Pergamon

Bioorganic & Medicinal Chemistry Letters 9 (1999) 1019–1022

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Affinity of 5-thio-L-fucose-containing Lewis X (Le^X) trisaccharide analogs to anti-Le^X monoclonal antibody

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Received 25 December 1998; accepted 24 February 1999

Abstract

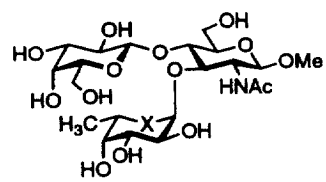
5-Thiofucose-containing Le^X trisaccharide analogs Galβ(1,4){5SFucα(1,3)}GlcNAc-OMe (**2**) and Galβ(1,4){5SFucβ(1,3)}GlcNAc-OMe (**4**) were synthesized via 5-thiofucosylation of methyl 2-azido-lactoside derivative **6** by the trichloroacetimidate method. Inhibitory activity of these analogs for the binding of Le^X to anti-Le^X antibody was evaluated by enzyme immunoassay, indicating that anti-Le^X strictly recognizes α-configuration of the fucose moiety and its binding pocket includes no advantageous region, such as hydrophobic area, for recognizing the ring sulfur atom of 5-thiofucosyl Le^X analog **2**.

Keywords: Carbohydrates; Glycosides; Immunological activity

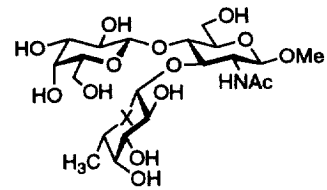
Several 5-thiosugar-containing di-[1-5] and trisaccharide analogs,[6,7] in which the ring oxygen atom of a monosaccharide unit at the non-reducing end is replaced with sulfur atom,[8] have been synthesized since the time when 5'-thio-lactosamine was synthesized[9] in 1992 as the first example. These analogs exhibited resistance to glycosidases,[2,3,9] and only recently has this promising property begun to be applied to developing glycosidase resistant drugs.[10,11] Although these analogs are desired to be better ligands of oligosaccharide-binding proteins than the ring oxygen counterparts, the binding of 5-thiofucose-containing H-type 2 analog to anti-H-type 2 antibody is only the one, reported to date,[6] which exceeds the

0960-894X/99/$ - see front matter
PII: S0960-894X(99)00124-9

binding between the natural type oligosaccharide and protein in its magnitude. This strong binding is presumably due to an attractive interaction between the ring sulfur atom and the hydrophobic region in the binding pocket of the antibody. The mechanism of strong inhibitory activity of 5-thiofucose[12] toward α-fucosidases has been similarly explained.[13]

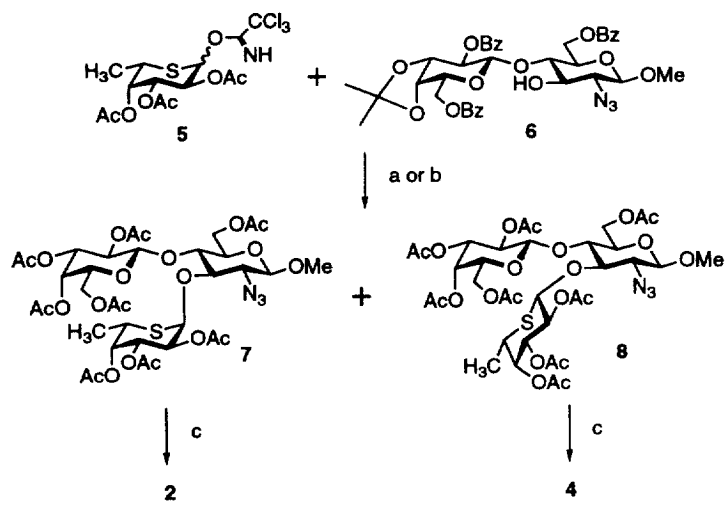


1 X = O
2 X = S



3 X = O
4 X = S

In this paper, we study the interaction between 5-thiofucose-containing Le^X trisaccharide analog (**2**) and anti-Le^X antibody to assess if replacing the fucose moiety of oligosaccharides with 5-thiofucose could be a general strategy in developing the strong inhibitors of the binding between fucose-containing oligosaccharides and receptor proteins. Though we have reported the synthesis of compound **2**,[6] we exploited the more straightforward route to **2** by employing the most efficient method for the synthesis of Le^X[14] reported by Windmüller and Schmidt.



