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Synthesis of a Thio-Linked Analogue of Sialyl Lewis X

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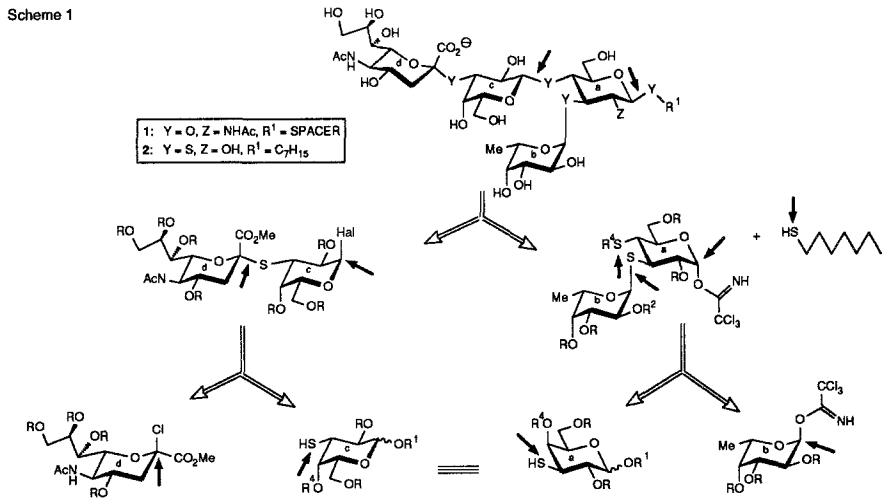
Abstract: Thiolinked sialyl Lewis X analogue **2** was obtained from neuraminic acid, D-galactose, and L-fucose. Galactose was transformed into 3-thiogalactose building block **6** and also into the required 3,4-dithioglucose moiety. Thioglycoside bond formation was performed via base-promoted S-glycosylation [Neu5Acα(2-3S)Gal and Galβ(1-4S)Glc linkages] and via acid catalyzed S-glycosylation [Fucα(1-3S)Glc and Glcβ-(1-1S)heptyl linkages].

The sialyl Lewis X (sLe^x) epitope Neu5Acα(2-3)Galβ(1-4)[Fucα-(2-3)]GlcNAc has become a prominent target because of its implication in inflammation through binding to selectins[1]. Several approaches to the synthesis of the basic structure (**1**, Scheme 1) have been investigated[2]; also a great variety of structural analogues for pharmacological studies have been prepared[3,4].

Scheme 1



To circumvent enzymatic hydrolysis in the course of in vivo experiments, thioglycosides have proven to be much more stable to glycosidase action than glycosides[5]. Therefore, we initiated a program to synthesize entirely sulfur connected sLe^x ligated to a heptylthio spacer (Scheme 1, **2**), thus to compare the conformation and the relative binding to selectins with the natural epitope[6]. Because replacement of the GlcNAc residue by a Glc residue is not essential for selectin binding[4] the synthesis of this thioanalogue is reported here.

