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Preliminary Communication

Synthesis of a Dimeric Lewis-x Hexasaccharide as a *p*-Trifluoroacetamidophenylethyl β-Glycoside

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Lewis-x type glycolipids, have been found in various types of human cancer cells [1]. They have trisaccharide repeating unit: -D-Galβ1-4-[L-Fucα1-3-]D-GlcNAcβ1-3. We now report the synthesis of the dimeric Lewis-x hexasaccharide **14**.

The synthesis was based on thioglycosides as building blocks. Thioglycosides are very useful in oligosaccharide synthesis [2], since they are stable under most reaction conditions and can be activated at the anomeric center by treatment with methyl triflate [3], dimethyl(methylthio)sulfonium triflate (DMTST) [4] or bromine [5, 6]. All these activation methods were used in the present synthesis.

The strategy was to synthesise the disaccharide **6**, which has a thioethyl group in the 1-position, a *p*-methoxybenzyl group in the 3-position and a chloroacetyl group in the 3'-position. The thioethyl group in **6** was converted into a *p*-nitrophenylethyl group and the chloroacetyl group was removed to give the disaccharide **8**, which was coupled with **6**, giving the linear tetrasaccharide **9**. Finally the *p*-methoxybenzyl groups in **9** were removed and the tetrasaccharide **10** was difucosylated, resulting in the hexasaccharide **11**. The following steps were performed:

Methyl 4,6-*O*-benzylidene-1-thio-β-D-galactopyranoside [7] was chloroacetylated in the 3-position selectively using chloroacetyl chloride (1.1 equiv.) and pyridine (5 equiv.) in dichloromethane at 0°C, giving compound **1** in 60% yield; R_F 0.76 in toluene/ethyl acetate, 3/1 by vol, $[\alpha]_{578}$ +87° (*c* 0.5, chloroform). ^{1}H-NMR: δ 4.98 ($J_{2,3}$ 9.8 Hz, $J_{3,4}$ 3.4 Hz, H-3) Acetylation of **1** using acetyl chloride (2 equiv.) and pyridine (5 equiv.) in dichloromethane at 0°C gave compound **2** in 89% yield; R_F 0.54 in toluene/ethyl acetate, 1/1 by vol, $[\alpha]_{578}$ +60° (*c* 0.5, chloroform). ^{1}H-NMR: δ 5.07 ($J_{2,3}$ 10 Hz, $J_{3,4}$ 3.7 Hz, H-3), 5.52 ($J_{1,2}$ 10 Hz, H-2). Treatment of **2** with bromine and tetraethylammonium bromide in dichloromethane at room temperature gave the α-bromide **3** in 84% yield; R_F 0.71 in toluene/ethyl acetate, 3/1 by vol. ^{13}C-NMR: δ 89.87 (C-1).

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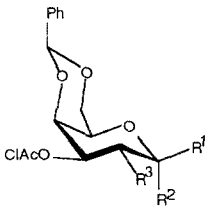
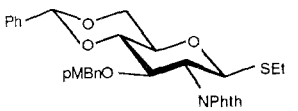


1 $R^1 = SCH_3$, $R^2 = H$, $R^3 = OH$
2 $R^1 = SCH_3$, $R^2 = H$, $R^3 = OAc$
3 $R^1 = H$, $R^2 = Br$, $R^3 = OAc$



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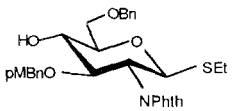

