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Glycosidations with Thioglycosides Activated by Sulfuryl Chloride/Trifluoromethanesulfonic Acid: Synthesis of a Human Blood Group B Trisaccharide Glycoside

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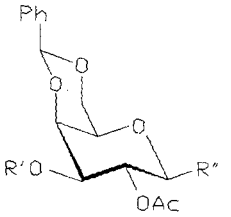
The trisaccharide 2-(*p*-trifluoroacetamidophenyl)ethyl 2-*O*-(α-L-fucopyranosyl)-3-*O*-(α-D-galactopyranosyl)-β-D-galactopyranoside, corresponding to the human blood group B determinant, was synthesized. Thioglycosides activated by sulfuryl chloride/trifluoromethanesulfonic acid were used as glycosyl donors in the construction of the three glycosidic linkages.

The blood group A and B determinant trisaccharides have, in recent years, become increasingly demanded for biomedical purposes. These purposes include, e.g., production of monoclonal antibodies using trisaccharide-protein conjugates, or preparation of columns with immobilized carbohydrates to be used for specific adsorption of antibodies. Consequently, several chemical syntheses of the A and B trisaccharides (or derivatives of them) have been reported [1-13]. However, since some biomedical applications require substantial amounts of trisaccharide, improved synthetic procedures are still needed, especially such which use cheap reagents and give rise to crystalline intermediates.

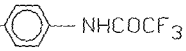
We now report synthesis of the 2-(*p*-trifluoroacetamidophenyl)ethyl β-glycoside **5** of the B trisaccharide. The synthetic pathway utilized thioglycoside [14] synthons, and these were activated for glycosidation with the newly developed [15], relatively cheap reagent sulfuryl chloride/trifluoromethanesulfonic acid. All intermediates were crystallized from the crude reaction mixtures.

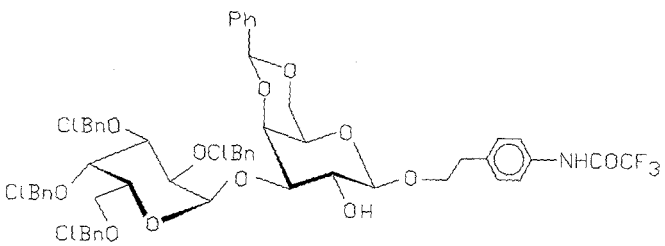
Results

The starting material for the β-galactosyl unit was ethyl 2-*O*-acetyl-4,6-*O*-benzylidene-3-*O*-*p*-chloroacetyl-1-thio-β-D-galactopyranoside **1**. It was prepared in 60% yield from ethyl 1-thio-β-D-galactopyranoside [16] as described [17] for the corresponding methyl 1-thioglycoside.



1: R' = ClAc
R'' = SEt

2: R' = H
R'' = OCH_2CH_2  $NHCOCF_3$



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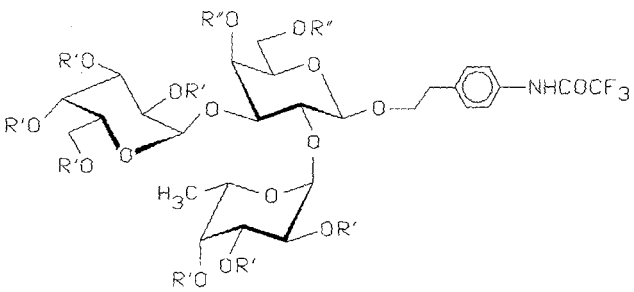

4: R' = ClBn
R'' = CHPh

5: R' = R'' = H

Compound **1** was treated with 2-(*p*-trifluoroacetamidophenyl)ethanol [18] and sulfuryl chloride/trifluoromethanesulfonic acid [15] in tetrahydrofuran-ethyl acetate at 0°C. The product mixture was directly de-chloroacetylated by treatment with aqueous pyridine [19] to give a mixture, from which **2** could be crystallized in 52% yield.

Compound **2** was used as glycosyl acceptor in a sulfuryl chloride/trifluoromethanesulfonic acid promoted glycosidation, employing ethyl 2,3,4,6-tetra-*O*-*p*-chlorobenzyl-1-thio-β-D-galactopyranoside [20] as glycosyl donor, tetrahydrofuran as solvent, and a reaction temperature of -40°C to -8°C. The resulting product mixture was directly de-*O*-acetylated with methanolic sodium methoxide to give a mixture, from which **3** could be crystallized in 73% yield.

