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Synthesis of 2-(*p*-Trifluoroacetamidophenyl)ethyl *O*-α-L-Fucopyranosyl-(1-3)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1-3)-*O*-β-D-galactopyranosyl-(1-4)-β-D-glucopyranoside, a Tetrasaccharide Fragment of a Tumour-associated Glycosphingolipid

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The tetrasaccharide 2-(*p*-trifluoroacetamidophenyl)ethyl *O*-α-L-fucopyranosyl-(1-3)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1-3)-*O*-β-D-galactopyranosyl-(1-4)-β-D-glucopyranoside was synthesized from thioglycoside intermediates. The key step was a methyl triflate promoted glycosidation of a lactose-derived 3',4'-diol with a disaccharide thioglycoside to give a β(1-3)-linked tetrasaccharide derivative in 67% yield.

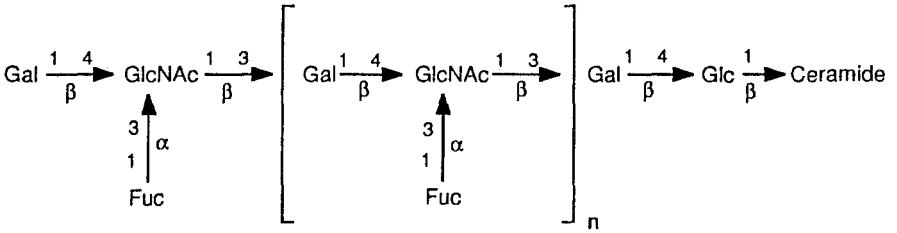
In 1984, Hakomori *et al.* [1] reported the isolation and characterization of glycosphingolipids from adenocarcinoma tissue having the general structure **1**. These structures were not present to any appreciable extent in normal tissue, and were therefore regarded as tumour-associated. Other, related structures have also been reported [2] to occur more frequently in tumour tissue.

As part of a programme aimed at synthesis of tumour-associated carbohydrate structures, we have synthesized the tetrasaccharide **9** which is a partial structure of the glycolipid **1**. This tetrasaccharide will be used together with other oligosaccharides to determine more precisely the specificity of monoclonal antibodies that recognize structures such as **1**.

The synthesis of **9** was based on thioglycosides [3] as building blocks. An AB + CD strategy was adopted, where, in the key step, the known [4] derivative **2** was condensed with the lactose derivative **5**.

Results and Discussion

Methyl 4-*O*-(β-D-galactopyranosyl)-1-thio-β-D-glucopyranoside [5] was treated with 2,2-dimethoxypropane and *p*-toluenesulfonic acid in dimethylformamide and the product mixture was directly treated with benzoyl chloride in pyridine. The benzoylated



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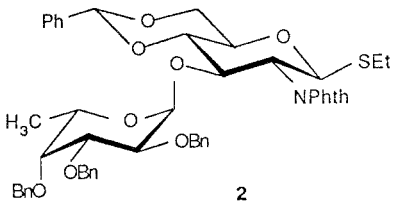
3′,4′acetal **3** was isolated in 78% yield; the yield of the corresponding benzoylated 4′,6′-acetal was 20%. The ^{13}C chemical shifts of the acetal carbons (110.9 and 98.9, respectively) in the two isomers were in accordance with the rules postulated by Buchanan *et al.* [6]. Methyl triflate-promoted glycosidation of 2-(*p*-nitrophenyl)ethanol with **3** gave **4** in 92% yield. Compound **4** was treated with aqueous acetic acid to give the diol **5** in 98% yield. In the ^1H-NMR spectrum of **5** in deuterochloroform, scalar coupling was observed between OH-3′ and H-3′ and between OH-4′ and H-4′. This verified that no acyl migration had taken place during hydrolysis of **4**. Glycosidation of **5** with **2** [4], using methyl triflate as promoter, gave the tetrasaccharide derivative **6** in 67% yield. The newly formed 1-3 linkage in **6** was demonstrated by ^1H-NMR spectroscopy, where scalar coupling was observed, in deuterochloroform solution, between H-4′ and 4′OH. The 4,6-benzylidene acetal in **6** was removed by treatment with aqueous acetic acid, and the product mixture was directly treated first with hydrazine hydrate (to remove the phthalimido group), then with a mixture of acetic anhydride and pyridine. This gave compound **7** in 76% yield. Treatment of **7** with aluminium amalgam [7] and then with trifluoroacetic anhydride/pyridine converted the nitro group in **7** to a *p*-trifluoroacetamido group; the yield of **8** was 72%. Finally, **8** was treated with sodium methoxide in methanol followed by hydrogen/palladium on carbon to give the target tetrasaccharide glycoside **9** in 62% yield.

