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Synthesis of 5-Thio-L-fucose-containing Blood Group Antigens H-type 2 and Lewis X (Le^x)

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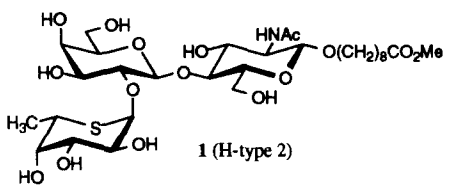
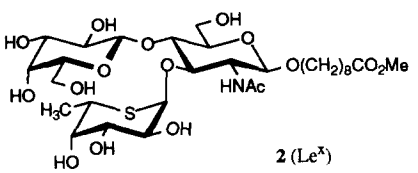
Abstract: Two blood group antigens, H-type 2 and Lewis X trisaccharides containing 5-thio-L-fucose instead of L-fucose, were synthesized. 2-Azido-2-deoxy-lactose derivative **11** was used as the common disaccharide intermediate. 5-Thio-L-fucosylation of the 2'-OH and 3-OH groups of 1,6-anhydro-2-azido-2-deoxy-lactose derivative by a trichloroacetimidate method gave α-linked trisaccharides stereoselectively.

Interest in cell-surface oligosaccharides is growing because of the important roles these compounds play in a number of biological phenomena such as cell adhesion.[1] Since 5-thio-L-fucose, which contains a sulfur atom instead of the ring oxygen, was found to be a potent competitive inhibitor of bovine α-L-fucosidases,[2] we have been interested in various 5-thio-L-fucose-containing oligosaccharide mimics. Glycosylation with 2-*O*-acetyl-5-thioaldopyranosyl trichloroacetimidates gave axial glycoside[3,4] predominantly. Therefore, 5-thio-α-L-fucoside can be easily constructed. We synthesized[4] several disaccharides having 5-thio-α-L-fucosyl residue at the non-reducing end using the peracetylated 5-thio-L-fucopyranosyl trichloroacetimidate. Now we describe here synthesis of two 5-thio-L-fucose-containing blood group antigens related to H-type 2 and Lewis X (Le^x).



1 (H-type 2)

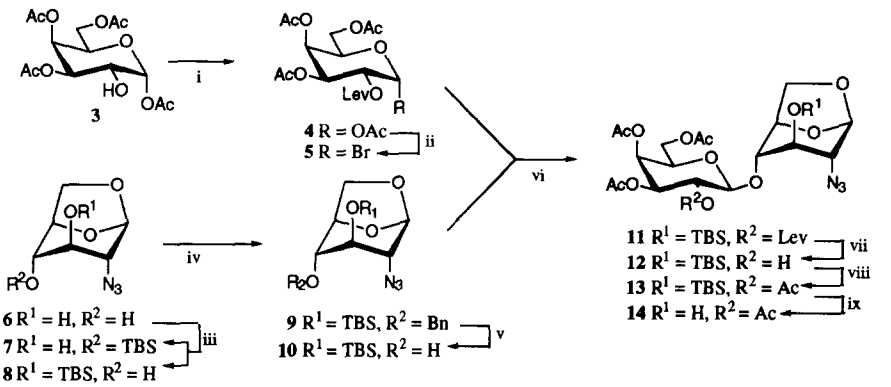


2 (Le^x)

Figure 1. Two target neotrisaccharides

Our designed common intermediate of the *N*-acetyllactosamine moiety is 2-azido-2-deoxylactose derivative **11**, whose 3-OH and 2'-OH groups are protected with *tert*-butyldimethylsilyl (TBS) and levulinoyl (Lev) groups, respectively. These two protective groups can be removed in the presence of an acetyl group, and the levulinoyl group has the ability to form a β-galactosidic bond by anchimeric assistance. 1,3,4,6-Tetra-*O*-acetyl-α-D-galactopyranose **3** was levulinoylated[5] with levulinic acid and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) and then converted into glycosyl bromide **5** by treatment with HBr–acetic acid. The 2-azido-2-deoxy-glucosyl acceptor was synthesized from 1,6-anhydro-2-azido-2-deoxy-β-D-glucopyranose **6** derived from D-glucal.[6] Compound **6** was treated with TBSCl and imidazole to give 4-*O*-TBS derivative **7** and 3-*O*-TBS derivative **8** in 9:1 ratio in 89% yield. These two isomers could be separated by

