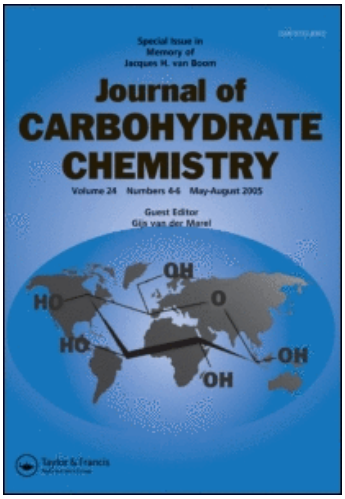

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Large Scale Synthesis of Two Trisaccharide Spacer Glycosides Corresponding to the Blood Group A and B Determinants Using Thioglycosides and Dimethyl(Thiomethyl)Sulfonium Tetrafluoroborate (DMTSB) as Promoter

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LARGE SCALE SYNTHESIS OF TWO TRISACCHARIDE SPACER GLYCOSIDES CORRESPONDING TO THE BLOOD GROUP A AND B DETERMINANTS USING THIOGLYCOSIDES AND DIMETHYL(THIOMETHYL)SULFONIUM TETRAFLUOROBORATE (DMTSB) AS PROMOTER

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ABSTRACT

Two trisaccharide spacer glycosides, *p*-trifluoroacetamidophenylethyl 3-*O*-(2-acetamido-2-deoxy-α-D-galactopyranosyl) - 2 - *O* - (α-L-fucopyranosyl)-β-D-galactopyranoside and *p*-trifluoroacetamidophenylethyl 2-*O*-(α-L-fucopyranosyl)-3-*O*-(α-D-galactopyranosyl)-β-D-galactopyranoside, corresponding to the human blood group A and B determinants, were synthesized. A key

fucosylgalactosyl disaccharide derivative was glycosylated with galactos-aminyl or galactosyl donors, respectively. Dimethyl(thiomethyl)sulfonium tetrafluoroborate was used for thioglycoside activation in all but one coupling reaction. The small scale procedure was further developed for use in a large scale (10-100 g final product) synthesis. Large scale syntheses of some crystalline thioglycoside building blocks are also described.

INTRODUCTION

The blood group A and B determinant trisaccharides have, in recent years, become increasingly in demand for biomedical purposes. Consequently, several chemical syntheses of the A or B tri- or tetrasaccharides (or derivatives of them) have been reported.[1-14,39] These biomedical purposes include, e.g., production of monoclonal antibodies using trisaccharide-protein conjugates[15] or preparation of columns with immobilized carbohydrates to be used for specific adsorption of antibodies.[16-18]

However, since our biomedical applications required substantial amounts of these trisaccharides, provided with a spacer suitable for coupling to a solid matrix, improved synthetic procedures were needed which used short synthetic paths, cheap reagents, and gave rise to crystalline intermediates. Such procedures would be suited for large scale preparations.

We have previously[19] reported a simple and efficient synthesis of the blood group B trisaccharide glycoside **15**, where the synthetic strategy was based on fucosylation of a digalactosyl derivative. This strategy gave satisfactory yields in all steps. Many intermediates were crystalline, which facilitated isolation and scale-up. However, if feasible, the alternative synthetic strategy, encompassing glycosylation of a fucosylgalactosyl derivative, should represent a more rational approach if *both* the A and B trisaccharide derivatives are desired. We have previously reported[20] such an approach in a preliminary communication. We now report full experimental details for the synthesis of the human blood group A and B trisaccharide derivatives **12** and **15**, respectively, and the adaptation of this synthesis to large scale synthesis. Thioglycosides[21] and a recently described[22] glycosylation reagent, dimethyl(thiomethyl)sulfonium tetrafluoroborate (DMTSB) were used in all but one of the glycosylation reactions.

RESULTS AND DISCUSSION

Small scale synthesis:

The DMTSB promoter reagent used here[22] is similar in performance to the widely used dimethyl(thiomethyl)sulfonium trifluoromethanesulfonate (DMTST).[21,22,23] The advantage lies in its ease of preparation and handling. DMTSB is a stable, relatively non-hygroscopic crystalline solid, commercially available or easily prepared[24] from trimethyloxonium tetrafluoroborate and dimethyl disulphide. Preparation of DMTST[22] requires the use of the highly toxic methyl triflate. DMTST is also highly hygroscopic. Thus, the use of DMTSB in large scale preparations is preferred. The reaction of DMTSB with thioglycosides has been shown to yield glycosyl fluorides[25] and glycosylations with this reagent probably proceed via these intermediates in most cases.

The starting material for the β-galactosyl unit was ethyl 4,6-*O*-benzylidene-1-thio-β-D-galactopyranoside[26] **1** which on treatment with benzoyl chloride in pyridine gave the 3-*O*-benzoate **2** (83 %). Treatment of **2** with bromoacetyl bromide and 2,4,6-trimethylpyridine/*N*,*N*-dimethylaminopyridine in dichloromethane gave **3a** (94 %). The thioethyl group was replaced by a *p*-trifluoroacetamidophenylethyl group by treatment of **3a** with *p*-trifluoroacetamidophenylethanol[27] **4** and DMTSB in dichloromethane-acetonitrile to give β-glycoside (60 %) **5a**. The 2-bromoacetyl group in **5a** was removed by treatment with pyridine-water to give **6** (70 %). Fucosylation of **6** using ethyl 2,3,4-tri-*O*-*p*-chlorobenzyl-1-thio-β-L-fucopyranoside[28] **7** and DMTSB in dichloromethane gave the disaccharide **8** (61 %). In the NMR spectrum of **8**, signals from, *inter alia*, H-1 (d 4.64, $J_{1,2}$ 7.8 Hz), H-2 (dd 4.40, $J_{2,3}$ 9.9 Hz), H-3 (dd 5.35, $J_{2,3}$ 9.9, $J_{3,4}$ 3.7 Hz) and H-1′ (d 5.40, $J_{1,2}$ 3.7 Hz) were present, which verified the expected anomeric configuration and the 2-*O*-fucosyl-3-*O*-benzoyl substitution pattern. The β-fucoside could also be isolated in ≈15 percentage yield. Debenzoylation of **8** with sodium methoxide in methanol then gave the key fucosylgalactosyl disaccharide derivative **9** (77%).

To obtain the A-trisaccharide glycoside, glycosylation of compound **9** with *p*-methylphenyl 2-azido-3,4,6-tri-*O*-*p*-chlorobenzyl-2-deoxy-1-thio-β-D-galactopyranoside[29] **10a** or the corresponding glycosyl bromide **10b** was investigated using various promoters and reaction conditions. Use of DMTSB and the thioglycoside donor gave in this case unsatisfactory yields (<30%). Some

