O-(2,3-di-O-benzoyl-6-O-benzyl-β-D-galactopyranosyl)-(1-4)-6-O-benzyl-2-deoxy-3-O-p-methoxybenzyl-2-phthalimido-β-D-glucopyranoside (**15**)

Methyl triflate (216 µl, 1.97 mmol) was added to a stirred mixture of **10** (1.00 g, 0.845 mmol), **9** (471 mg, 0.704 mmol) [6], 2,6-di-t-butyl-4-methylpyridine (123 mg, 0.598 mmol) and 4 Å molecular sieves (2.7 g) in diethyl ether (20 ml) at room temperature. After 10 h no **9** remained as shown by TLC, and piperidine (1 ml) was added. The mixture was stirred for 20 min, filtered through a layer of Celite and concentrated. Column chromatography (toluene/dichloromethane/ethyl acetate, 40/20/6 by vol) yielded amorphous **15** (1.20 g, 95%) (R_F 0.27).

$[\alpha]_D$ +17°. NMR data (C^2HCl_3): ^{13}C, δ 35.3 (OCH_2CH_2), 54.6, 55.3 (C-2, OMe), 67.0, 67.2, 67.6, 68.7, 71.9, 72.1, 73.1, 73.2, 73.5, 73.9, 74.0 (C-6,6',6'', OCH_2CH_2, OCH_2Ph), 69.2, 70.7, 73.3, 74.3, 74.6, 74.7, 75.1, 75.6, 76.6, 77.0, 78.9 (C-3,4,5,2',3',4',5',2'',3'',4'',5''), 98.2, 99.8, 99.8 (C-1,1',1''), 113.2, 158.5 (aromatic C MBn), 122.4–146.7 (aromatic C), 165.3, 166.2 (C=O OBz), 167.1, 167.4 (C=O NPhth); ^1H, δ 2.71 (dd, $J_{5'',6''a}$ 4.9 Hz, $J_{6''a,6''b}$ 8.0 Hz, H-6''a), 3.22 (dd, $J_{5'',6''b}$ 9.8 Hz, H-6''b), 3.36 (m, $J_{4,5}$ 10.0 Hz, $J_{5,6a}$ 1.8 Hz, $J_{5,6b}$ 3.3 Hz, H-5), 3.57 (dd, $J_{6a,6b}$ 10.8 Hz, H-6a), 3.68 (dd, H-6b), 3.68 (dd, $J_{5',6'a}$ 5.8 Hz, $J_{6'a,6'b}$ 9.5 Hz, H-6'a), 3.74 (broad dd, $J_{4',5'}$ 1.2 Hz, $J_{5',6'b}$ 8.1 Hz, H-5'), 3.86 (m, H-2'',3'',4''), 4.08 (dd, H-6'b), 4.14 (dd, $J_{3,4}$ 7.9 Hz, H-4), 4.78 (d, $J_{1',2'}$ 7.7 Hz, H-1'), 4.92 (d, $J_{1,2}$ 8.0 Hz, H-1), 4.96 (d, $J_{1'',2''}$ 2.4 Hz, H-1''), 5.07 (dd, $J_{2',3'}$ 10.8 Hz, $J_{3',4'}$ 2.9 Hz, H-3'), 5.73 (dd, H-2').

Table 1. ^{1}H- and ^{13}C-NMR shifts of compound **19** from spectra run in ^2H_2O. Chemical shifts are given in ppm. Coupling constants (Hz) for the ^{1}H-NMR spectrum are in parentheses.

^{1}H-NMR	*H-1* ($J_{1,2}$)	*H-2* ($J_{2,3}$)	*H-3* ($J_{3,4}$)	*H-4* ($J_{4,5}$)	*H-5* ($J_{5,6a}$)	*H-6a* ($J_{5,6b}$)	*H-6b* ($J_{6a,6b}$)
GlcNAc	4.47 (8.2[a])	3.64[a] (n.d.[b])	3.62[a] (n.d.)	3.68[a] (n.d.)	3.55 (5.0)	3.84 (2.2)	4.00 (12.2)
β-Gal	4.51 (7.7)	3.56 (10.2)	3.74 (3.2)	4.03 (<1)	3.77 (4.6)	3.82 (7.6)	3.89 (11.6)
α-Gal	4.94 (3.9)	3.83 (10.5)	3.88 (3.2)	4.02 (1.6)	4.35 (6.3)	3.69* (6.3)	3.71* (n.d.)
CH_3CONH	1.75						
OCH_2CH_2	2.87	2.94					
OCH_2CH_2	3.83	4.22					
Ar	7.33	7.48					
^{13}C-NMR	*C-1*		*C-2*	*C-3*	*C-4*	*C-5*	*C-6*
GlcNAc	101.7		55.9	73.2	79.6	75.6	60.9
β-Gal	104.0		71.7	73.0	78.1	76.2	61.1
α-Gal	101.1		69.3	69.9	69.7	71.6	61.3
CH_3CONH	22.8						
OCH_2CH_2	35.2						
OCH_3CH_2	71.0						
CF_3CO	116.8 (*J* 286 Hz)						
Ar	123.1, 130.4, 133.6, 138.7						
CF_3CO	157.8 (*J* 38 Hz)						
CH_3CONH	175.0						

[a] Approximate value due to strong coupling effects.
[b] n.d. not determined.