An attempt to prepare **9** from the protected tetrasaccharide thioglycoside **10** was also made. (Compound **10**, in turn, was prepared by a route analogous to the one used for preparation of **7**). However, when glycosidation of alcohols such as methanol or 2-(*p*-trifluoroacetamidophenyl)ethanol was attempted with **10**, only low yields (<20%) of desired β-glycosides were obtained, regardless of the glycosidation method used. This is in accordance with the results reported [8] by Ogawa *et al.* who obtained only low yields of glycosides when treating ceramide alcohols with acetylated oligosaccharide donors. Formation of other products, derived from decomposition of intermediate orthoesters, was the reason for the low yields obtained. Ogawa suggested the use of other protecting groups in the 2-position, that would impose other properties on the orthoesters or supress their formation. It is interesting to note here, that we have previously [5] carried out similar glycosidations to the one attempted in this work, but then with a 2-*O*-benzoylated thioglycoside derivative, which gave satisfactory yields. The conclusion here must be that, as shown [9, 10] before, a benzoyl group in the 2-position of a glycosyl donor is more advantageous than an acetyl group.



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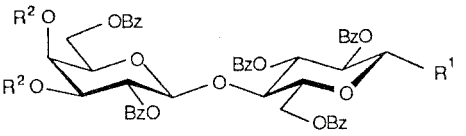
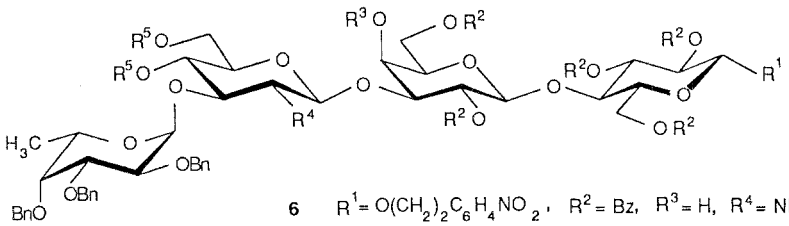