Scheme 1. Synthetic scheme of 5-thiofucose-containing Le^X trisaccharide analogs **2** and **4**. a) 1.1 equiv. of **6**, 0.33 equiv. of $BF_3{\cdot}OEt_2$, MS4A, CH_2Cl_2, -20 °C; 50% TFA/CH_2Cl_2; NaOMe; Ac_2O-Py; **7** (11% based on **5**), **8** (9% bsed on **5**). b) 1.7 equiv. of **5**, 1.0 equiv. of $ZnCl_2{\cdot}OEt_2$, MS4A, $(CH_2Cl)_2$, RT; 50% TFA/CH_2Cl_2; NaOMe; Ac_2O-Py; **7** (19% based on **6**). c) H_2S/Py-H_2O; Ac_2O-Py; NaOMe; **2** (48%) or **4** (30%).

2-Azido-lactoside derivative **6** was synthesized from lactal derived azidolactose by the same method developed by Windmüller and Schmidt[14] for the synthesis of the thexyldimethylsilyl glycoside. Compound **6** was subjected to 5-thiofucosylation, in which the 1-*O*-trichloroacetimidate **5** and $BF_3{\cdot}OEt_2$ were used as a glycosyl donor and a promoter, respectively, to give both α- (**7**) and β-anomers (**8**) in almost equal quantity with poor yields, after deisopropylidenation, deacylation, and acetylation (Scheme 1). Most of the glycosyl donor **5** decomposed to give structurally undefined multiproducts. Formation of the significant amount of the β-anomer was unexpected, because per-*O*-acetylated 5-thioglycosyl donors usually afford the α-glycosides as major isomer despite the presence of a participating acetate at the 2-position of the glycosyl donor.[8] The α-glycoside formation from per-*O*-acetylated 5-thioglucosyl donors was ascribed by Mehta and coworkers[2] to the greater thermodynamic stability of the axially oriented aglycon of 5-thiohexopyranosides as compared to their ring oxygen counterparts. Partial formation of the β-glycosides from the 5-thiofucosyl donor, then, might indicate that this donor is more reactive than 5-thioglucosyl donors and the glycosylation reaction is in part under the kinetic control that exclusively affords the β-glycosides, as suggested from the Leffler-Hammond Principle.[15] Indeed, the use of $ZnCl_2{\cdot}OEt_2$,[14] the milder promoter that will increase the thermodynamic contribution, permitted an α-selective 5-thiofucosylation of compound **6**, though the yield was also poor as shown in Scheme 1.

Since the β-5-thiofucosyl Le^X analog **8** was obtained, we determined to also investigate the binding between this analog and anti-Le^X. Thus, the compounds **7** and **8** were subjected to final process, i.e., reduction of the azido group, *N*-acetylation, and de-*O*-acetylation, to give Le^X analogs **2**[1] and **4**,[2] respectively. Le^X derivative **1** was prepared by the method of Windmüller and Schmidt[14] and the β-fucosyl isomer of Le^X **3** was similarly synthesized by β-selective fucosylation of compound **6** using $BF_3{\cdot}OEt_2$ as a promoter. The rather low-field resonance (δ 3.89-4.08 ppm) of the 5-thiofucose unit of compound **2** as compared to that of 5-thiofucose (δ 3.64 ppm) [12] indicates the location of this residue in vicinal position to the galactose unit, as discussed for the Le^X structure.[16] Thus it is suggested that the conformation of compound **2** resembles that of compound **1**.