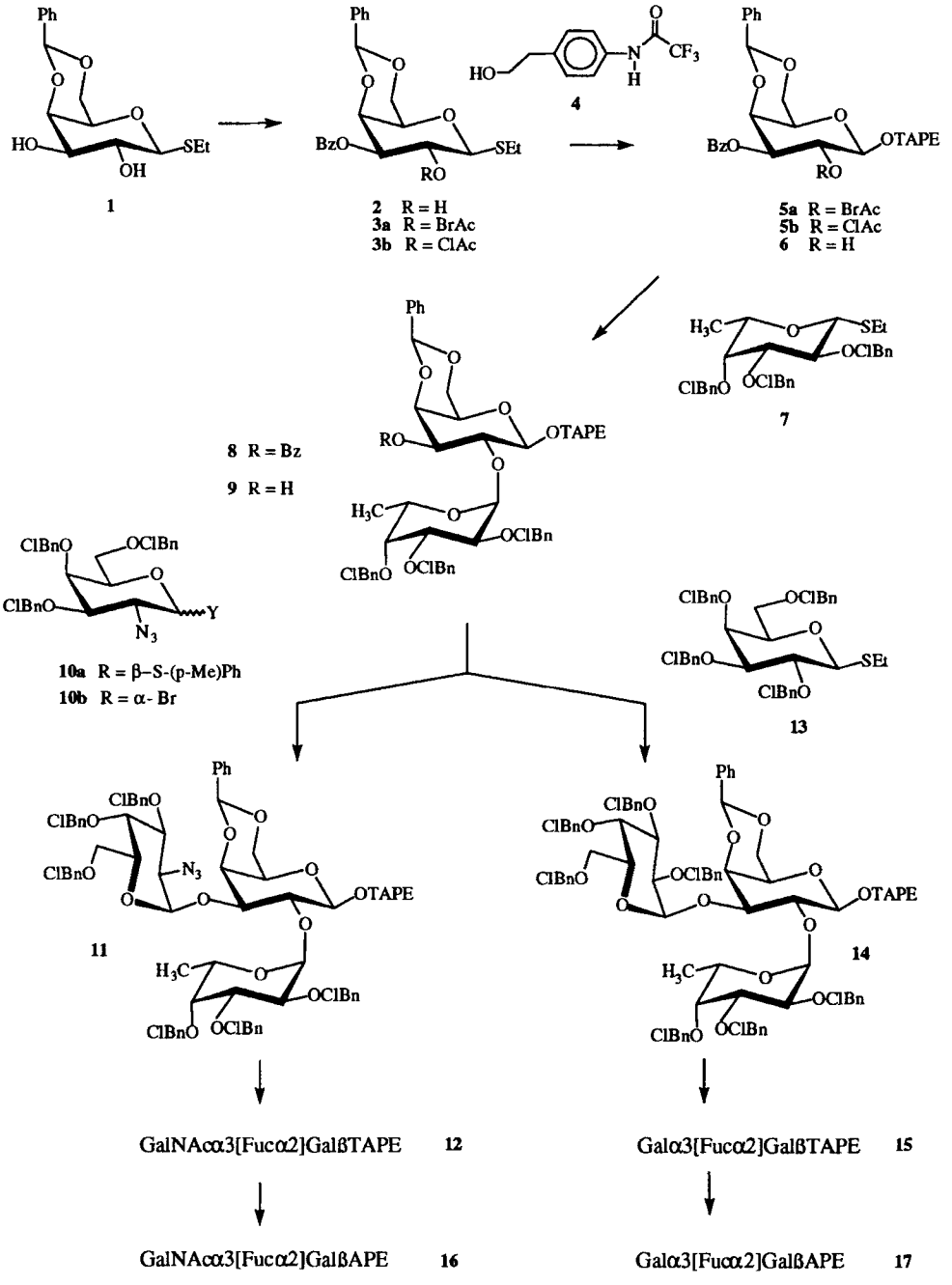
side products isolated from the reaction mixture were, according to NMR, the two disaccharides **18a** and **18b** where the 4,6-benzylidene had migrated to the 3,4-position. This was probably due to the acidic (liberated BF_3) reaction conditions. The corresponding two α-6-O-galactosaminylated trisaccharides **19a** and **19b** containing the 3,4-benzylidene group were also isolated together with **11**. The assignments of *endo* and *exo* were made according to previously reported typical NMR data[30,31,32,33,34,35] for 1,3-dioxolan benzylidene derivatives. The best glycosylation results were obtained using silver triflate and the glycosyl bromide **10b**. The α/β product ratio was increased in polar solvents. Thus, glycosylation of compound **9** with 2-azido-3,4,6-tri-*O*-*p*-chlorobenzyl-2-deoxy-β-D-galactopyranosyl bromide **10b** in tetrahydrofuran:dioxane gave a 4:1 α/β mixture of trisaccharide derivatives (64 %). The anomeric mixture was not resolved at this stage. Catalytic hydrogenation of the mixture (Pd/C) followed by *N*-acetylation with acetic anhydride gave an α/β mixture of trisaccharide derivatives from which the desired compound **12** could be isolated by chromatography (54 %).

To obtain the B-trisaccharide glycoside, compound **9** was glycosylated with ethyl 2,3,4,6-tetra-*O*-*p*-chlorobenzyl-1-thio-β-D-galactopyranoside **13** in dichloromethane-tetrahydrofurane, using DMTSB as promoter, to give compound **14** (57 % yield). Catalytic hydrogenation (Pd/C) of **14** then gave the target B-trisaccharide glycoside **15** (90 % yield). Compounds **14** and **15** displayed physical data identical with those reported.[19]

Large scale synthesis:

When considering large scale synthesis factors like raw material prices, time, waste and health factors must be considered. Usually oligosaccharide synthesis is performed in mg or at most gram quantities in university laboratories as exemplified above,[1-14] where these factors are of minor importance. A large scale synthetic process should include the following features: A minimum number of steps; crystalline intermediates; cheap and commercially available or easily obtainable reagents and chemicals; stoichiometrically amounts of reagents; concentrated solutions; absence of extreme reaction conditions such as long reaction times and low temperatures; absence of dangerous chemicals; minimum waste problems, and especially, a minimum number of chromatographic purifications.

The overall strategy, based on our previous work, was to carry out as many steps as possible without subsequent purification. If this was not possible, due for instance to the presence of accumulated side products or incompatible



BrAc = bromoacetyl; ClAc = chloroacetyl; ClBn = *p*-chlorobenzyl;

TAPE = 2-(*p*-trifluoroacetamidophenyl)ethyl; APE = 2-(*p*-aminophenyl)ethyl

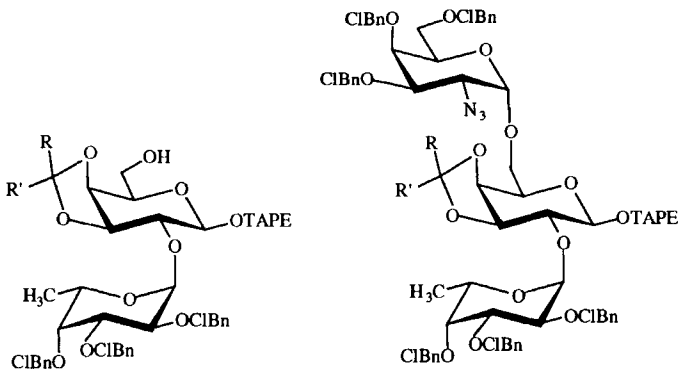


Fig 1. Side products in the DMTSB-promoted glycosidation of 9 and 10.

impurities, crystallization was performed. Chromatography was only used as the last alternative. The purity of the intermediates were of less importance compared to that of the final products.

According to these guidelines the following changes of the small scale procedure were made: In general the reactions were performed in more concentrated solutions. The preparation of the monosaccharides **7, 13** were modified for large scale synthesis (see experimentals).