3 R^1= SMe, R^2= $(CH_3)_2C<$

4 R^1= $O(CH_2)_2C_6H_4NO_2$, R^2= $(CH_3)_2C<$

5 R^1= $O(CH_2)_2C_6H_4NO_2$, R^2= H

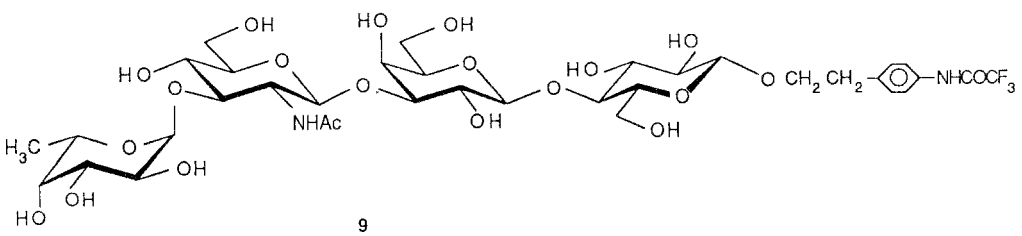


6 R^1= $O(CH_2)_2C_6H_4NO_2$, R^2= Bz, R^3= H, R^4= NHPhth, R^5= C_6H_5-CH<

7 R^1= $O(CH_2)_2C_6H_4NO_2$, R^2=R^3= R^5= Ac, R^4= NHAc

8 R^1= $O(CH_2)_2C_6H_4NHCOCF_3$, R^2=R^3= R^5= Ac, R^4= NHAc

10 R^1= SCH_3, R^2= R^3= R^5= OAc, R^4= NHAc



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Experimental

General Methods

Melting points are corrected. Concentrations were performed at 1-2 kPa at $<40°C$ (bath). Optical rotations were recorded at 22-24°C for 0.5-1.0% solutions in chloroform, unless otherwise stated, using a Perkin-Elmer 241 polarimeter. NMR spectra were recorded at 25°C for solutions in C^2HCl_3 unless otherwise stated using JEOL GXS 270 or Bruker AM 500 instruments. The following reference signals were used: Trimethylsilane δ 0.00 (^{13}C and ^1H in C^2HCl_3), Me_2CO δ 2.225 (^1H in ^2H_2O), dioxane δ 67.4 (^{13}C in ^2H_2O). Only selected NMR data are reported. All ^1H assignments were corroborated by 2-D COSY decoupling experiments. The FAB-MS spectra were recorded with a VG ZAB-SE mass spectrometer. The primary beam consisted of xenon atoms with a maximum energy of 8 KeV. The samples were dissolved in thioglycerol and the positive ions were extracted and accelerated over a potential of 10 kV. TLC was performed on silica gel F_{254} (Merck, Darmstadt, W. Germany) with detection by UV light when applicable or by charring with sulfuric acid. Column chromatography was performed on silica gel 60 (0.04-0.063 mm, Merck) with loadings in the range 1/25-1/100 and elution toluene/ethyl acetate mixtures unless otherwise stated. Organic solutions were dried over $MgSO_4$. Molecular sieves (4Å, Union Carbide, obtained from Fluka, Buchs, Switzerland) were desiccated in a vacuum at 300°C and ground immediately before use.

*Methyl 4-O-(2,6-di-O-benzoyl-3,4-O-isopropylidene-β-D-galactopyranosyl)-2,3,6-tri-O-benzoyl-1-thio-β-D-glucopyranoside (**3**)*

A solution of methyl 4-*O*-(β-D-galactopyranosyl)-1-thio-β-D-glucopyranoside [5] (10.0 g) in *N,N*-dimethyl formamide (100 ml) was heated to 80°C, then *p*-toluenesulfonic acid (100 mg) was added followed by 2,2-dimethoxypropane (8.4 ml) in three portions with five min intervals. Heating was continued for 1 h, then the mixture was cooled and triethylamine (2 ml) was added. Concentration and co-concentration twice with toluene left a residue, which was dissolved in pyridine (100 ml). The solution was cooled in ice while benzoyl chloride (32 ml) was added dropwise. The mixture was stirred overnight at room temperature, then water (5 ml) was added, and stirring was continued for 30 min. The mixture was partitioned between dichloromethane and water, the organic layer was washed with water, 1 M aqueous sulfuric acid, aqueous sodium hydrogen carbonate, and water. Drying and concentration left a residue, which was purified by chromatography. The first material to be eluted was a compound (5.2 g, 20%) which, according to its ^{13}C-NMR spectrum, was the 4',6'-isomer of **3**. NMR data: ^{13}C, δ 11.5 (SCH_3), 19.0, 28.5 (CH_3), 61.6, 62.5 (C-6, C-6'), 83.0 (C-1), 98.9 (acetal C), 101.2 (C-1'). The second compound eluted was pure **3** (19.5 g, 78%). Crystallization from ethanol gave material with m.p. 171-172°C, $[\alpha]_D$ +49°. Analytical data: calculated for $C_{51}H_{48}O_{15}S$: C, 65.7; H, 5.2; S, 3.4. Found: C, 65.5; H, 5.1; S, 3.1 NMR data: ^{13}C, δ 11.6 (SCH_3), 26.1, 27.4 (CH_3), 62.8, 62.9 (C-6, C-6'), 83.3 (C-1), 100.0 (C-1'), 110.9 (acetal C).