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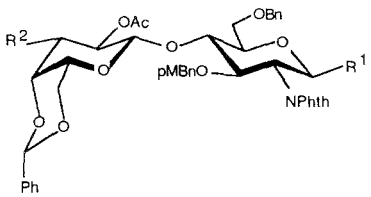
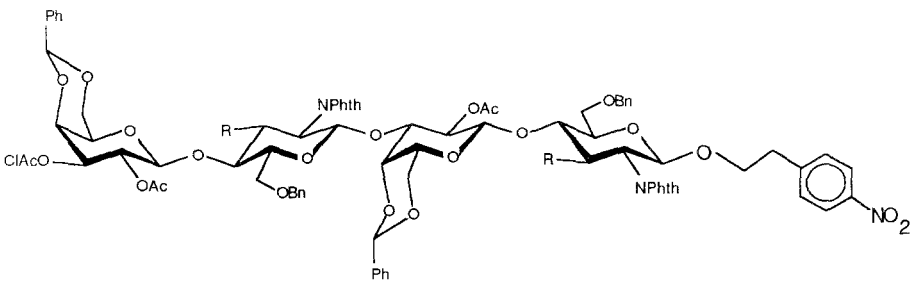
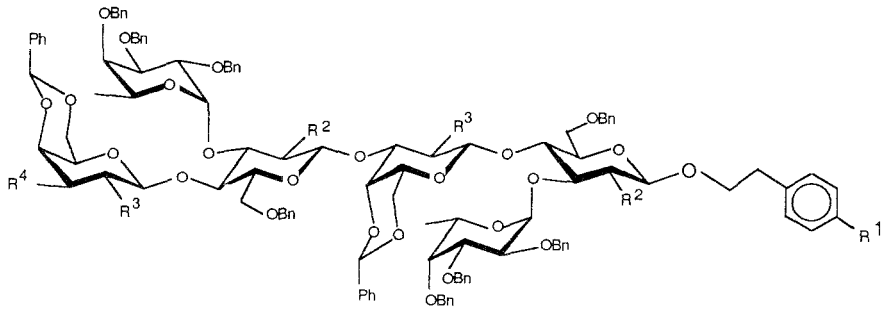


6 R^1 = SEt, R^2 = OClAc
7 $R^1 = O(CH_2)_2$-Ph-NO_2, R^2 = OClAc
8 $R^1 = O(CH_2)_2$-Ph-NO_2, R^2 = OH



9 R = OpMBn
10 R = OH

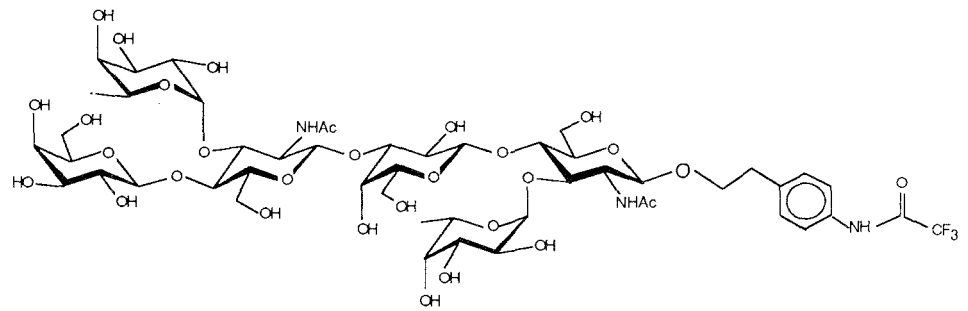
Ethyl 4,6-*O*-benzylidene-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside [8] was *p*-methoxybenzylated using *p*-methoxybenzyl chloride and sodium hydride in dimethylformamide, giving compound **4** in 76% yield; R_F 0.67 in toluene/ethyl acetate, 3/1 by vol, $[\alpha]_{578}$ +59° (*c* 0.5, chloroform). The 4,6-benzylidene acetal in **4** was opened by treatment with sodium cyanoborohydride and HCl-saturated diethylether in tetrahydrofuran at room temperature, giving the 4-OH compound **5** in 70% yield; R_F 0.38 in toluene/ethyl acetate, 3/1 by vol, $[\alpha]_{578}$ +39° (*c* 1.0, chloroform).