Compound **6** was made in four steps/three pot reactions from **1** with purification by crystallization in the last step. The major differences were that the 2-*O*-bromoacetyl derivatives **3a** and **5a** were substituted by the chloroacetyl analogs **3b**[36] and **5b** because the bromoacetyl group did not show any advantage in the synthesis. Chloroacetyl chloride is also considerably cheaper than bromoacetyl bromide. Collidine and dimethylaminopyridine was replaced by the cheaper pyridine and used in nearly equivalent amounts relative to the acid chlorides. The crude **3b** was glycosylated with **4** using an equivalent amount of DMTSB in dichloromethane/diethyl ether, instead of dichloromethane/acetonitrile. The former solvent mixture presumably stabilized the intermediate glycosyl fluoride,[25] and thus increased the yield. Deacetylation of crude **5b** was performed more concentrated, with a modified work-up followed by crystallization to give **6** in ≈45% yield from **1** and with a purity of ≈85-90%.

Disaccharide **9** was obtained in a two steps/one pot reaction. Crude **6** was fucosylated with **7** in THF instead of dichloromethane and with a slight excess of **7** and DMTSB. Debenzoylation of **8** was performed by directly adding >4 equivalents of sodium methoxide/eq. DMTSB to the reaction mixture. Crude **9** was obtained in ≈90% yield and with a purity of ≈90%.

Compound **11** was made essentially as described before but with a change of solvents. In the scale-up, using the small scale procedure, we encountered severe problems during work-up. The solution was almost impossible to filter even when Celite was used as filtration aid. This was presumably due to colloidal AgBr which was formed when the glycosidation was performed in THF-dioxane. Changing of the solvent system to ethyl acetate:dioxane 2.5:1 solved the problem with colloidal AgBr. The selectivity decreased somewhat to a 3:1 α/β-mixture of **11** which was not resolved at this stage. Mercury salts like $Hg(CN)_2$, $HgBr_2$ were not used because of environmental reasons, although the results indicates that their reactivity could give good α-selectivity and chemical yields.[37]

Compound **14** was made as described for the small scale procedure. The large difference in crystallinity between **11** and **14** is noteworthy despite their close resemblance in structure. While **14** crystallized easily in quantitative yield in high purity from impure reaction mixtures, **11** resisted numerous attempts at crystallization even with purified material.

Hydrogenation of **11** and **14**, to give **12** and **15**, respectively, were performed in ethanol:ethyl acetate at atmospheric pressure and 60 °C. Our general experience with deprotection of benzyl groups by catalytic hydrogenation is that, especially on a larger scale, higher pressures than atmospheric can be avoided. Careful purification of the starting compound and warming of the hydrogenation reaction mixture are more efficient ways to achieve a fast and reproducible reaction. In this case, the presence of a basic ion exchanger was necessary to neutralize liberated hydrogen chloride from the hydrogenolysis of the *p*-chlorobenzyl groups.

To make possible attachment to solid matrixes, the *N*-trifluoroacetyl groups in **12** and **15** were removed by treatment with aqueous ammonia at 50 °C. The crude products were purified by adsorption/desorption on reversed phase C-18 silica, to give **16** and **17** in >95% yield and >98% purity (by NMR and HPLC).

In conclusion, the A and B trisaccharide glycosides **16** and **17** were synthesized from a common disaccharide intermediate **6** in acceptable to good yields under conditions which potentially permit their syntheses on a large scale.

EXPERIMENTAL

The general methods were the same as those reported[29] with the following additions. NMR spectra were recorded at 300 K for solutions in $CDCl_3$ or D_2O using a Bruker AM 500 instrument. The following reference signals were used, unless otherwise stated: $CDCl_3$ δ 77.0 (^{13}C in $CDCl_3$); Me_4Si δ 0.00 (^1H in $CDCl_3$). Column chromatography was performed on silica gel (0.035-0.070mm or 0.020-0.040mm, Matrex LC 60A, Grace, W.Germany) and elution with toluene:ethyl acetate or toluene:dichloromethane:ethyl acetate mixtures unless otherwise stated. Molecular sieves (4Å, Union Carbide, powder, Fluka) were dried *in vacuo* at ca 300 °C overnight. Dichloromethane, tetrahydrofuran, diethyl ether and pyridine were bought puriss, absolute, over 4Å molecular sieves (Fluka, Switzerland). Ethyl acetate and dioxane were dried over 4Å molecular sieves when necessary.

Small scale procedure:

Ethyl 3-*O*-Benzoyl-4,6-*O*-benzylidene-1-thio-β-D-galactopyranoside (2). Compound **2** was prepared in 83% crystalline yield from ethyl 4,6-*O*-benzylidene-1-thio-β-D-galactopyranoside[26] **1** as described[38] for the corresponding methyl 1-thioglycoside. Mp 123.5-125 °C $[\alpha]_D^{22}$ +55.4°. NMR data: ^{13}C, δ 15.3 (CH_2CH_3), 23.4 (CH_2CH_3), 66.7 (C-2), 69.2 (C-6), 70.0 (C-5), 74.0 (C-4), 75.3 (C-3), 86.0 (C-1), 100.9 (PhCH), 166.4 (C=O). ^1H, δ 1.36 (t, CH_3), 2.79, 2.87 (m, SCH_2), 3.64 (br s, H-5), 4.04 (dd, $J_{5,6}$ 1.8 Hz, $J_{6,6}$ 12.2 Hz, H-6), 4.23 (dt, $J_{OH,2}$ 2.1 Hz, $J_{2,3}$ 9.8 Hz, H-2), 4.37 (dd, H-6'), 4.49 (d, $J_{1,2}$ 9.8 Hz, H-1), 4.53 (br d, H-4), 5.16 (dd, $J_{3,4}$ 3.7 Hz, H-3), 5.50 (s, CHPh).

Ethyl 3-*O*-Benzoyl-4,6-*O*-benzylidene-2-*O*-bromoacetyl-1-thio-β-D-galactopyranoside (3a). Bromoacetyl bromide (320 μL) dissolved in dichloromethane (2 mL) was added dropwise to a cooled (-5 °C) and stirred solution of **2** (1.02 g), *s*-collidine (1.18 mL), and 4-dimethylaminopyridine (30 mg) in dichloromethane (17 mL) during 5 min. The mixture was diluted with dichloromethane and washed with water, 1 M H_2SO_4, saturated aqueous sodium hydrogen carbonate and water, dried and treated with silica gel (3 g) for 20 min. The mixture was filtered and concentrated. The product was purified by crystallisation from dichloromethane and light petroleum to give **2** (1.23 g, 94 %), mp 126-127 °C, $[\alpha]_D^{30}$ +79°. NMR data: ^{13}C, δ 14.8 (CH_3CH_2), 22.9 (SCH_2), 25.1 ($BrCH_2$), 68.3 (C-2), 69.1(C-6), 69.8 (C-5), 73.4 (C-3), 73.7(C-4), 82.3 (C-1), 101.0 (PhCH), 165.9, 166.0 (C=O). ^1H, δ 1.32 (t, CH_3), 2.76, 2.91 (m, SCH_2), 3.67

(br s, H-5), 3.76 (s, $BrCH_2CO$), 4.05 (dd, H-6), 4.37 (dd, H-6'), 4.57 (br d, H-4), 4.59 (d, $J_{1,2}$ 9.8 Hz, H-1), 5.25 (dd, $J_{3,4}$ 3.7 Hz, H-3), 5.50 (s, CHPh), 5.70 (t, $J_{2,3}$ 9.8 Hz, H-2).

Anal. Calcd for $C_{24}H_{25}BrO_7S$: C, 53.6; H, 4.7; Br, 14.9. Found: C, 53.7 H, 4.7; Br, 14.9.