*2-(p-Nitrophenyl)ethyl 4-O-(2,6-di-O-benzoyl-3,4-O-isopropylidene-β-D-galactopyranosyl)-2,3,6-tri-O-benzoyl-β-D-glucopyranoside (**4**)*

Methyl triflate (0.59 ml) was added, at 50°C, to a stirred mixture of **3** (1.0 g), 2-(*p*-nitrophenyl)ethanol (0.27 g), molecular sieves (0.7 g), and toluene (15 ml). After 3 h at

50°C, triethylamine (0.3 ml) was added, and stirring was continued for 30 min at room temperature. The mixture was then filtered, concentrated, and the residue was purified by column chromatography to give pure **4** (1.04 g, 92%). Crystallization from ethanol gave material with m.p. 186-187°C, $[\alpha]_D$ +43°. Analytical data: calculated for $C_{58}H_{53}O_{18}N$: C, 66.2; H, 5.1; N, 1.3. Found: C, 65.9; H, 5.0; N, 1.3. NMR data: ^{13}C, δ 26.1, 27.4 (CH_3), 35.5 (Ph**C**H_2CH_2), 62.4, 62.8 (C-6, C-6'), 100.2, 100.9 (C-1, (C-1'), 110.9 (acetal C).

*2-(p-Nitrophenyl)ethyl-4-O-(2,6-di-O-benzoyl-β-D-galactopyranosyl)-2,3,6-tri-O-benzoyl-β-D-glucopyranoside (**5**)*

A solution of **4** (1.0 g) in 80% aqueous acetic acid (30 ml) was heated to 80°C until no starting material remained (3 h). The solution was then cooled and partitioned between ice-cold water and dichloromethane. The organic layer was washed with aqueous sodium hydrogen carbonate and water, dried, and concentrated. The residue was pure **5** (0.94 g, 97%). Crystallization from ethanol-water gave material with m.p. 171-172°C, $[\alpha]_D$ +44°. Analytical data: calculated for $C_{55}H_{49}O_{18}N$: C, 65.3; H, 4.9; N, 1.4. Found: C, 64.8; H, 4.8; N, 1.3. NMR data: ^{13}C, 35.5 (Ph**C**H_2CH_2), 62.1, 62.5 (C-6, C-6'), 100.7, 101.1 (C-1, C-1'); ^1H, δ 2.95 (d, $J_{4',OH}$ 5.8 Hz, OH-4'), 3.24 (d, $J_{3',OH}$ 8.2 Hz, OH-3'), 3.67 (m, H-3'), 3.79 (m, H-4'), 4.58 (d, $J_{1,2}$ 7.9 Hz, H-1), 4.61 ($J_{1',2'}$ 7.9 Hz, H-1'), 5.27 (dd, $J_{2',3'}$ 9.8 Hz, H-2'), 5.38 (dd, $J_{2,3}$ 10.1 Hz, H-2), 5.62 (dd, $J_{3,4}$ 8.9 Hz, H-3).

*2-(p-Nitrophenyl)ethyl O-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-(1-3)-O-(4,6-O-benzylidene-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1-3)-O-(2,6-di-O-benzoyl-β-D-galactopyranosyl)-(1-4)-2,3,6-tri-O-benzoyl-β-D-glucopyranoside (**6**)*