11 $R^1 = NO_2$, R^2 = NPhth, R^3 = OAc, R^4 = OClAc

12 $R^1 = NO_2$, R^2 = NHAc, $R^3 = R^4$ = OH

13 $R^1 = NHCOCF_3$, R^2 = NHAc, $R^3 = R^4$ = OH



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The bromide **3** was coupled with the glycosyl acceptor **5** in the presence of silver triflate and 2,6-di-*tert*-butyl-4-methylpyridine (DTBMP) in dichloromethane at -40°C, giving the disaccharide **6** in 72% yield; R_F 0.36 in toluene/ethyl acetate, 3/1 by vol, $[\alpha]_{578}$ +44° (*c* 0.5, chloroform). ^{13}C-NMR: δ 81.05 (C-1), 100.15 (C-1'). The thioethyl group in **6** was converted into a *p*-nitrophenylethyl group by treatment with *p*-nitrophenethyl alcohol using methyl triflate as glycosidation promotor (DTBMP was used as acid acceptor), giving a 70% yield of **7**; R_F 0.22 in toluene/ethyl acetate, 3/1 by vol, $[\alpha]_{578}$ +14° (*c* 0.5, chloroform). ^{13}C-NMR: δ 98.20 (C-1), 100.20 (C-1'). Treatment of **7** with hydrazine acetate in ethyl acetate/methanol, 1/1 by vol, at room temperature removed the chloroacetyl group, giving the 3'-OH compound **8** in 83% yield; R_F 0.33 in toluene/ethyl acetate, 1/1 by vol.

DMTST promoted glycosidation of **6** with **8**, using DTBMP as acid acceptor, was carried out at room temperature in dichloromethane, to give the tetrasaccharide **9** in 63% yield; R_F 0.43 in toluene/ethyl acetate, 1/1 by vol, $[\alpha]_{578}$ -16° (*c* 0.5, chloroform). ^{13}C-NMR: δ 98.14 (C-1), 99.14 (C-1''), 100.44, 100.58 (C-1' and C-1'''). The two *p*-methoxybenzyl groups in **9** were removed by treatment with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) in water-dichloromethane at room temperature, giving the diol **10** in 82% yield. Compound **10** was difucosylated with 2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl bromide [8] in the presence of silver triflate-collidine in dichlormethane at -25°C, resulting in the hexasaccharide **11**