The binding abilities of compounds **1**, **2**, **3**, and **4** to anti-Le^X mouse monoclonal antibody[17] were evaluated by enzyme immunoassay as follows. Le^X-BSA conjugate was immobilized on a plastic multiwell plate (1.5 pmol/well) and the Le^X analogs then anti-Le^X were added into the wells. After 90 min shaking, each well was washed with buffer, and peroxidase-labeled anti-mouse IgM antibody was added. Bound anti-Le^X was monitored by

[1]**2**: ^{1}H NMR (D_2O, 25°C, HOD=4.8ppm) δ 4.94 (d, 1H, $J_{1'',2''}$ 2.9 Hz, H-1''), 4.53 (d, 1H, $J_{1,2}$ 8.4 Hz, H-1), 4.47 (d, 1H, $J_{1',2'}$ 7.9 Hz, H-1'), 4.24 (dd, 1H, $J_{2,3} = J_{3,4}$ 9.8 Hz, H-3), 4.08-3.89 (m, 9H, H-3,4,6a,6b,4',2'',3'',4'',5''), 3.80-3.74 (m, 2H, H-6'a,6'b), 3.69 (dd, 1H, $J_{2',3'}$ 9.9, $J_{3',4'}$ 3.5 Hz, H-3'), 3.67-3.63 (m, 2H, H-5,5'), 3.55 (s, 3H, OMe), 3.50 (dd, 1H, H-2'), 2.10 (s, 3H, NAc), 1.20 (d, 3H, $J_{5'',6''}$ 7.2 Hz, H-6''); ESI Mass (m/z) 582.3 (M + Na).

[2]**4**: ^{1}H NMR (D_2O, 35°C, OMe=3.55ppm) δ 4.82 (d, 1H, $J_{1'',2''}$ 9.0 Hz, H-1''), 4.60 (d, 1H, $J_{1',2'}$ 7.8 Hz, H-1'), 4.54 (d, 1H, $J_{1,2}$ 8.4 Hz, H-1), 4.08 (dd, $J_{5,6a}$ 2.1, $J_{6a,6b}$ 12.5 Hz, H-6a), 4.02-3.92 (m, 5H, H-3,4,6b,4',4''), 3.89 (dd, 1H, $J_{5',6'a}$ 7.0, $J_{6'a,6'b}$ 11.1 Hz, H-6'a), 3.83-3.73 (m, 4H, H-2,5',6'b,2''), 3.73 (dd, 1H, $J_{2',3'}$ 9.9, $J_{3',4'}$ 3.5 Hz, H-3'), 3.68-3.62 (m, 1H, H-5), 3.59 (dd, 1H, H-2'), 3.55 (s, 3H, OMe), 3.53 (dd, 1H, $J_{2'',3''}$ 9.8, $J_{3'',4''}$ 3.1 Hz, H-3''), 3.26 (dq, 1H, $J_{4'',5''}$ 1.5, $J_{5'',6''}$ 7.0 Hz, H-5''), 2.12 (s, 3H, NAc), 1.27 (d, 3H, H-6''); ESI Mass (m/z) 582.3 (M + Na).

measuring OD at 490 nm. Inhibitory activity of the synthesized analogs was evaluated as % inhibition at 5 mM: 59, 21, 5.4, and 9.8 % for compounds **1**, **2**, **3**, and **4**, respectively. These results show that mouse anti-Le^X antibody strictly recognizes the anomeric configuration of the fucose moiety of Le^X trisaccharide and its binding pocket includes no advantageous region, such as hydrophobic area, for recognizing the ring sulfur atom of 5-thiofucosyl Le^X analog **2**, if we assume that the conformations of compounds **1** and **2** are similar as discussed above. Thus we could conclude that replacing the fucose moiety of oligosaccharides with 5-thiofucose is not always a good strategy in developing the strong inhibitors of the fucose-recognizing antibody.

Acknowledgments

This work was supported by Grant-in-aid (No. 09780523) for Scientific Research From Japanese Ministry of Education, Science, Sports and Culture.

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