Compound **3** was then used as glycosyl acceptor in another sulfuryl chloride/trifluoromethanesulfonic acid promoted glycosidation, now using ethyl 2,3,4-tri-*O*-*p*-chlorobenzyl-1-thio-β-L-fucopyranoside [20] as glycosyl donor, tetrahydrofuran as solvent and a reaction temperature of -30°C. Compound **4** was obtained in 59% yield after crystallization. Chromatography of the mother liquor on silica gel gave an additional 14% of **4**, along with 7% of the corresponding β-isomer.

Finally, **4** was hydrogenolyzed over Pd/C in the presence of sodium acetate to give, after purification by adsorption-washing-desorption on a C-18 reversed phase column, crystalline compound **5** in 88% yield.

Experimental

General Methods

Melting points are corrected. Concentrations were performed at <40°C bath temperature. Optical rotations were recorded 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 spectrometer. The following reference signals were used: Me_4Si δ 0.0 (^1H in C^2HCl_3, acetone-d_6: ^{13}C in acetone-d_6), $CHCl_3$ δ 77.0 (^{13}C in C^2HCl_3, Me_2CO δ 2.225 (^1H in ^2H_2O), and external dioxan δ 67.4 (^{13}C in ^2H_2O). Only selected NMR data are reported. In the assignments below, atoms of 3-linked galactose and 2-linked fucose carry the 'and " superscripts respectively. The FAB-MS spectrum was recorded with a VG ZAB-SE mass spectrometer. Silica gel 60 F-254 (Merck, Darmstadt, W. Germany) was used for TLC with detection by u.v. light or by charring with sulfuric acid. Column chromatography was performed on silica gel (Matrex 60A, 35-70 μm, Grace, Worms, W. Germany). Organic solutions were dried over $MgSO_4$. Powdered molecular sieves (4Å, Fluka, Buchs, Switzerland) were used without further treatment. Sulfuryl chloride/triflic acid reagent [15] was made 1 M in toluene containing 10% diethylether by vol and kept at -18°C under nitrogen. Glycosidations were performed under nitrogen.

*Ethyl 2-O-Acetyl-4,6-O-benzylidene-3-O-chloroacetyl-1-thio-β-D-galactopyranoside (**1**)*

Compound **1** was prepared in 60% yield from ethyl 1-thio-β-D-galactopyranoside [16] as described [17] for the corresponding methyl 1-thioglycoside.

M.p. 132-133°C, $[\alpha]_D$ +37°. ^{1}H-NMR (C^2HCl_3): δ 4.45 (d J_{12} 9.8 Hz, H-1); 5.48 (t J_{23} 9.8 Hz, H-2); 5.03 (dd J_{34} 3.7 Hz, H-3); 4.45 (dd J_{45} <1 Hz, H-4); 3.57 (m J_{56a} 1.52 Hz, J_{56b} 1.84 Hz, H-5); 4.35 (dd J_{6a6b} 12.5 Hz, H-6a); 4.02 (dd, H-6b); 1.29 (t CH_2-C**H**$_3$); 2.72, 2.87 (m C**H**$_2$-CH_3); 5.48 (s C**H**-Ph); 7.42 (m H-Ar).
^{13}C-NMR (C $^2HCl_3$): δ 14.7, 22.9 (SEt); 20.8 (OAc); 40.6 (ClAc); 69.0 (C-6), 66.3, 69.5, 73.3, 74.6 (C-2,3,4,5); 82.7 (C-1); 101.1 (**C**H-Ph); 126.3-137.3 (C-Ar); 167.0, 169.3 (CO).
Analytical data. Calculated for $C_{19}H_{23}ClO_7S$ (430.9): C, 53.0; H, 5.4. Found: C, 52.8; H, 5.2.

*2-(p-Trifluoroacetamidophenyl)ethyl 2-O-Acetyl-4,6-O-benzylidene-β-D-galactopyranoside (**2**)*

To a solution of **1** (3.47 g) and 2-(*p*-trifluoroacetamidophenyl) ethanol [18] (2.07 g) in 50 ml tetrahydrofuran/ethyl acetate, 5/1 by vol, containing molecular sieves (7 g) was added sulfuryl chloride/trifluoromethanesulfonic acid reagent [15] (16 ml) during 5 min at 0°C. Then ethyl acetate (100 ml), saturated aqueous sodium hydrogencarbonate (200 ml) and pyridine (10 ml) was added, and the mixture was filtered. The organic layer was concentrated to 50 ml. Aqueous pyridine (80% by vol, 120 ml) [19] was added and the clear solution was kept at room temperature for 16 h, then partitioned between dichloromethane and ice-cooled 1 M sulfuric acid. The organic layer was washed with water and dried. Compound **2** crystallized upon concentration. Yield 2.22 g (52%). Chromatography of the mother liquor in toluene/ethyl acetate, 1/1 by vol, yielded more **2** (0.55 g, 13%).