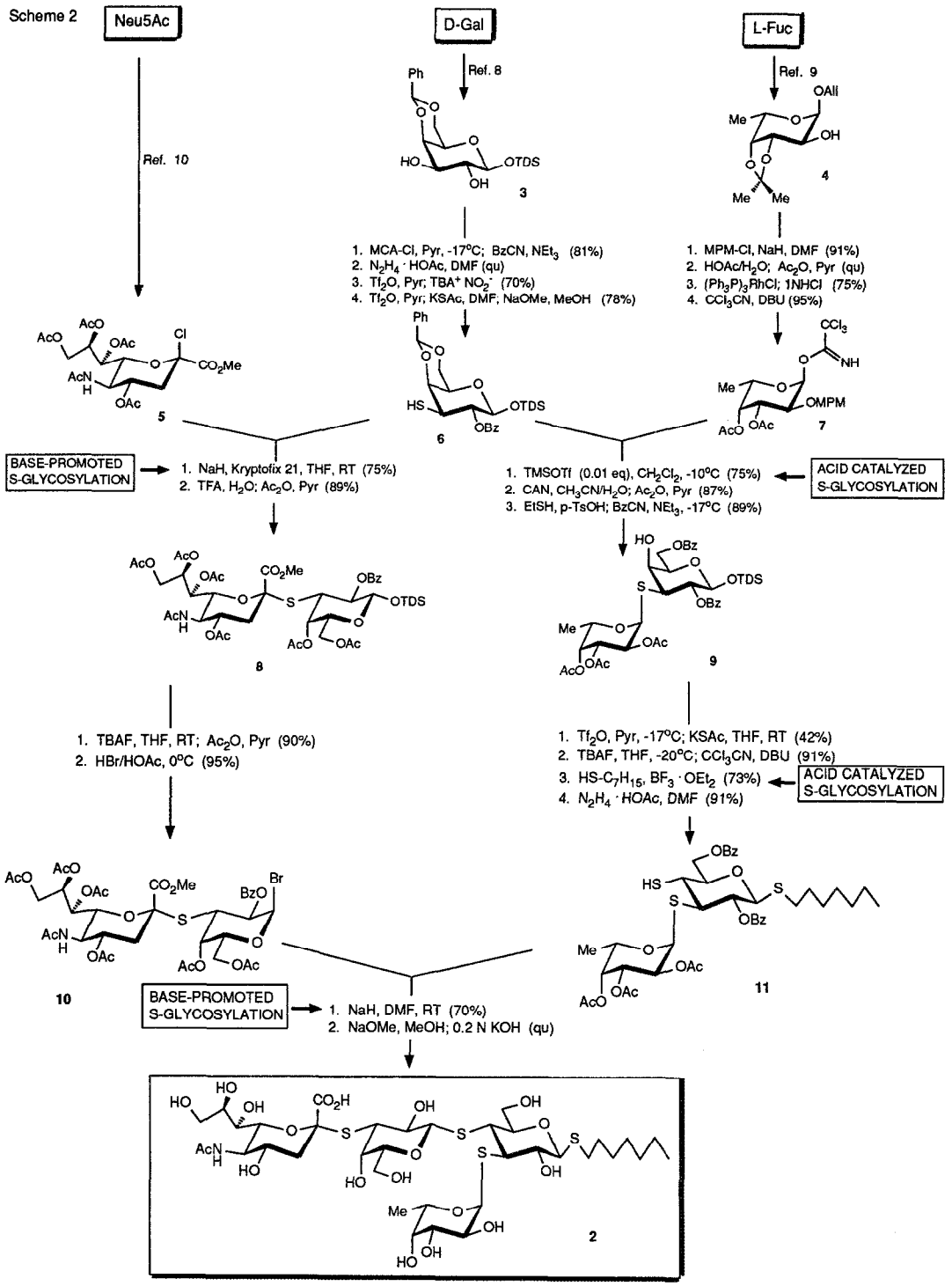
As previously shown, equatorial thioglycoside bond formation can be readily based on S_N2 displacement in halogenoses[5] in which the halogen atom generally adopts axial position. Yet, the question remains if firstly thiosugar formation and reaction with the halogenose ("base-promoted S-glycosylation") is superior to the most commonly applied procedure, encomprising first halogenose transformation into an anomeric thiol and then "anomeric S-alkylation" with an O-activated sugar. Because also ready access to axial thioglycosides is required, alternatively, thioglycoside bond formation with the help of O-glycosyl trichloroacetimidates (fucose residue and spacer attachment) was considered ("acid catalyzed S-glycosylation")[7]. Yet, a nonparticipating 2-O-benzyl group at the fucose residue, permitting α-glycoside formation, or any other O-benzyl protection requiring at a later stage hydrogenolytic O-debenzylation is not compatible with the presence of thio-linkages. Therefore, besides O-acyl protection cleavable under mild base conditions, acid labile protective groups (for instance, alkylidene) or protective groups cleavable with a mild oxidizing agent (for instance, *p*-methoxybenzyl = MPM) have to be employed. Also, the chances for vicinal thioglycoside formation at the glucose moiety (bilding block **a**) has to be explored because thioethers possessing a leaving group in β-position tend to β-elimination. This may be even more so for a sterically and stereoelectronically disfavored situation.