(*p*-Trifluoroacetamidophenyl)ethyl 3-*O*-Benzoyl-4,6-*O*-benzylidene-2-*O*-bromoacetyl-β-D-galactopyranoside (5a). Compound **3a** (1.50 g) and 2-(*p*-trifluoroacetamidophenyl) ethanol[21] **4** (725 mg) were dissolved in dichloromethane-acetonitrile (3:1, 60 mL). Molecular sieves (4Å, 4.5 g) was added and the mixture was stirred for 1 h, then DMTSB[24] (930 mg) was added and the mixture was stirred for 30 min, when TLC indicated complete reaction. The mixture was filtered through a layer of celite and diluted with dichloromethane, washed with saturated aqueous sodium hydrogen carbonate and water, dried and concentrated. The product was purified by chromatography (toluene/ethyl acetate 4:1) to give **3** (1.18 g, 60%). NMR data: ^{13}C, δ 25.3 ($BrCH_2$), 35.4 (Ar-CH_2), 68.8, 69.7 (C-6, OCH_2CH_2), 66.4, 70.1, 72.3, 73.4 (C-2, C-3, C-4, C-5), 100.5 (C-1), 100.8 (Ph-CH), 165.8, 166.0 (C=O). ^1H, δ 2.91 (m, $-CH_2CH_2Ph$), 3.57 (br s, H-5), 3.59 (br s, $BrCH_2$), 4.10 (dd, H-6), 4.35 (dd, H-6'), 4.53 (br d, H-4), 4.59 (d, $J_{1,2}$ 7.9 Hz, H-1), 5.18 (dd, $J_{2,3}$ 10.4 Hz, $J_{3,4}$ 3.7 Hz, H-3), 5.51 (s, CHPh), 5.59 (q, H-2).

(*p*-Trifluoroacetamidophenyl)ethyl 3-*O*-Benzoyl-4,6-*O*-benzylidene-β-D-galactopyranoside (6). Compound **5a** (1.18 g) was dissolved in pyridine-water (8:2, 50 mL) and stirred overnight. The mixture was diluted with dichloromethane and washed with water, 1 M H_2SO_4, saturated aqueous sodium hydrogen carbonate and water, dried and concentrated. The product was purified by crystallisation from dichloromethane and light petroleum to give **6** (684 mg, 70%) m.p 224-225 °C $[\alpha]_D^{30}$ +79.2°. NMR data: ^{13}C, δ 35.5(Ar-CH_2CH_2), 66.6 (C-2), 68.7, 69.0 (C-6, O-CH_2CH_2), 70.2, 73.5, 74.0 (C-3,C-4,C-5), 100.7 (Ph-CH), 103.2 (C-1), 165.8 (C=O). ^1H, δ 2.97 (m, $-CH_2CH_2Ph$), 3.57 (br s, H-5), 3.74 (m, OCH_2CH_2-), 4.09 (dd, H-6), 4.15 (dt, $J_{OH,2}$ 2.2 Hz, H-2), 4.24 (m, OCH_2CH_2), 4.34 (dd, H-6'), 4.41 (d, $J_{1,2}$ 7.6 Hz, H-1), 4.48 (br d, H-4), 5.11 (dd, $J_{2,3}$ 10.2 Hz, $J_{3,4}$ 3.7 Hz, H-3), 5.51 (s, CHPh).

Anal. Calcd for $C_{30}H_{28}F_3NO_8$: C, 61.3; H, 4.8; N, 2.4. Found: C, 61.0; H, 4.8; N, 2.3.

(*p*-Trifluoroacetamidophenyl)ethyl 3-*O*-Benzoyl-4,6-*O*-benzylidene-2-*O*-(2,3,4-tri-*O*-*p*-chlorobenzyl-α-L-fucopyranosyl)-β-D-galactopyranoside (8). Compound **6** (706 mg) and ethyl 2,3,4-tri-*O*-*p*-chlorobenzyl-1-thio-β-L-

fucopyranoside[28] **7** (1.00 g) were dissolved in dichloromethane (40 mL) and molecular sieve (4Å, 2.5 g) was added. The mixture was stirred for 1 h, DMTSB (409 mg) was added and the mixture was stirred for 30 min when TLC indicated complete reaction. The mixture was filtered through a layer of celite and diluted with dichloromethane, washed with saturated aqueous sodium hydrogen carbonate and water, dried and concentrated. The product was purified by chromatography (toluene:ethyl acetate 4:1) to give **8** (800 mg, 60%) as an amorphous solid. NMR data: ^{13}C, δ 16.5 (C-6'), 35.3 ($CH_2\mathbf{C}H_2Ph$), 66.3, 66.5, 69.0, 69.2 (C-6, OCH_2CH_2), 71.2, 71.7 (2), 72.2 (2), 73.5, 74.1(2), 75.8, 78.2, 79.0, 97.0 (C-1') 100.7 (Ph-**C**H), 101.8 (C-1), 165.8 (C=O).^1H, δ 1.07 (d, $J_{5,6}$ 5.6 Hz, CH_3), 2.94 (m, $OCH_2\mathbf{C}H_2Ph$), 3.44 (br d, H-4'), 3.58 (br s, H-5), 3.71 (dd, $J_{3,4}$ 2.9 Hz, H-3'), 3.75 (m, OCH_2CH_2), 3.82 (dd, $J_{2,3}$ 10.2 Hz, H-2'), 4.09 (dd, H-6), 4.40 (dd, $J_{2,3}$ 9.9 Hz, H-2), 4.54 (br d, H-4), 4.64 (d, $J_{1,2}$ 7.8 Hz, H-1), 5.35 (dd, $J_{2,3}$ 9.9 Hz, $J_{3,4}$ 3.7 Hz, H-3), 5.40 (d, $J_{1,2}$ 3.7Hz, H-1'), 5.50 (s, Ph-**C**H).

The β-fucoside was isolated in approximately 15% yield. NMR data: 1H, d 1.10 (d, $J_{5,6}$ 6.4 Hz, CH_3), 2.82 (tr, $OCH_2\mathbf{C}H_2Ph$), 3.22 (q, H-5'), 3.36 (dd $J_{2,3}$ 9.7, $J_{3,4}$ 3.0 Hz, H-3'), 3,36 (br d, H-4'), 3.55 (br s, H-5), 3.60/4.10 (m, OCH_2CH_2), 3.60(m, H-2), 4.10 (m, H-6), 4.31 (m, 2H, H-2, H-6'), 4.54 (d, $J_{1,2}$ 7.8 Hz, H-1), 4.55 (H-4), 4.62 (d, $J_{1,2}$ ≈7.5 Hz, H-1'), 5.11 (dd, $J_{2,3}$ 9.9 Hz, $J_{3,4}$ 3.8 Hz, H-3), 5.51 (s, Ph-**C**H). ^{13}C, δ 16.6 (C-6'), 35.5 ($CH_2\mathbf{C}H_2Ph$), 68.9, 70.4, 71.9, 73.9, 74.0 (C-6, OCH_2CH_2, 3xClPh$\mathbf{C}H_2$), 100.6, 102.6, 103.1 (C-1, C-1', Ph**C**H), 166.4 (C=O).