M.p. 200-204°C, $[\alpha]_D$ +14.8°. ^{1}H-NMR (acetone-d_6): δ 4.49 (d J_{12} 7.9 Hz, H-1); 5.10 (dd J_{23} 10.1 Hz, H-2); 3.80 (dd J_{34} 3.7 Hz, H-3); 5.62 (s C**H**-Ph). ^{13}C-NMR (acetone-d_6): δ 20.9 (OAc); 35.9 (**C**H$_2$-Ph); 67.3, 71.4, 72.6, 76.8 (C-2,3,4,5); 69.5, 70.1, (C-6, O-**C**H$_2$-CH_2); 101.3, 101.5 (C-1, **C**H-Ph); 116.8 (q *J* 289 Hz, CF_3); 121.4-139.4 (C-Ar); 155.3 (q *J* 37.7 Hz, **C**OCF_3); 170.2 (**C**OCH_3).

Analytical data. Calculated for $C_{25}H_{26}F_3NO_8$ (525.5): C, 57.1; H, 5.0; N, 2.7. Found: C, 56.9; H, 4.9; N, 2.6.

*2-(p-Trifluoroacetamidophenyl)ethyl 4,6-O-Benzylidene-3-O-(2,3,4,6-tetra-O-p-chlorobenzyl-α-D-galactopyranosyl)-β-D-galactopyranoside (**3**)*

To a solution of **2** (1.45 g) and ethyl 2,3,4,6-tetra-*O*-*p*-chlorobenzyl-1-thio-β-D-galactopyranoside [20] (2.20 g) in tetrahydrofuran (50 ml) containing molecular sieves (14 g) was added sulfuryl chloride/trifluoromethanesulfonic acid reagent (5.52 ml) at -40°C. The temperature was raised to -8°C during 10 min, and saturated sodium hydrogencarbonate (100 ml) and pyridine (5 ml) were added. The molecular sieves were filtered off and washed with ethyl acetate (30 ml). The organic layer was dried and concentrated to 20 ml. Methanolic sodium methoxide (0.05 M, 200 ml) was added and the mixture was kept at room temperature for 16 h. The solution was then neutralized with Dowex 50 (H^+) resin. Compound **3** crystallized upon concentration, and was recrystallized from diethyl ether-heptane. Yield 2.31 g (73%). Chromatography of the mother liquor in toluene/ethyl acetate, 1/1 by vol, yielded more **3** (0.11 g, 4%).

M.p. 182-184°C, $[\alpha]_D$ +64°. ^{1}H-NMR (C^2HCl_3): δ 4.23 (d *J* 7.6 Hz, H-1); 5.20 (d *J* 3.4 Hz, H-1'); 4.48 (s, **C**H-Ph). ^{13}C-NMR (C^2HCl_3): δ 35.6 (CH_2-**C**H$_2$-Ph): 66.5-78.4 (C-2,3,4,5,2',3',4',5'); 68.9-74.0 (O-**C**H$_2$-Ph, O-**C**H$_2$-CH_2, C-6,6'); 93.6 (C-1'); 101.2 (**C**H-Ph); 103.1 (C-1); 115.6 (q *J* 289 Hz, CF_3); 120.5-137.6 (C-Ar); 154.2 (q *J* 36.2 Hz, (**C**OCF_3).
Analytical data. Calculated for $C_{57}H_{54}Cl_4F_3NO_{12}$ (1143.8): C, 59.9; H, 4.8; N, 1.2. Found: C, 59.7; H, 4.8; N, 1.2.

Table 1. ^{1}H- and ^{13}C-NMR shifts of compound **5.** Spectra run in $^{2}H_2O$ at 300 K. Chemical shifts given in ppm. Coupling constants (Hz) given for the ^{1}H-NMR spectrum.

H-NMR-Data	H1	H2	H3	H4	H5	H6a	H6b
β-Gal	4.64	3.79	3.96	4.28	3.72	3.78	3.83
	7.8_{12}	9.7_{23}	3.1_{34}	1.7_{45}	4.3_{56a}	11.5_{6a6b}	—
					7.6_{56b}		
α-Gal	5.23	3.86	3.86	3.97	4.18	3.73	3.73
	3.2_{12}	N.D.[a]	N.D.	N.D.	N.D.	N.D.	—
α-Fuc	5.21	3.65	3.34	3.14	3.79	0.94	—
	4.0_{12}	10.3_{23}	3.1_{34}	1.8_{45}	N.D.	6.6_{6a6b}	—
^{13}C-NMR Data	C1	C2	C3	C4	C5	C6	
β-Gal	100.6	71.8	76.3	63.4	74.6	60.9	
α-Gal	92.9	68.0	69.4	69.2	71.0	61.2	
α-Fuc	98.2	67.6	69.5	71.5	66.4	14.9	
O-**C**H$_2$-CH$_2$	68.1						
CH$_2$-**C**H$_2$-Ph	33.6						
CF$_3$	116.0 (J 287 Hz)						
Ar	122.1-137.4						
COCF$_3$	156.8 (J 37.2 Hz)						