For the synthesis of target molecule **2** thiodisaccharides **ab** and **cd** were regarded as versatile intermediates because they contain an identical 3-thiogalactose building block (**a** = **c**, see retrosynthesis in Scheme 1). This building block can be prepared from D-galactose via known thexyldimethylsilyl (TDS) 4,6-O-benzylidene-galactopyranoside **3**[8] (Scheme 2). The desired regio- and stereoselective introduction of the thio group was performed via treatment with mono-chloroacetyl chloride (MCA-Cl) in pyridine at -17°C (→ 3-O-acylation), then with benzoyl cyanide in the presence of triethylamine (→ 2-O-benzoylation), and removal of the MCA group with hydrazinium acetate; the obtained 3-O-unprotected intermediate was treated with trifluoromethanesulfonic anhydride (Tf_2O) in the presence of pyridine and then with tetrabutylammonium nitrite to give clean inversion of the 3-hydroxy group, thus providing the corresponding *gulo* derivative; ensuing activation with Tf_2O in pyridine, then reaction with potassium thioacetate and selective cleavage of the S-acetyl group with sodium methoxide in methanol afforded **6** in good overall yield.

Fucosyl donor **7** (building block **b**) was prepared from known **4**[9]; treatment with MPM-Cl in the presence of sodium hydride led to 2-O-MPM protection. Ensuing acid catalyzed removal of the isopropylidene group and then reaction with Ac_2O in pyridine furnished the 3,4-di-O-acetyl derivative. The allyl group was cleaved by treatment with Wilkinson's catalyst and then with acid. The 1-O-unprotected compound obtained was cleanly transformed with trichloroacetonitrile in the presence of DBU into **7**.

Then the reaction of 3-thiogalactose **6** with known halogenose of N-acetyl neuraminic acid (Neu5Ac)[10] was investigated. Various approaches (including transformation into the anomeric thiol[11] and anomeric S-alkylation with the activated *gulo* derivative) exhibited that "base promoted S-glycosylation" in the presence of Kryptofix 21 in THF afforded by far the best results in thioglycoside bond formation. Acid catalyzed removal of the O-benzylidene group and ensuing O-acetylation led to the desired thiodisaccharide **8** in high yield.

α-Selective S-glycosylation of **6** with donor **7** was readily achieved with TMSOTf as catalyst at -10°C. Oxidative removal of the MPM group with ceric ammonium nitrate (CAN) and then O-acetylation furnished a fully O-acetyl protected fucose moiety. The O-benzylidene group could be cleaved by treatment with *p*-toluenesulfonic acid (*p*-TsOH) in the presence of ethylmercaptan; regioselective 6-O-benzoylation with benzoyl cyanide/triethylamine gave the desired thiodisaccharide **9** in high overall yield.


Scheme 2
Neu5Ac
D-Gal
L-Fuc
Ref. 10
Ref. 8
Ref. 9
1. MCA-Cl, Pyr, -17°C; BzCN, NEt3 (81%)
2. N2H4 · HOAc, DMF (qu)
3. Tf2O, Pyr; TBA+ NO2- (70%)
4. Tf2O, Pyr; KSAc, DMF; NaOMe, MeOH (78%)
1. MPM-Cl, NaH, DMF (91%)
2. HOAc/H2O; Ac2O, Pyr (qu)
3. (Ph3P)3RhCl; 1NHCl (75%)
4. Cl3CN, DBU (95%)
BASE-PROMOTED S-GLYCOSYLATION
1. NaH, Kryptofix 21, THF, RT (75%)
2. TFA, H2O; Ac2O, Pyr (89%)
1. TMSOTf (0.01 eq), CH2Cl2, -10°C (75%)
2. CAN, CH3CN/H2O; Ac2O, Pyr (87%)
3. EtSH, p-TsOH; BzCN, NEt3, -17°C (89%)
ACID CATALYZED S-GLYCOSYLATION
1. TBAF, THF, RT; Ac2O, Pyr (90%)
2. HBr/HOAc, 0°C (95%)
1. Tf2O, Pyr, -17°C; KSAc, THF, RT (42%)
2. TBAF, THF, -20°C; Cl3CN, DBU (91%)
3. HS-C7H15, BF3 · OEt2 (73%)
4. N2H4 · HOAc, DMF (91%)
ACID CATALYZED S-GLYCOSYLATION
BASE-PROMOTED S-GLYCOSYLATION
1. NaH, DMF, RT (70%)
2. NaOMe, MeOH; 0.2 N KOH (qu)
2