(*p*-Trifluoroacetamidophenyl)ethyl 4,6-*O*-Benzylidene-2-*O*-(2,3,4-tri-*O*-*p*-chlorobenzyl-α-L-fucopyranosyl)-β-D-galactopyranoside (9). Disaccharide derivative **8** (500 mg) was dissolved in dichloromethane-methanol (3:2, 25 mL), sodium methoxide in methanol (0.5 M, 4 mL) was added. The mixture was stirred for 40 h, neutralized with Dowex 50 H^+, filtered and concentrated to yield **9** (350 mg, 77%) as an amorphous solid. $[\alpha]_D^{30}$ -58°. NMR data: ^{13}C, δ 16.7 (C-6'), 35.5 ($OCH_2\mathbf{C}H_2$), 66.6 (C-5), 66.7 (C-5'), 69.1(C-6), 69.4 (OCH_2CH_2), 72.0, 72.7, 74.1 (3xAr$\mathbf{C}H_2$), 73.5, 77.4 (C-2,C-3), 75.4 (C-4), 76.7 (C-2'), 78.1(C-4'), 79.2 (C-3'), 98.7 (C-1') 101.4 (Ph-**C**H), 101.6 (C-1), .^1H, δ 1.10 (d, J 6.4 Hz, CH_3), 2.92 (m, $CH_2\mathbf{C}H_2Ar$), 3.42 (br s, H-5), 3.56 (br d, H-4'), 3.70 (m, 1H, $\mathbf{C}H_2CH_2Ar$) 3.82 (dd, $J_{3,4}$ 2.9 Hz, H-3'), 3.98 (dd, $J_{2,3}$ 10.1 Hz, H-2'), 4.04 (dd, $J_{5,6}$ 1.7 Hz, H-6), 4.10 (H-5'), 4.12 (m, 1H, CH_2CH_2Ar), 4.20 (br d, H-4), 4.30 (dd, $J_{6,6'}$ 12.3 Hz, H-6'), 4.42 (d, $J_{1,2}$ 7.5 Hz, H-1), 5.29 (d, $J_{1,2}$ 3.7 Hz, H-1'), 5.54 (s, Ph**C**H).

(*p*-Trifluoroacetamidophenyl)ethyl 3-*O*-(2-Azido-3,4,6-tri-*O*-*p*-chlorobenzyl-2-deoxy-α-D-galactopyranosyl) - 4,6-*O*-benzylidene - 2-*O*-(2,3,4-tri-*O*-*p*-chlorobenzyl-α-L-fucopyranosyl)-β-D-galactopyranoside (11). *p*-Methylphenyl

2-azido-3,4,6-tri-*O*-*p*-chlorobenzyl-2-deoxy-1-thio-β-D-galactopyranoside[29] **10a** (1.56 g) was dissolved in THF:1,4-dioxane (2.5:1, 28 mL) and molecular sieves (4Å, 10 g) was added. The mixture was stirred for 40 min and then cooled to (-5 °C). Bromine (130 μL) was added dropwise and the mixture was stirred for 45 min at -10 °C - 0 °C. Cyclohexene was added until the brown colour of the solution disappeared. *S*-collidine (275 μL) and disaccharide derivative **9** (1.00 g) were added. The mixture was stirred for 30 min and then cooled to -35 °C. Silver triflate (637 mg) slurried in toluene (2.5 mL) was added dropwise. The solution was stirred for 1.5 h at -35 °C- -25 °C. The mixture was diluted with dichloromethane and filtered through a layer of celite and washed with sodium thiosulfate, water, saturated aqueous sodium hydrogen carbonate and water, dried and concentrated. The product was purified by chromatography (toluene:dichloromethane:ethyl acetate 8:6:1) to give **11** (990 mg, 64%) as an α/β mixture 4:1. .NMR data α-isomer: ^{13}C, δ 16.8(C-6'), 35.4 (Ar-**C**H$_2$), 59.9 (C-2"), 66.2, 66.4 (C-2), 69.2 (C-6), 69.3(C-6"), 69.9 (O**C**H$_2$CH$_2$), 70.1(C-5"), 71.4, 71.5, 72.7, 72.8, 73.5, 73.8, 74.3, 75.5 (C-3), 76.5 (C-2'), 78.0(C-5), 79.5 (C-3'), 93.8 (C-1"), 97.4 (C-1') 100.6 (Ph**C**H), 102.4 (C-1). ^{1}H, δ 1.09 (d, $J_{5,6}$ 6.7Hz, C**H$_3$**), 3.37 (br d, H-4'), 3.62 (br d, H-4"), 3.77 (dd, $J_{2,3}$ 10.5 Hz, H-2"), 3.81 (dd, $J_{3,4}$ 2.8 Hz, H-3'), 3.85 (dd, $J_{3,4}$ 2.8 Hz, H-3"), 3.88 (dd, $J_{3,4}$ 3.7 Hz, H-3), 3.93 (dd, $J_{2,3}$ 10.4 Hz, H-2'), 4.09 (dd, $J_{2,3}$ 9.5 Hz, H-2), 4.32 (br d, H-4), 4.38 (d, $J_{1,2}$ 7.6 Hz, H-1), 5.23 (d, $J_{1,2}$ 3.5 Hz, H-1"), 5.36 (d, $J_{1,2}$ 3.6 Hz, H-1"), 5.53 (s, C**H**Ph).

From a glycosidation using DMTSB and **10a**, approximately 20-30% of **11** was isolated, together with disaccharide products **18a.** NMR data: ^{13}C, δ 16.5 (C-6'), 35.4 (CH$_2$**C**H$_2$Ph), 62.6 (C-6), 69.1, 72.2, 72.3, 72.7 (3 x **C**H$_2$PhCl + **C**H$_2$CH$_2$Ph), 96.1 (C-1'), 100.7 (C-1), 103.5 (**C**HPh) and **18b.** NMR data: ^{13}C, δ 16.5 (C-6'), 35.4 (CH$_2$**C**H$_2$Ph), 62.3 (C-6), 69.1, 72.1, 72.3, 74.2 (3 x **C**H$_2$PhCl + **C**H$_2$CH$_2$Ph), 95.3 (C-1'), 101.0 (C-1), 105.2 (**C**HPh). Also isolated were trisaccharide derivatives **19a**. NMR data: ^{1}H, δ 1.06 (d, $J_{5,6}$ 6.5 Hz, CH$_3$), 2.88 (tr, 2H, OCH$_2$C**H$_2$**Ar), 4.94 (d, $J_{1,2}$ 2.6 Hz, H-1"), 5.48 (d, $J_{1,2}$ 3.8 Hz, H-1'), 6.09 (s, PhC**H**), ^{13}C, δ 16.5 (C-6'), 35.4 (CH$_2$**C**H$_2$Ph), 59.6 (C-2"), 96.2 (C-1'), 98.1 (C-1"), 100.6 (C-1), 103.5 (**C**HPh), and **19b**, NMR-data: ^{1}H δ 1.03 (d, $J_{5,6}$ 6.5 Hz, CH$_3$), 2.84 (tr, 2H, OCH$_2$C**H$_2$**Ar), 4.97 (d, $J_{1,2}$ 2.7 Hz, H-1"), 5.40 (d, $J_{1,2}$ 3.7 Hz, H-1') 5.89 (s, PhC**H**); ^{13}C δ 17.0 (C-6'), 35.4 (CH$_2$**C**H$_2$Ph), 60.1 (C-2"), 96.0 (C-1'), 98.7 (C-1"), 101.4 (C-1), 105.7 (**C**HPh).

FAB-MS of **19b** showed an [M-H]$^-$ cluster-ion m/z = 1562 (MW calcd for $C_{77}H_{73}Cl_6F_3N_4O_{15}$ = 1564.1; exact mass = 1560.3).