A mixture of **2** [4] (200 mg), **5** (236 mg), molecular sieves (700 mg) and toluene (6 ml) was stirred at 50°C while methyl triflate (128 µl) was added. Stirring was continued at 50°C for 2 h, then triethylamine (150 µl) was added and the mixture was stirred an additional 30 min at room temperature. Filtration and concentration left a residue, which was purified by column chromatography to give pure **6** (282 mg, 67%), $[\alpha]_D$ +8°. NMR data: ^{13}C, δ 16.4 (C-6'''), 35.5 (Ph**C**H_2CH_2), 55.3 (C-2''), 62.2, 62.5 (C-6, C-6'), 99.5, 99.7, 100.6, 100.7, 101.1 (C-1, C-1', C-1'', C-1''', Ph**C**H); ^1H, δ 0.83 (d, $J_{5,6}$ 6.4 Hz, H-6), 3.39 (d, OH-4'), 3.58 (m, H-4'), 3.72 (dd, $J_{3',4'}$ 3.4 Hz, H-3'), 4.45 (d, $J_{1',2'}$ 8.0 Hz, H-1'), 4.48 (d, $J_{1'',2''}$ 8.5 Hz, H-1''), 4.49 (d, $J_{1,2}$ 7.9 Hz, H-1), 4.65 (d, $J_{1''',2'''}$ 2.7 Hz, H-1'''). 5.26 (dd, $J_{2',3'}$ 9.9 Hz, H-2'), 5.31 (dd, $J_{2,3}$ 9.8 Hz, H-2), 5.48 (s, PhCH), 5.55 (dd, $J_{3,4}$ 8.9 Hz, H-3).

*2-(p-Nitrophenyl)ethyl O-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-(1-3)-O-(2-acetamido-4,6-di-O-acetyl-2-deoxy-β-D-glucopyranosyl)-(1-3)-O-(2,4,6-tri-O-acetyl-β-D-galactopyranosyl)-(1-4)-2,3,6-tri-O-acetyl-β-D-glucopyranoside (**7**)*

A solution of **6** (160 mg) in 80% aqueous acetic acid (12 ml) was heated to 80°C until no starting material remained (2 h), the solution was then concentrated and the residue co-concentrated twice with ethanol. Aqueous ethanol (90%, 16 ml) and hydrazine hydrate (0.73 ml) were added, and the solution was refluxed overnight, then concentrated to dryness and mixed with pyridine (8 ml) and acetic anhydride (8 ml). After standing overnight at room temperature, the mixture was concentrated and the residue was purified by column chromatography. Pure **7** (97 mg, 76%) was obtained, $[\alpha]_D$ -15°. NMR-data: ^{13}C, δ 16.4 (C-6'''), 23.1 (**C**H_3CONH), 35.8 (Ph**C**H_2CH_2), 57.4 (C-2''), 61.7, 61.8, 62.0 (C-6, C-6', C-6''),

100.2, 100.4, 100.7, 100.9 (C-1, C-1',C-1'', C-1'''); ^{1}H, δ 1.04 (d, J 6.4 Hz, H-6'''), 3.70 (dd, $J_{2',3'}$ 10.4, $J_{3',4'}$ 3.3 Hz, H-3'), 4.31 (d, $J_{1',2'}$ 7.8 Hz, H-1'), 4.42 (d, $J_{1,2}$ 7.5 Hz, H-1), 4.80 (d, $J_{1''',2'''}$, 3.4 Hz, H-1'''), 4.84 (dd, $J_{2,3}$ 10.0 Hz, H-2), 4.88 (d, $J_{1'',2''}$ 8.2 Hz, H-1''), 4.93 (dd, H-2'), 5.10 (dd, $J_{3,4}$ 8.3 Hz, H-3), 5.30 (dd, $J_{4,5}$ 1.2 Hz, H-4'), 5. 62 (d, J 6.7 Hz, NH).

*2-(p-Trifluoroacetamidophenyl)ethyl O-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-(1-3)-O-(2-acetamido-4,6-di-O-acetyl-2-deoxy-β-D-glucopyranosyl)-(1-3)-O-(2,4,6-tri-O-acetyl-β-D-galactopyranosyl)-(1-4)-2,3,6-tri-O-acetyl-β-D-glucopyranoside (**8**)*

A solution of **7** (70 mg) in 5 ml tetrahydrofuran/water, 9/1 by vol, was treated with aluminium amalgam [7] (50 mg) at room temperature for 3 h, then the mixture was filtered through Celite and concentrated. The residue was dissolved in pyridine (2 ml), cooled in ice and stirred, while trifluoroacetic anhydride (78 μl) was added. Stirring was continued overnight at room temperature and the mixture was then partitioned between dichloromethane and aqueous sodium hydrogen carbonate. The organic layer was washed with water, dried, and concentrated. Purification by column chromatography gave **8** (52 mg, 72%), $[\alpha]_D$ -15°. NMR data: ^{13}C, δ 100.2, 100.5, 100.7, 100.9 (C-1, C-1', C-1'',C-1'''), 116.5 (q, CO**C**F$_3$), 154.6 (q, **C**OCF$_3$).