[a] N.D. = not determined

*2-(p-Trifluoroacetamidophenyl)ethyl 4,6-O-Benzylidene-3-O-(2,3,4,6-tetra-O-p-chlorobenzyl-α-D-galactopyranosyl)-2-O-(2,3,4-tri-O-p-chlorobenzyl-α-L-fucopyranosyl)-β-D-galactopyranoside (**4**)*

To a solution of **3** (0.77 g) and ethyl 2,3,4-tri-*O*-*p*-chlorobenzyl-1-thio-β-L-fucopyranoside [Lönn H, Nilsson S, Norberg T; unpublished results] (0.59 g) in tetrahydrofuran (40 ml) containing molecular sieves (3.5 g) was added sulfuryl chloride/trifluoromethanesulfonic acid reagent (1.35 ml) at -30°C. The temperature was raised to -10°C during 10 min, then saturated sodium hydrogencarbonate (200 ml) and pyridine (2 ml) was added together with ethyl acetate (100 ml). The mixture was filtered and the organic layer was dried. The solvents were evaporated, and the residual syrup taken up in chloroform-heptane. Crystals of pure **4** were obtained upon standing (664 mg, 59%).
M.p. 77-79°C, $[\alpha]_D$ -9.6°. ^{1}H-NMR (C^2HCl_3): δ 4.39 (d *J* 7.7 Hz, H-1); 5.34 (d *J* 3.7 Hz, H-1'); 5.50 (d *J* 3.7 Hz, H-1''); 5.43 (s, C**H**-Ph); 1.13 (d *J* 6.3 Hz, H-6''). ^{13}C-NMR (C^2HCl_3): 16.8 (C-6''); 35.4 (CH_2-**C**H_2-Ph); 66.1-79.7 (C-2,3,4,5,2',3',4',5',2'',3'',4'',5''); 69.3-74.2 (O-**C**H_2-Ph, O-**C**H_2-CH_2, C-6,6'); 91.8 (C-1'); 97.3 (C-1''); 101.2 (CH-Ph); 102.0 (C-1); 115.6 (q *J* 289 Hz, CF_3); 120.5-137.3 (C-Ar); 154.6 (q *J* 36.2 Hz, CO-CF_3).
Analytical data. Calculated for $C_{84}H_{79}Cl_7F_3NO_{16}$ (1663.6): C, 60.6; H, 4.8; N, 0.8. Found: C, 59.7; H, 4.7; N, 0.8. Chromatography of the mother liquor from the crystallization using toluene/ethyl acetate, 2/1 by vol, as eluent yielded more **4** (158 mg, 14%), unreacted **3** (46 mg, 6%) and the corresponding β-isomer of **4** as a syrup (81 mg, 7%). ^{13}C-NMR (C^2HCl_3): δ 93.1, 101.1, 102.4, 103.9, (**C**H-Ph, C-1, C-1', C-1'').

*2-(p-Trifluoroacetamidophenyl)ethyl 2-O-(α-L-fucopyranosyl)-3-O-(α-D-galactopyranosyl)-β-D-galactopyranoside (**5**)*

A solution of **4** (50 mg) in 5 ml ethyl acetate/ethanol/acetic acid/water, 4/2/1/1 by vol, containing sodium acetate (36 mg) was hydrogenolyzed over Pd/C (10%, 50 mg) at atmospheric pressure for 8 h. The mixture was filtered, concentrated, taken up in water (0.5 ml) and passed through a C-18 column (Bond Elut, Analytichem International, Harbor City, CA, USA, 1 g column) in water. Soluble salts were washed out with water (10 ml), and the product was then eluted off the column with 50% aqueous methanol (5 ml). Concentration and lyophilization yielded 18 mg (88%) of pure **5**, which could be crystallized from water (0.1 ml).
M.p. 150-154°C, $[\alpha]_D$ -5.1° (c = 0.88, ^2H_2O). ^1H- and ^{13}C-NMR data: see Table 1.
Analytical data. Calculated for $C_{28}H_{40}F_3NO_{16}$ (703.6): C, 47.8; H, 5.7; N, 2.0. Found: C, 46.1; H, 5.7; N, 1.9. FAB-MS of **5** showed an M+1 ion of m/z 704.

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