(*p*-Trifluoroacetamidophenyl)ethyl 3-*O*-(2,3,4,6-Tetra-*O*-*p*-chlorobenzyl-α-D-galactopyranosyl)-4,6-*O*-benzylidene-2-*O*-(2,3,4-tri-*O*-*p*-chlorobenzyl-α-L-

fucopyranosyl)-β-D-galactopyranoside (14). Compound **9** (0.93 g) and ethyl 2,3,4,6-tetra-*O*-*p*-chlorobenzyl-1-thio-β-D-galactopyranoside[26] **13** (0.93 g) in dichloromethane/tetrahydrofuran (26 mL, 20:1 v/v) were stirred with molecular sieves (4Å, 1 g) for 20 min. DMTSB (250 mg) was added and the mixture was stirred for 9 h. The mixture was worked up in the same way as for compound **8** to give crude product, which was crystallized from ethanol to give **14** (0.82 g, 57%) with spectral and physical data identical with those reported.[19]

Anal. Calcd for $C_{84}H_{79}Cl_7F_3NO_{16}$: C, 60.6; H, 4.8; N, 0.8; Cl, 14.9. Found: C, 60.3; H, 5.0; N, 1.0; Cl, 14.8.

(*p*-Trifluoroacetamidophenyl)ethyl 3-*O*-(2-Acetamido-2-deoxy-α-D-galactopyranosyl)-2-*O*-(α-L-fucopyranosyl)-β-D-galactopyranoside (12). An α/β mixture of trisaccharide **11** (ratio 2.7:1,190 mg), sodium acetate (200 mg) and palladium on charcoal (10%, 400 mg) were dissolved in acetic acid-ethanol (2:1, 9 mL). The mixture was hydrogenated at 60 psi for 40 h. The mixture was then filtered and concentrated. The residue was dissolved in water and applied to a column of Bond-Elut (C-18, 3 g), washed with water and eluted with methanol. The eluant was concentrated and the residue was dissolved in methanol (8 mL) and acetic anhydride (2 mL) was added. The mixture was stirred for 1 h and was then concentrated. The residue was purified with chromatography (ethyl acetate:acetic acid:methanol:water, 17:3:3:2). The fractions that contained the product were further purified with gel chromatography (Biogel P2) to give, after freeze drying, **12** (36 mg, 55%) $[\alpha]_D^{31}$ +28.5°. NMR data; see table 1 and 2.

(*p*-Trifluoroacetamidophenyl)ethyl 2-*O*-(α-L-fucopyranosyl)-3-*O*-(α-D-galactopyranosyl)-β-D-galactopyranoside (15). Compound **14** was hydrogenated as previously reported[19] to give **15** in 80-90% yield with spectral and physical data identical with those reported. $[\alpha]_D$ -5.1° (c = 0.88, H_2O). NMR data see Table 1 and 2 .

FAB-MS of **5** showed an M+1 ion of m/z = 704 (calculated m.w. = 703.6).

Large scale procedures:

Ethyl 2,3,4-Tetra-*O*-*p*-chlorobenzyl-1-thio-β-D-galactopyranoside (13). β-D-Galactose pentaacetate (1952 g, 5 mol) was dissolved in dichloromethane (3.5 L). Ethanethiol (561 mL, 7.8 mol) was added in one portion, followed by BF_3-etherate (246 mL, 2 mol) in two portions. The mixture was stirred at room temperature for 1 h. Aqueous sodium hydrogen carbonate (3.5 L) was carefully added while stirring the mixture, and an additional 75 g solid sodium hydrogen carbonate was carefully added. The organic phase was separated and washed with aqueous sodium hydrogen carbonate (2 L), water (2 L) and dried (Na_2SO_4).

Table 1: ^1H NMR data for compound 12, 16, 15, 17 in deuterium oxide solution (acetone δ_H = 2.225, J in Hz)

Entry:		12	16	15	17
β-Gal	1($J_{1,2}$)	4.63(7.9)	4.61(7.8)	4.64(7.8)	4.62(7.8)
	2($J_{2,3}$)	3.77(9.8)	3.76	3.79(9.7)	3.79
	3($J_{3,4}$)	3.76(3.6)	3.86	3.96(3.1	3.96
	4($J_{4,5}$)	4.22	4.22	4.28(1.7)	4.27
	5($J_{5,6;5,6'}$)	3.68	3.66	3.72(4.3;7.6)	3.69
	6($J_{6,6'}$)	3.76	3.77	3.78(11.5)	3.83
	6′	3.81	3.81	3.83	3.83
α-Fuc	1($J_{1,2}$)	5.23(4.1)	5.24(4.1)	5.21(4.0)	5.21(4.1)
	2($J_{2,3}$)	3.64(10.4)	3.65	3.65(10.3)	3.66
	3($J_{3,4}$)	3.33(3.3)	3.41	3.34(3.1)	3.41
	4($J_{4,5}$)	3.14(<1.0)	3.23	3.14(1.8)	3.23
	5	3.78	3.83	3.79	3.84
	6($J_{5,6}$)	0.97(6.6)	1.00	0.94(6.6)	0.99
α-GalNAc /	1($J_{1,2}$)	5.16(3.8)	5.18(3.7)	5.23(3.2)	5.23(3.0*)
α-Gal	2($J_{2,3}$)	4.23(10.0)	4.23	3.86	3.86
	3($J_{3,4}$)	3.85(3.0)	3.87	3.86	3.87
	4($J_{4,5}$)	3.97	3.98	3.97	3.96
	5($J_{5,6;5,6'}$)	4.19	4.20	4.18	4.19
	6($J_{6,6'}$)	3.75	3.75	3.73	3.73
	6′	3.75	3.75	3.73	3.73
$COCH_3$			2.03		
O-CH_2-CH_2			4.06/4.21		4.05/4.19
OCH_2-CH_2-Ar			2.82/2.92		2.82/2.92
Ar-H			6.83		6.82
Ar-H′			7.15		7.14

* Measured, not true coupling, due to strong coupling effects between H_2 and H_3.

The solution was filtered and concentrated, at 40 °C, to give a crude oil. The oil was dissolved in methanol (2.5 L) and methanolic sodium methoxide (10%) was added until the pH was ≈ 12. The mixture was stirred over night, neutralized with Dowex $50H^+$ and filtered. The solids were washed with methanol and the combined filtrates were concentrated at 50 °C and coconcentrated with toluene (2x250 mL). The resulting warm oil was dissolved in acetone (p.a., 2.2 L), pyridine (50 mL) was added and the solution was heated to boiling. The solution was left to crystallize at 4 °C. The crystals were filtered off and washed with cold (4 °C) acetone (p.a., 1.2 L), to give ethyl 1-thio-β-D-galactopyranoside ≈75% (two steps).