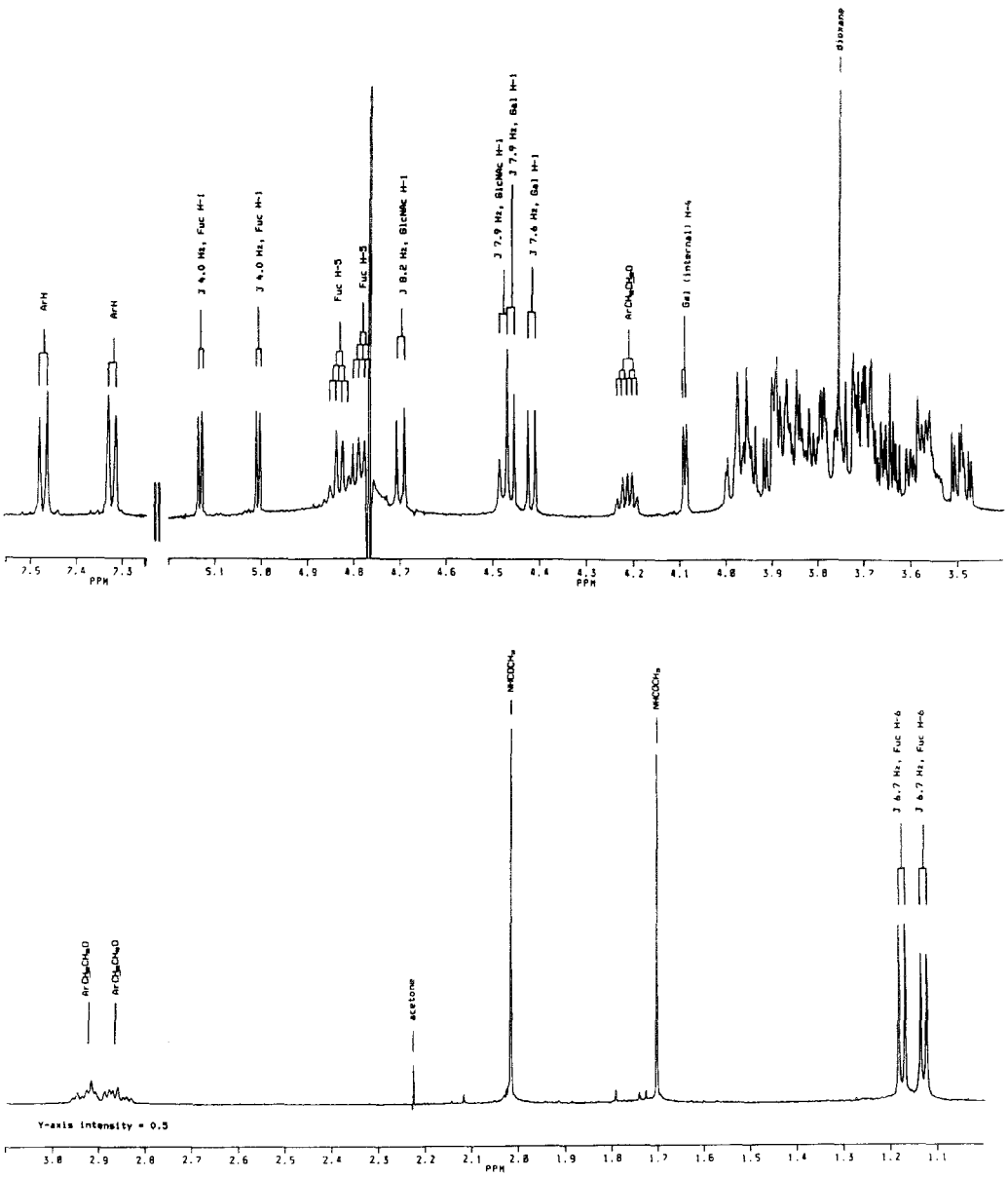

Figure 1. 500 MHz ^{1}H-NMR spectrum of **14** in ^2H_2O at 25°C. The chemical shifts are relative to internal acetone δ = 2.225.

in 70% yield; R_F 0.73 in toluene/ethyl acetate, 1/2 by vol, $[\alpha]_{578}$ -91° (*c* 0.5, chloroform). ^{13}C-NMR: δ 97.49 and 97.67 (2 C-1, Fuc), 98.10, 98.89, 99.58, 99.61 (C-1, C-1', C-1'' and C-1''').

The phthalimido groups in **11** were removed by treatment with hydrazine acetate in boiling ethanol, giving free amino groups which were *N*-acetylated by acetic anhydride in dichloromethane/methanol, 1/1 by vol, giving compound **12** in 62% yield; R_F 0.52 in toluene/ethyl acetate/methanol, 6/20/3 by vol. Reduction of the nitro group by treatment with aluminium amalgam in tetrahydrofuran/water, 9/1 by vol, followed by trifluoroacetylation with trifluoroacetic anhydride and de-*O*-trifluoroacetylation with sodium methoxide in methanol gave compound **13** in 55% yield; R_F 0.58 in toluene/ethyl acetate/methanol, 6/20/3 by vol. Finally, compound **13** was hydrogenated over Pd/C (10%) in a mixture of ethyl acetate/ethanol/water, 12/3/2 by vol, giving the deprotected hexasaccharide **14** in 96% yield; R_F 0.72 in ethyl acetate/acetic acid/methanol/water, 4/3/3/2 by vol, $[\alpha]_{578}$ -67° (*c* 0.5, water). The positive ion Fast Atom Bombardment-MS of **14** showed an M+H ion at m/z 1256. The ^{1}H-NMR of **14** is shown in Fig. 1. Full experimental details for the preparation of **14** and other related oligosaccharides including elemental analysis data will be published in the near future.

References

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