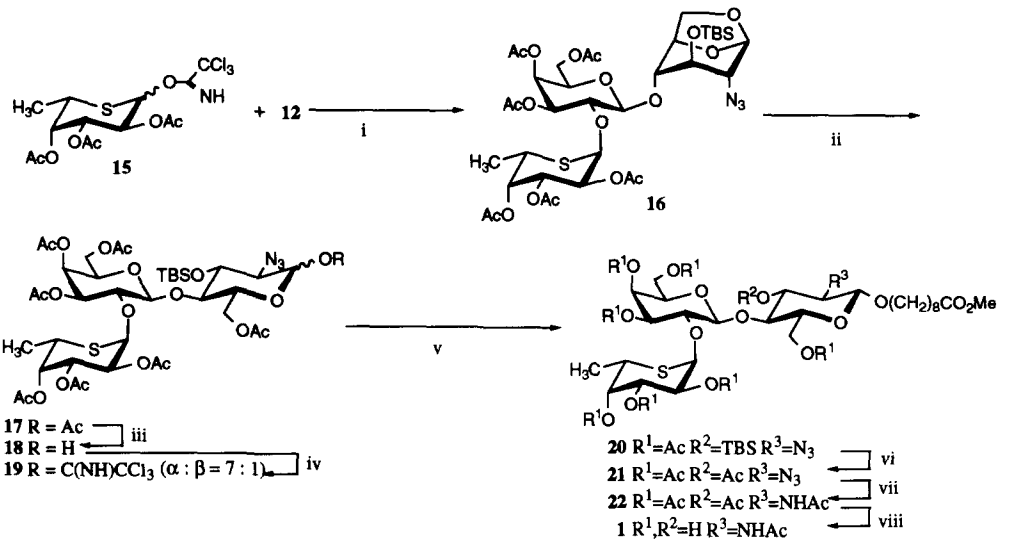
column chromatography on silica gel, but benzylation of the mixture with benzyl bromide and BaO, $Ba(OH)_2$ gave 1,6-anhydro-2-azido-4-*O*-benzyl-3-*O*-*tert*-butyldimethylsilyl-2-deoxy-β-D-glucopyranose **9**[7] as the sole product in 92% yield. Highly efficient debenzylation of **9** with $TiCl_4$[8] gave an acceptor **10** in 91% yield. Glycosylation of **10** with galactosyl bromide **5** in the presence of $AgClO_4$ and Ag_2CO_3 gave the desired disaccharide **11** in 60% yield along with a byproduct of orthoester in 33% yield. It is confirmed by ^1H NMR data of **11** ($J_{1',2'}$ 7.9 Hz) that the orientation of the newly formed glycosidic bond is β.