Table 2: ^{13}C NMR data for compound 12, 16, 15, 17 in deuterium oxide solution (dioxane δC = 67.4, J in Hz)

Entry:		12	16	15	17
β-Gal	1	101.6	101.6	100.6	101.6
	2	72.5	72.5	71.8	72.7
	3	76.8	76.8	76.3	77.3
	4	63.9	63.9	63.4	64.4
	5	75.8	75.8	74.6	75.5
	6	61.8	61.8	60.9	61.8
α-Fuc	1	99.0	99.0	98.2	99.1
	2	68.5	68.5	67.6	68.6
	3	70.4	70.4	69.5	70.4
	4	72.4	72.4	71.5	72.7
	5	67.5	67.5	66.4	67.4
	6	15.9	15.9	14.9	15.9
α-Gal(GalNAc)	1	92.2	92.2	92.9	93.9
	2	50.3	50.3	68.0	70.3
	3	68.6	68.6	69.4	70.4
	4	69.3	69.3	69.2	70.1
	5	71.9	71.9	71.0	72.0
	6	62.2	62.2	61.2	62.2
$COCH_3$		22.8	22.8		
$COCH_3$		175.6	175.6		
O-CH_2-CH_2		69.2	69.8	68.1	69.6
OCH_2-CH_2-Ar		33.6	34.2	33.6	34.1
Ar		123.2-138.5	117.6-145.0	122.1 - 137.4	117.6-145.0
$COCF_3$		157.8 (J 37 Hz)		156.8(J 37 Hz)	
$COCF_3$		116.8 (J 288 Hz)		116.0 (J 287 Hz)	

To a mixture of sodium hydroxide (2407 g, 40 mol) in water (4 L), ethyl 1-thio-β-D-galactopyranoside (300 g, 1.34 mol) was added. The solution was added to a solution of *p*-chlorobenzyl chloride (1098 g, 6.8 mol) and tetrabutylammonium hydrogensulfate (227 g, 0.67 mol) in dichloromethane (2 L). The mixture was stirred vigorously overnight. The organic phase was separated, washed with 1 M H_2SO_4 (2 L), saturated aqueous sodium hydrogen carbonate (2 L) and water (2 L), dried (Na_2SO_4) and concentrated. The residue was dissolved in boiling absolute ethanol (5 L) and allowed to crystallize at room temperature. Yield of **12** ≈ 65% (600-650g), with identical physical properties with those reported.[26]

Ethyl 2,3,4-Tri-*O*-*p*-chlorobenzyl-1-thio-β-L-fucopyranoside (7). L-Fucose (951 g, 5.5 mol) was added to a mixture of acetic anhydride (4.2 L, 44 mol) and pyridine (2.1 L, 26 mol) at such speed that the solution began to boil and kept boiling. After 2 h the mixture was concentrated and coconcentrated with xylene (3x250 mL). The resulting oil was dissolved in dichloromethane (2.7 L) and ethanethiol (610 mL, 8.5 mol) was added. Boron trifluoride etherate (829 mL, 6.6 mol) was added dropwise during 15 min at such speed that the solution began to boil and kept boiling. After 30 min ice water and sodium hydroxide (at least 200 g) was added to pH ≈7. The organic phase was washed with water twice, dried (Na_2SO_4) and concentrated at 30-60 °C. The crude oil was dissolved in methanol (2.0 L) and sodium methoxide solution (10%) was added to ≈pH 11. The mixture was neutralized after 3h with Dowex 50 H^+, filtered, concentrated and coconcentrated with toluene (2x250 mL). The resulting oil was heated to 100 °C in toluene (3.0 L) and solid potassium hydroxide (2.2 kg) was added and the mixture was heated to boiling. *p*-Chlorobenzyl chloride (1.62 kg, 10 mol) dissolved in a small amount of warm toluene was added slowly. When TLC indicated complete reaction the mixture was cooled to room temperature and filtered. The organic phase was washed with 1 M H_2SO_4 (2x1 L), saturated aqueous sodium hydrogen carbonate (1 L) and water (1 L), dried with $MgSO_4$ and concentrated. The residue was dissolved in abs ethanol (4 L) and allowed to crystallize first in room temperature overnight and thereafter at 4 °C until completion. Yield 1.0 kg ≈30% over four steps. The product showed identical physical and spectral data to those reported.[28]

(*p*-Trifluoroacetamidophenyl)ethyl 3-*O*-Benzoyl-4,6-*O*-benzylidene-β-D-galactopyranoside (6). Compound **1** (200 g, 0.64 mol) was dissolved in dichloromethane (900 mL) and pyridine (160 mL, 2.0 mol) and the mixture was stirred and cooled to -20 °C. Benzoyl chloride (86 mL, 0.74 mol) was added slowly during 30 min. Thereafter chloroacetyl chloride (60 mL, 0.74 mol) was added when TLC showed disappearance of **1** and the temperature was allowed to rise. When TLC showed complete reaction the mixture was diluted with cold 1 M H_2SO_4 (≈600 mL) or until pH was acidic. The organic phase was washed with 1 M H_2SO_4 (2x400 mL), saturated aqueous sodium hydrogen carbonate (2x500 mL) and water (1 L) and dried first with $MgSO_4$ and then, after filtration, with powdered 4 Å molecular sieves (40 g). The solution was filtered into a dry round bottom vessel, under nitrogen atmosphere, and was diluted with diethyl ether to a final volume of 2.4 L. (The solution should be 40% dichloromethane in ether (v/v)). 2-(*p*-Trifluoroacetamidophenyl) ethanol[27] **4** (160 g, 0.68 mol) and

4Å molecular sieves (40 g) was added. After stirring for 15 min, DMTSB (130 g, 0.66 mol) was added and the stirring was continued for 2 h until TLC indicated complete reaction. The solution was filtered through Celite and washed with saturated aqueous sodium hydrogen carbonate (1x1000 mL + 2x600 mL). The organic phase was concentrated to an oil (≈500 g) which was dissolved in pyridine-water (8:2, 1.6 L) and stirred for 15-24 h. The solution was concentrated and coconcentrated twice with toluene - methanol (250 + 100 mL) then with toluene (100 mL). Ethyl acetate (1.3 L) was added and the mixture was heated to boiling. The mixture was then cooled while stirring. The solution was subsequently decanted. This procedure was repeated once more and the solids were then removed by filtration (contains pyridinium salts). The combined organic supernatants were concentrated to ≈ 1.5 L and filtered through a short column of silica gel (800mL; 7.5x12cm). The column was washed with ethyl acetate (700 mL) and the eluate was concentrated to ≈700 mL, then petroleum ether (40-60°, ≈250 mL) was added. The precipitated crystals were removed by filtration. A second crop was obtained from ethylacetate - petroleum ether (4 °C). The total yield of **6** was 201 g (≈47%). The purity, according to NMR was ≈90%. The material was used directly in the next step without further purification.

(*p*-Trifluoroacetamidophenyl)ethyl 4,6-*O*-benzylidene-2-*O*-(2,3,4-tri-*O*-*p*-chlorobenzyl-α-L-fucopyranosyl)-β-D-galactopyranoside (9). Compound **6** (100 g, ≈0.17 mol) and fucoside **7** (110 g, 0.19 mol) was dissolved in THF (500 mL). The mixture was cooled to 0 °C and 4Å molecular sieves (20 g) was added and the mixture was stirred for 15 min. DMTSB (36.8 g, 0.19 mol) was added and the stirring was continued for 1 h. A methanolic solution of sodium methoxide (20.6 g, 0.896 mol sodium, in 240 mL methanol) was then added. TLC indicated complete reaction after 1 h. The mixture was filtered through Celite and the solids were washed with ethyl acetate (600 mL). The organic phase was washed with 1M H_2SO_4 (2x500 mL), saturated aqueous sodium hydrogen carbonate (2x500 mL), dried with $MgSO_4$ and concentrated to ≈500 mL. The solution was then filtered through a short column of silica (650 mL; 6x12cm) and the column was washed with ethyl acetate (600 mL). The eluate was concentrated to ≈350 mL and petroleum ether (40-60°, 500 mL) was added. The solution was left overnight at 4 °C and the jelly-like solids were filtered off thoroughly to remove the solvents. The solids were dried in vacuum to give **9** (171 g, 90-95%) with a purity, according to NMR, of ≈90%.