The following compound was also isolated, when the condensation was performed without the addition of 2,6-di-t-butyl-4-methylpyridine:

2-(p-Nitrophenyl)ethyl O-(2,3,4,6-tetra-O-p-chlorobenzyl-α-D-galactopyranosyl)-(1-4)-O-(2,3-di-O-benzoyl-6-O-benzyl-β-D-galactopyranosyl)-(1-4)-6-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (**16**). R_F 0.18 (toluene/dichloromethane/ethyl acetate, 40/20/6 by vol). NMR data (C^2HCl_3): ^{13}C, δ 35.4 (OCH_2CH_2), 55.7 (C-2), 67.3, 68.0, 68.4, 68.8, 71.8, 72.1, 72.8, 73.0, 73.3, 74.1 (C-6,6′,6″, OCH_2CH_2, OCH_2Ph), 69.4, 69.6, 69.7, 74.0, 74.1, 74.3, 74.7, 75.0, 76.3, 78.5 (C-3,5,2′,3′,4′,5′,2″,3″,4″,5″), 82.4 (C-4), 98.1 (C-1), 100.1 (C-1″), 101.8 (C-1′), 122.9–146.8 (aromatic C), 165.3, 166.2 (C=O OBz); ^{1}H, δ 3.74 (dd, $J_{3,4}$ 8.1 Hz, $J_{4,5}$ 9.7 Hz, H-4), 3.88 (dd, $J_{1'',2''}$ 3.7 Hz, $J_{2'',3''}$ 10.3 Hz, H-2″), 3.97 (broad m, H-4″), 4.04 (dd, $J_{3'',4''}$ 2.8 Hz, H-3″), 4.15 (dd, $J_{1,2}$ 8.5 Hz, $J_{2,3}$ 10.8 Hz, H-2), 4.34 (d, $J_{3',4'}$ 3.0 Hz, H-4′), 4.48 (dd, H-3), 4.74 (d, $J_{1',2'}$ 7.9 Hz, H-1′), 4.83 (d, H-1″), 5.08 (d, H-1), 5.14 (dd, $J_{2',3'}$ 10.7 Hz, H-3′), 5.76 (dd, H-2′).

2-(p-Nitrophenyl)ethyl O-(2,3,4,6-tetra-O-p-chlorobenzyl-α-D-galactopyranosyl)-(1-4)-O-(6-O-benzyl-β-D-galactopyranosyl)-(1-4)-2-acetamido-6-O-benzyl-2-deoxy-3-O-p-methoxybenzyl-β-D-glucopyranoside (**17**)

Hydrazine hydrate (1.42 ml, 29 mmol) and acetic acid (1.25 ml, 22 mmol) were added to a mixture of **15** (0.72 g, 0.41 mmol) in 45 ml toluene/95% ethanol, 1/30 by vol. The mixture was refluxed overnight, then cooled, concentrated and coevaporated with toluene and ethanol. The residue was acetylated with 20 ml acetic anhydride/pyridine, 1/1 by vol, at room temperature. After 3 h the reaction mixture was concentrated, coevaporated with xylene and partitioned between toluene and water. The organic layer was dried and concentrated. Methanol (20 ml) and sodium methoxide in methanol (2.5 ml, 0.2 M) were added to the residue. The solution was refluxed overnight, and then neutralized with Dowex 50 (H^+) resin, filtered and concentrated. The residue was partitioned between ethyl acetate and water. The organic layer was dried and concentrated. Column chromatography (ethyl acetate) gave **17** as a syrup (0.44 g, 73%).

$[\alpha]_D$ +27°. NMR data (C^2HCl_3): ^{13}C, 23.5 (CH_3CONH), 99.8, 100.3, 102.9 (C-1,1′,1″), 170.0 (CH_3CONH).

2-(p-Trifluoroacetamidophenyl)ethyl O-(2,3,4,6-tetra-O-p-chlorobenzyl-α-D-galactopyranosyl)-O-(6-O-benzyl-β-D-galactopyranosyl)-(1-4)-2-acetamido-6-O-benzyl-2-deoxy-3-O-p-methoxybenzyl-β-D-glucopyranoside (**18**)

A solution of **17** (0.41 g, 0.28 mmol) in ethyl acetate (10 ml) was hydrogenated over platinum oxide (90 mg) at atmospheric pressure. After 1 h the mixture was filtered and concentrated. The residue was dissolved in pyridine (8 ml), cooled to −25°C under nitrogen and trifluoroacetic

anhydride (0.47 ml, 3.3 mmol) was added dropwise. The solution was allowed to attain room temperature. Water (0.25 ml) was added at 0°C, and after stirring at room temperature overnight the solution was concentrated and coevaporated with toluene and ethanol. The residue was dissolved in ethyl acetate and washed with water, cold 1 M sulfuric acid, saturated sodium hydrogen carbonate and water. The organic layer was dried and concentrated. Column chromatography (toluene/ethyl acetate, 1/5 by vol) gave **18** as a syrup (0.34 g, 79%).

$[\alpha]_D$ +26°. NMR data (C^2HCl_3): ^{13}C, δ 99.8, 100.3, 102.9 (C-1,1',1''), 115.8 (q, J 288 Hz, CF_3CO), 154.8 (q, J 38 Hz, CF_3CO).

2-(p-Trifluoroacetamidophenyl)ethyl O-α-D-galacto-pyranosyl-(1-4)-O-β-D-galactopyranosyl-(1-4)-2-acetamido-2-deoxy-β-D-glycopyranoside (**19**)

A solution of **18** (35 mg, 0.022 mmol) in 95% ethanol (3 ml) was hydrogenated over Pd/C (10%, 30 mg) at atmospheric pressure for 3 h. A drop of pyridine was added and the mixture was filtered and evaporated. The residue was purified on a Bio-Gel P-2 column, using water as eluant. After lyophilization **19** was obtained as an amorphous powder (15 mg, 88%).

$[\alpha]_D$ +34° (c = 0.2, H_2O). NMR data are shown in Table 1. FAB-MS of **19** showed an M + 1 ion of m/z 761. (The nuclide mass sum of **19** is 760.25).

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