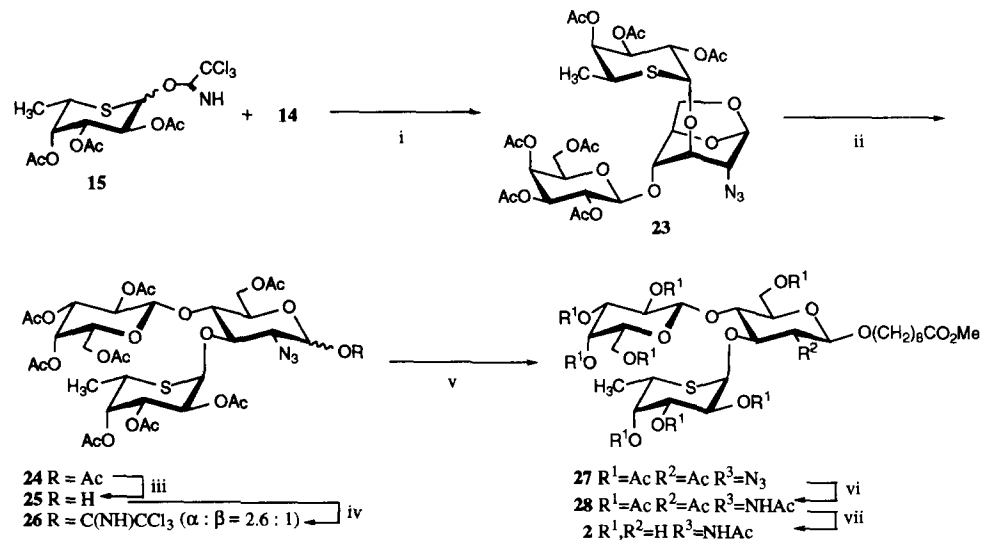
Scheme 1. i) LevOH, EDC, CH_2Cl_2, quant. ii) HBr–AcOH, CH_2Cl_2. iii) TBSCl, imidazole, DMF, 89%. iv) BnBr, $Ba(OH)_2 \cdot 8H_2O$, BaO, DMF, 92%. v) $TiCl_4$, CH_2Cl_2, 91%. vi) $AgClO_4$, Ag_2CO_3, CH_2Cl_2, 60%. vii) $H_2NNH_2 \cdot AcOH$, EtOH, 88%. viii) Ac_2O, Pyridine, 93%. ix) TBAF, THF, quant.

The levulinoyl group of **11** was removed with $H_2NNH_2 \cdot AcOH$ to give 2'-OH derivative **12** (88%), which was used for the synthesis of the H-type 2 analog **1**. Furthermore, **12** was converted to 3-OH derivative **14** via acetylation and removal of the TBS group with tetra-n-butylammonium fluoride (TBAF) and used for the synthesis of the Le^x analog **2**. 5-Thio-L-fucosylation was carried out as communicated previously[4] with the 2,3,4-tri-*O*-acetyl-5-thio-L-fucopyranosyl trichloroacetimidate **15** in the presence of $BF_3 \cdot OEt_2$ as a catalyst at -20°C. Reaction of **15** with the 2'-OH derivative **12** gave exclusively 5-thio-α-L-fucosyl trisaccharide **16**[9] in 75% yield. The 1,6-anhydro ring of the trisaccharide **16** was acetolysed with Ac_2O and TFA to give **17** quantitatively. Then the anomeric acetyl group was removed and converted into trichloroacetimidate **18** with trichloroacetonitrile and DBU. The α-imidate was separated to construct β-glycoside. Glycosylation of 8-methoxycarbonyloctanol with **18α** in the presence of $BF_3 \cdot OEt_2$ gave the spacer-linked trisaccharide **20** in 91% yield. Its TBS group was removed with TBAF, and acetylated, then the azido group was reduced with H_2S and *N*-acetylated. Finally, de-*O*-acetylation with sodium methoxide gave the target H-type 2 trisaccharide **1**[10] in 37% yield from **20**.

5-Thio-L-fucosylation of the 3-OH derivative **14** under the same conditions as for **12** gave 5-thio-α-L-fucoside **23**[11] in 54% yield. The trisaccharide **23** was converted into the glycosyl imidate **26** by the same procedure as for conversion of **16** into **18**. Glycosidation with 8-methoxycarbonyloctanol with **26α** in the presence of $BF_3 \cdot OEt_2$ gave the spacer-linked trisaccharide **27** in 64% yield. Then the azido group was reduced with H_2S and *N*-acetylated and finally de-*O*-acetylated with sodium methoxide to give the target Le^x trisaccharide **2**[12] in 76% yield from the protected trisaccharide **27**.