(*p*-Trifluoroacetamidophenyl)ethyl 3-*O*-(2-Azido-3,4,5-tri-*O*-*p*-chlorobenzyl-2-deoxy-α-D-galactopyranosyl)-4,6-*O*-benzylidene- 2 - *O* - (2,3,4-tri-*O*-*p*-chlorobenzyl-α-L-fucopyranosyl)-β-D-galactopyranoside (11). Compound **10a** (30 g, 43.8 mmol) was dissolved in ethyl acetate:1,4-dioxane (2:1, 300 mL) and molecular sieves (4Å, 20g) were added. The mixture was stirred for 20 min and then cooled to (≈-10 °C). Bromine (2.45 mL, 47.8 mmol) was added dropwise and the mixture was stirred for 45 min at -10 °C - 0 °C. Cyclohexene was added until the brown colour of the solution disappeared. *S*-collidine (5.16 mL, 38.7 mmol) and disaccharide derivative **9** (30 g, ≈90% pure, ≈26.7 mmol) were added and the mixture was stirred for 30 min and then cooled to -40 - -30°C. Silver triflate (12 g, 46 mmol), slurried in toluene, was added dropwise. The solution was stirred for 30 min at -35 °C - -25 °C. Dichloromethane (200 mL) and 1M aqueous sodium thiosulfate (300 mL) were added and the mixture was stirred for 30 min. The mixture was filtered through a layer of Celite. The Celite was washed with dichloromethane (400 mL). To the resulting eluates was 1M aqueous sodium thiosulfate (100 mL) added. The aqueous phase was separated and the organic phase was washed with 1M aqueous sodium thiosulfate (500 mL) and saturated aqueous sodium hydrogen carbonate (600 mL), dried and concentrated. The product was purified by chromatography on silica gel (20-45 μm; toluene:dichloromethane:ethyl acetate; 5.5:2:1). All fractions containing the desired product were collected to give **11** in a mixture with the β-isomer, ≈3:1 (yield of α-anomer ≈40%, by NMR).

(*p*-Trifluoroacetamidophenyl)ethyl 3-*O*-(2-Acetamido-2-deoxy-α-D-galactopyranosyl)-2-*O*-(α-L-fucopyranosyl)-β-D-galactopyranoside (12). An α/β mixture of trisaccharide **11** (ratio 3:1, 30 g), and palladium on charcoal (10%, 30 g) were dissolved in ethyl acetate-ethanol (1:9, 700 mL) together with Amberlite IRA 93 (75 g in free amino form). The mixture was hydrogenated at 60 °C at atmospheric pressure for 3.5 h. The mixture was filtered while still hot. And the solids were extracted, first with hot ethanol, then hot ethanol/water-mixtures at least three times. Acetic anhydride (20 mL) was then added. Pyridine (≈10 mL) was added when TLC (EtOAc:HOAc:MeOH:H_2O 12:3:3:2) indicated complete reaction, followed by xylene, and the mixture was concentrated. The residue was purified with chromatography on silica gel (20-45 μm; EtOAc:HOAc:MeOH:H_2O; 15:3:3:2). The fractions that contained product were further purified by preparative reversed phase HPLC to give, after lyophilization, **12** (7g, 65%).

(*p*-Trifluoroacetamidophenyl)ethyl 3-*O*-(2,3,4,6-Tetra-*O*-*p*-chlorobenzyl-α-D-galactopyranosyl) - 4,6-*O*-benzylidene - 2 - *O* - (2,3,4-tri-*O*-*p*-chlorobenzyl-α-L-fucopyranosyl)-β-D-galactopyranoside (14). Compound **14** was prepared essentially as already described starting from crude **9** (70 g, ≈70 mmol) and **13** (70 g, 97 mmol) and 1.3 L of solvent, yield 50-55% after 5 recrystallizations from ethanol (1.5 L). Purity >98% (NMR).

(*p*-Trifluoroacetamidophenyl)ethyl 2-*O*-(α-L-Fucopyranosyl)-3-*O*-(α-D-galactopyranosyl)-β-D-galactopyranoside (15). Compound **15** was hydrogenated as described for **12** but starting from **14** and omitting the acetylation step. The appearance of a thick slurry in the beginning of the reaction is not a problem, if sufficient stirring is provided. The filtrates were concentrated, redissolved in water and filtered through a 0.45 μm filter and lyophilized without chromatography to give **15** (>90% yield, purity >95% by NMR).

(*p*-Aminophenyl)ethyl 3-*O*-(2-Acetamido-2-deoxy-α-D-galactopyranosyl)-2-*O*-(α-L-fucopyranosyl)-β-D-galactopyranoside (16). Compound **12** (5 g, 6.7 mmol) was dissolved in concentrated aqueous ammonia (100 mL) and heated to 50 °C for 1-1.5 h. The reaction mixture was cooled, then applied to a column of C18-silica (125 g, 45-60 μm). The column was washed with 5 column volumes degassed distilled water. The product was eluted with 30 % methanol in water. The methanol was removed by evaporation and the product was lyophilized. This solid was dissolved in endotoxin-free water and filtered through a 0.45 μm filter, then lyophilized to give **16** (4.18 g, 96%, purity 98.8% according to HPLC, and with no impurity >0.5%). $[\alpha]_D^{22}$ +38.4° (*c* 1.0, H_2O). Elemental analysis showed S<0.03%, Si 0.3%. NMR showed F<0.01%. Sugar analysis showed the presence of only *N*-acetylgalactosamine, galactose and fucose.

Anal. Calcd for $C_{28}H_{44}N_2O_{15}x2H_2O$: C, 49.1; H, 7.1; N, 4.1. Found: C, 49.4; H, 6.7; N, 3.9. FAB-MS showed an $(M+H)^+$ ion m/z = 648.3.

(*p*-Aminophenyl)ethyl 2-*O*-(α-L-Fucopyranosyl)-3-*O*-(α-D-galactopyranosyl) -β-D-galactopyranoside (17). Compound **17** was prepared from **15** (5 g, 7.1 mmol) as described for compound **16** in 96% yield with a purity of 99.4% according to HPLC. $[\alpha]_D^{22}$ +0.2° (*c* 1.0, H_2O). Elemental analysis showed S<0.03%, Si 0.13%. NMR showed F<0.01%. Sugar analysis showed the presence of only galactose and fucose.

Anal. Calcd for $C_{26}H_{41}NO_{15}xH_2O$: C, 49.9; H, 6.9; N, 2.2. Found: C, 49.4; H, 7.0; N, 2.1. FAB-MS showed an $(M+H)^+$ ion m/z = 607.3.

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36. Analytical data for ethyl 3-*O*-benzoyl-4,6-*O*-benzylidene-2-*O*-chloroacetyl-1-thio-β-D-galactopyranoside (**3b**): mp 127.5-128 °C $[\alpha]_D$ +72°. The NMR data were the same as for **3a** (± 0.02ppm for ^1H and ±0.2ppm for ^{13}C) except for: ^1H δ 4.01 (dd, 2H, J 14.8Hz, $ClCH_2$), and ^{13}C δ 40.6 ($ClCH_2$). FAB-MS of **3b** showed an $[M-H]^-$ ion of m/z = 491 and an $[M+Cl]^-$ ion of m/z = 527 (calculated exact mass = 492.1).
Anal. Calcd for $C_{24}H_{25}ClO_7S$: C, 58.5; H, 5.1; Cl, 7.2. Found: C, 58.3; H, 5.1; Cl, 7.2.

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39. After the submission of this manucript a disclosure of Chembiomed's effort for synthesing blood group trisaccharides in large scale was reported.
R. Ippolito, Glycotechnology conference; San Fransisco, 16-19 May 1993.