*2-(p-Trifluoroacetamidophenyl)ethyl O-(α-L-fucopyranosyl)-(1-3)-O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1-3)-O-(β-D-galactopyranosyl)-(1-4)-β-D-glucopyranoside (**9**)*

A solution of **8** (30 mg) in methanol (1 ml) was treated with methanolic sodium methoxide (0.5 M, 0.1 ml) until TLC indicated no further reaction, then the mixture was neutralized with acetic acid, concentrated, and taken up in aqueous ethanol (80%, 10 ml). The solution was hydrogenated over Pd/C (50 mg) at 400 kPa overnight. The residue was purified on a column of Bio-Gel P-2 (Bio-Rad, Richmond, CA, USA), using water as eluant, to give **9** (11 mg, 62%), $[\alpha]_D$ -34° (H_2O). NMR data (^2H_2O): ^{13}C, δ 16.0 (C-6''), 24.1 (**C**H$_3$CONH), 56.3 (C-2''), 60.9, 61.4, 61.8 (C-6, C-6', C-6''), 100.7, 102.8, 103.4, 103.8 (C-1, C-1', C-1'', C-1'''); ^{1}H, δ 1.16 (d, J 6.7 Hz, H-6'''), 2.02 (s, CH$_3$CONH), 2.98 (t, PhC**H**$_2$CH$_2$), 4.33 (q, H-5'''), 4.43 (d, $J_{1',2'}$ 7.6 Hz, H-1'), 4.49 (d, $J_{1,2}$ 7.9 Hz, H-1), 4.70 (d, $J_{1'',2''}$, 8.5 Hz, H-1''), 5.00 (d, $J_{1''',2'''}$ 4.3 Hz, H-1'''), 7.38, 7.40, 7.42, 7.44 (aromatic H). The FAB-MS spectrum of **9** showed an $(M+H)^+$ ion at m/z = 907. Methylation of **9** followed by hydrolysis with strong acid gave 2,3,6-tri-*O*-methylhexose, 2,4,6-tri-*O*-methylhexose, 4,6-di-*O*-methyl-2-*N*-methylacetamidohexose, and 2,3,4-tri-*O*-methyl-6-deoxyhexose, all identified as their alditol acetates by GLC-MS.

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References

1 Hakomori S, Nudelman E, Levery S, Kannagi R (1984) J Biol Chem 259:4672-80.
2 Hakomori S (1986) Chem Phys Lipids 42:203-33.

3 Fugedi P, Garegg PJ, Lönn H, Norberg T (1987) Glycoconjugate J 4:97-108.
4 Lönn H (1985) Carbohydr Res 139:105-13.
5 Leontein K, Nilsson M, Norberg T (1985) Carbohydr Res 144:231-40.
6 Buchanan JG, Chacon-Guertes ME, Edgar AR, Moorhouse SJ, Rawson DI, Wightman RH (1980) Tetrahedron Lett 21:1793-96.
7 Corey EJ, McCaully RJ, Sachdev HS (1970) J Am Chem Soc 92:2476-88.
8 Ogawa T (1987) Proc 9th Int Symp Glycoconjugates, eds. Montreuil J, Verbert A, Spik G, Fournet B, Secretariat, L. 25 (Plenary Lecture), Lille.
9 Garegg PJ, Norberg T (1979) Acta Chem Scand B33:116-17.
10 Garegg PJ, Konradsson P, Kvarnström I, Norberg T, Svensson SCT, Wigilius B (1985) Acta Chem Scand B39:569-77.