Scheme 2. i) BF_3•OEt_2, MS4A, CH_2Cl_2, -20°C, 75%. ii) Ac_2O, TFA, quant. iii) H_2NNH_2•AcOH, DMF, 86%. iv) CCl_3CN, DBU, CH_2Cl_2, 93%. v) $HO(CH_2)_8CO_2Me$, BF_3•OEt_2, MS4A, CH_2Cl_2, -20°C, 91%. vi) TBAF, THF then Ac_2O, pyridine, 69%. vii) H_2S, pyridine, H_2O then Ac_2O, pyridine, 95%. viii) NaOMe, MeOH, 62%



Scheme 3. i) BF_3•OEt_2, MS4A, CH_2Cl_2, -20°C, 54%. ii) Ac_2O, TFA, 94%. iii) H_2NNH_2•AcOH, DMF, 83%. iv) CCl_3CN, DBU, CH_2Cl_2, 76%. v) $HO(CH_2)_8CO_2Me$, BF_3•OEt_2, MS4A, CH_2Cl_2, -20°C, 64%. vi) H_2S, pyridine, H_2O then Ac_2O, pyridine, 97%. vii) NaOMe, MeOH, 78%

The H-type 2 trisaccharide analog **1** showed strong inhibitory activity[13] against hemagglutination reactions with *Ulex europaeus* lectin I (UEA-I) and the H-type 2 trisaccharide-specific monoclonal antibody (anti-H MoAb). These biological activities of 5-thio-L-fucose-containing trisaccharides will be reported in detail elsewhere.