For the ligation of **8** and **9** the TDS group in **8** was removed with tetrabutylammonium fluoride (TBAF) in THF; treatment with Ac_2O in pyridine gave a fully O-acylated intermediate which was stable enough to give with hydrogen bromide in acetic acid glycosyl bromide **10** in high yield. The introduction of the second mercapto group into **9** proved to be the most difficult problem because any activation of the axial hydroxy group led mainly to β-elimination; finally trifluoromethanesulfonate formation at -17°C and immediate reaction with potassium thioacetate in THF at room temperature gave the desired 4-acetylthio-glucose derivative. For the attachment of the spacer the 1-O-TDS group was removed with TBAF; ensuing treatment with CCl_3CN/DBU provided the trichloroacetimidate in high yield. S-Glycosylation of n-heptylmercaptan as acceptor in the presence of $BF_3{\cdot}OEt_2$ as catalyst gave due to neighboring group participation exclusively the β-thioglycoside. Treatment of this material with hydrazinium acetate led to selective removal of the S-acetyl group, thus affording thiodisaccharide **11**. Base promoted S-glycosylation with glycosyl bromide **10** (with sodium hydride as base) in DMF as solvent furnished the fully O-acylated tetrasaccharide in 70% yield which upon treatment with NaOMe/MeOH and then with KOH and ion exchange resin (Amberlite IR 120, H^+ form) afforded target molecule **2**. The structural assignments of **2** and all intermediates could be readily based on the ^1H NMR data[12].

In conclusion, a combination of base-promoted and acid-catalyzed S-glycosylation furnished a complex thioglycoconjugate possessing α-linkages to neuraminic acid and fucose, β-linkages to galactose and glucose, and vicinal branching. Based on this methodology various thioglycoconjugates should be accessible.

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12. ^1H NMR data of **2** (600 MHz, D_2O): δ = 0.71 (dd, J = 7.1 Hz, 3 H, CH_2CH_3), 1.09 (d, $J_{5b,6b}$ = 6.5 Hz, 3 H, 6b-H), 1.18 (m, 8 H, 4 CH_2), 1.47 (m, 2 H, SCH_2CH_2), 1.67 (dd, $J_{3d,3'd}$ = 12.4 Hz, $J_{3d,4d}$ = 11.8 Hz, 1 H, 3d-H), 1.87 (s, 3 H, CH_3CO), 2.52-2.68 (m, 3 H, 3'd-H, SCH_2CH_2), 2.72 (dd, $J_{2a,3a}$ = 9.7 Hz, $J_{3a,4a}$ = 12.2 Hz, 1 H, 3a-H), 3.02 (dd, $J_{3a,4a}$ = 12.2 Hz, $J_{4a,5a}$ = 10.6 Hz, 1 H, 4a-H), 3.22 (dd, $J_{2c,3c}$ = 10.9 Hz, $J_{3c,4c}$ = 2.8 Hz, 1 H, 3c-H), 3.33 (dd, $J_{1c,2c}$ = 9.3 Hz, $J_{2c,3c}$ = 10.9 Hz, 1 H, 2c-H), 3.39 (dd, $J_{1a,2a}$ = $J_{2a,3a}$ = 9.7 Hz, 1 H, 2a-H), 3.40-3.78 (m, 14 H), 3.80 (dd, $J_{5a,6a}$ = 4.9 Hz, $J_{6a,6'a}$ = 12.4 Hz, 1 H, 6a-H), 3.93 (dd, $J_{1b,2b}$ = 5.8 Hz, $J_{2b,3b}$ = 10.5 Hz, 1 H, 2b-H), 3.95 (dd, $J_{5a,6'a}$ = 2.1 Hz, $J_{6a,6'a}$ = 12.4 Hz, 1 H, 6'a-H), 4.35 (d, $J_{1a,2a}$ = 9.7 Hz, 1 H, 1a-H), 4.43 (dq, $J_{4b,5b}$ < 1 Hz, $J_{5b,6b}$ = 6.5 Hz, 1 H, 5b-H), 4.55 (d, $J_{1c,2c}$ = 9.3 Hz, 1 H, 1c-H), 5.62 (d, $J_{1b,2b}$ = 5.8 Hz, 1 H, 1b-H.

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