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9. ^{1}H NMR data for **16** (270 MHz, $CDCl_3$) δ 5.45–5.41 (m, 3 H, H-1, 3", 4"), 5.32 (bd, 1 H, H-4'), 5.26 (dd, 1 H, H-2"), 5.21 (d, 1 H, $J_{1",2"}$ 2.6 Hz, H-1"), 5.05 (dd, 1 H, $J_{3',4'}$ 3.3 Hz, H-3'), 4.67 (bd, 1 H, $J_{5,6b}$ 6.3 Hz, H-5), 4.57 (d, 1 H, $J_{1',2'}$ 7.9 Hz, H-1'), 4.29 (dd, 1 H, $J_{2',3'}$ 9.9 Hz, H-2'), 4.16–4.12 (m, 3 H, H-6a, 6'a, 6'b), 4.10–4.00 (m, 2 H, H-3, 5"), 3.90 (dt, 1 H, $J_{5',6'a} = J_{5',6'b}$ 6.6 Hz, H-5'), 3.81 (t, 1 H, $J_{6a,6b}$ 6.9 Hz, H-6b), 3.54 (s, 1 H, H-4), 3.18 (s, 1 H, H-2), 2.171, 2.166, 2.04, 2.00, 1.99, 1.98 (each s, 3 H × 6, Ac × 6), 1.14 (d, 1 H, $J_{5",6"}$ 6.9 Hz, H-6"), 0.91 (s, 9 H, tBu), 0.12 (s, 6 H, $SiMe_2$).
10. ^{1}H NMR data for **1** (400 MHz, D_2O) δ 5.21 (d, 1 H, $J_{1",2"}$ 3.2 Hz, H-1"), 4.57 (d, 1 H, $J_{1',2'}$ 6.4 Hz, H-1'), 4.55 (d, 1 H, $J_{1,2}$ 8.4 Hz, H-1), 4.10 (bs, 1 H, H-4"), 4.06 (m, 1 H, H-6a), 4.05 (dd, 1 H, $J_{2",3"}$ 10.2 Hz, H-2"), 4.00–3.92 (m, 4 H, H-2', 3', 4', CH_2O), 3.87 (dd, 1 H, $J_{5,6b}$ 6.3, $J_{6a,6b}$ 12.1 Hz, H-6b), 3.84 (dd, 1 H, $J_{3",4"}$ 2.8 Hz, H-3"), 3.90–3.73 (m, 5 H, H-2, 4, 5', 6'a, 6'b), 3.74, 3.40 (each s, 3 H, MeO), 3.71 (dd, 1 H, $J_{2,3}$ 8.7, $J_{3,4}$ 10.2 Hz, H-3), 3.67–3.60 (m, 1 H, CH_2O), 3.56–3.53 (m, 1 H, H-5), 3.46 (q, 1 H, $J_{5",6"}$ 7.2 Hz, H-5"), 2.44, 2.22 (each t, 2 H, *J* 7.3 Hz, CH_2CO), 2.09 (s, 3 H, Ac), 1.67–1.35, (m, 12 H, $(CH_2)_6$), 1.27, (d, 1 H, H-6").
11. ^{1}H NMR data for **23** (270 MHz, $CDCl_3$) δ 5.50 (bs, 1 H, H-4"), 5.45 (s, 1 H, H-1), 5.41 (bd, 1 H, $J_{3',4'}$ 3.6 Hz, H-4'), 5.30 (m, 2 H, H-2", 3"), 5.23 (dd, 1 H, $J_{2',3'}$ 10.5 Hz, H-2'), 5.05 (dd, 1 H, H-3'), 5.01 (bs, 1 H, H-1"), 4.72 (d, 1 H, $J_{1',2'}$ 7.9 Hz, H-1'), 4.61 (bd, 1 H, $J_{5,6b}$ 5.6 Hz, H-5), 4.20–4.16 (m, 3 H, H-3, 6'a, 6'b), 4.08, (d, 1 H, $J_{6a,6b}$ 6.9 Hz, H-6a), 3.94 (dt, 1 H, $J_{5',6'a} = J_{5',6'b}$ 6.6 Hz, H-5'), 3.82 (bs, 1 H, H-4), 3.77 (dd, 1 H, H-6b), 3.54 (q, 1 H, $J_{5",6"}$ 7.3 Hz, H-5"), 3.05 (s, 1 H, H-2), 2.18, 2.07, 2.00 (each s, 6 H, 9 H, 6 H, Ac × 7), 1.19 (d, 3 H, H-6").
12. ^{1}H NMR data for **2** (400 MHz, D_2O) δ 4.95 (d, 1 H, $J_{1",2"}$ 2.9 Hz, H-1"), 4.58 (d, 1 H, $J_{1,2}$ 8.7 Hz, H-1), 4.47 (d, 1 H, $J_{1',2'}$ 7.9 Hz, H-1'), 4.23 (t, 1 H, $J_{2,3} = J_{3,4}$ 9.5 Hz, H-3), 4.05 (bs, 1 H, H-4"), 4.04 (m, 1 H, H-6a), 4.02 (dd, 1 H, $J_{2",3"}$ 10.2, $J_{3",4"}$ 2.6 Hz, H-3"), 3.97–3.88 (m, 7 H, H-2, 4, 6b, 4', 2", 5", CH_2O), 3.79 (dd, 1 H, $J_{5',6'a}$ 7.6, $J_{6'a,6'b}$ 11.6 Hz, H-6'a), 3.74 (dd, 1 H, H-6'b), 3.72 (s, 3 H, MeO), 3.69 (dd, 1 H, $J_{3',4'}$ 3.5 Hz, H-3'), 3.65–3.59 (m, 3 H, H-5, 5', CH_2O), 3.49 (dd, 1 H, $J_{2',3'}$ 9.8 Hz, H-2'), 2.42, 2.20 (each t, 2 H, *J* 7.4 Hz, CH_2CO), 2.05 (s, 3 H, Ac), 1.64–1.30, (m, 12 H, $(CH_2)_6$), 1.19, (d, 1 H, $J_{5",6"}$ 7.2 Hz, H-6").
13. Minimum concentration needed for inhibition:

Inhibitors / Lectin or Antibody	UEA-I	anti-H MoAb
Fucα1→2Galβ1→4GlcNAcβO$(CH_2)_8CO_2Me$	0.313 mM	1.25 mM
5SFucα1→2Galβ1→4GlcNAcβO$(CH_2)_8CO_2Me$	1.25 mM	0.154 mM

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