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Sialyl Lewis X-Polysaccharide Conjugates : Targeting Inflammatory Lesions

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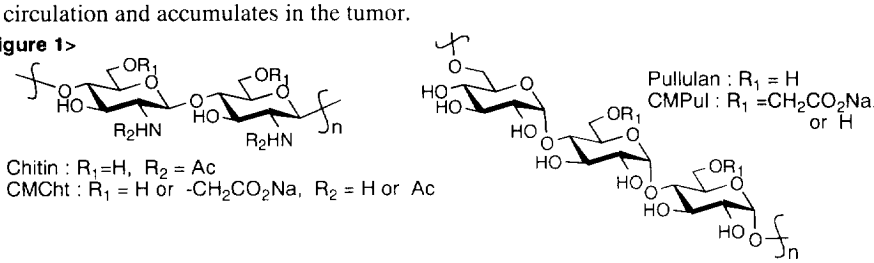
Abstract: A novel system for active targeting of inflammatory lesions has been established. A SLe^X-CMPul conjugate (**2**) showed accumulation that was 2.5-fold higher in inflammatory lesions *in vivo* than a SLN-CMPul conjugate (**4**) and 300-fold higher than monovalent SLe^X (**6**).

In the last decade, the widespread occurrence of oligosaccharides in nature and their importance in various biological processes are becoming increasingly evident,[2] but carbohydrate recognition has not been applied to targeting delivery to tissues or lesions, except for the interaction between galactose and the asialoglycoprotein receptor expressed on the liver.[3]

We focused on the interaction between sialyl Lewis X (SLe^X, Neu5Acα2→3Galβ1→4(Fucα1→3)GlcNAc) and E-selectin. SLe^X is known to be a ligand of the cell adhesion molecule called E-selectin, which is expressed on the surface of endothelial cells during inflammation.[4] As the interaction between SLe^X and E-selectin is essential for the initial stage of neutrophil infiltration into the inflammatory site, SLe^X and its derivatives, which block the interaction, should be useful as new anti-inflammatory agents.[5]

Moreover, SLe^X and its derivatives may be considered to be effective homing devices for active-targeting DDS (Drug Delivery System) to inflammatory lesions, since E-selectin is only expressed on such lesions. However, as carbohydrate recognition becomes understood, the affinity of carbohydrate for their protein has been shown to be relatively weak and carbohydrates to be generally sensitive to glycosidase *in vivo*. Furthermore, SLe^X should be rapidly filtered out at the glomerulus because of its high hydrophilicity and low molecular weight.[6] One way to solve these problems would be to support SLe^X on a macromolecule[7] which would be stabilize the sugar moiety and multivalent interaction of SLe^X with E-selectin expressed at the inflammatory lesion. We have already reported that some polysaccharides with molecular size above 70KDa such as carboxymethylchitosan (CMCht)[8] and carboxymethylpullulan (CMPul)[9] are useful as carriers in passive-targeting DDS to tumors.[9],[10] The doxorubicin-CMPul conjugate gives dramatic enhancement of the therapeutic index of antitumor effects.[9] This type of conjugate has high biocompatibility, is retained in blood circulation and accumulates in the tumor.

<Figure 1>



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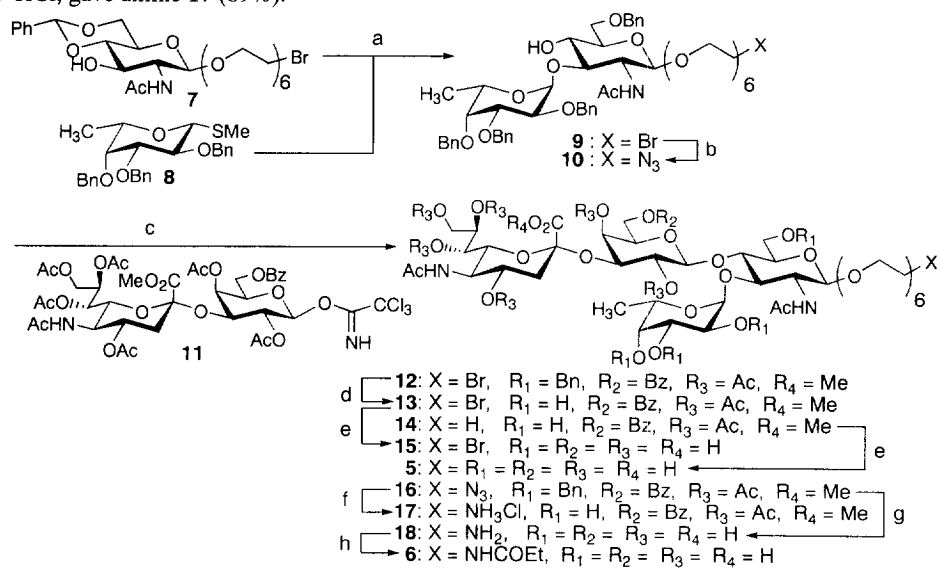
We report here the synthesis of SLe^X-polysaccharide (CMCht and CMPul) conjugates and evaluation of the possibility of using these conjugates as an active-targeting system for delivery to inflammatory lesions. We also synthesized SLN(Neu5Acα2→3Galβ1→4GlcNAc)-polysaccharide conjugates as the negative control. SLN is a trisaccharide without the fucose moiety from SLe^X and has been reported to not support E-selectin-mediated adhesion.[11]

The synthesis of a SLe^X-CMCht conjugate (**1**) is described in Scheme 1. Glycosylation of **7**[12] with **8**[13] in presence of $Me_2SSMe{\cdot}OTf$ [14] in CH_2Cl_2 at 0°C (95%, α:β = 15:1), and subsequent regioselective opening of benzylidene ring using $NaBH_3CN$-HCl[15], affords **9** (77%). However, there is a disadvantage with this ring opening reaction. $NaBH_3CN$ is a hazardous chemical that precludes its safe use on a large scale. We investigated this reaction with various acids and found that Et_3SiH-TfOH[16] is useful for this reaction. Using this system, compound **9** was cleanly obtained in 83% yield. Coupling of **9** with sialyl-galactose imidate (**11**)[17] in the presence of $BF_3{\cdot}OEt_2$ led to tetrasaccharide **12** (61%), which was hydrogenated with 10% Pd-C in MeOH to afford **13** (77%) and **14** (10%). Deacetylation of **13** followed by saponification of the methyl ester group gave **15** (93%). Similarly, **14** was converted to **5** (quant.).

Bromide (**15**) was reacted with CMCht[8] (molecular weight: about 100kDa) in 0.5% $NaHCO_3$-H_2O for 160 hr at 60°C (*N*-alkylation) to afford a SLe^X-CMCht conjugate (**1**). SLe^X content of **1** was 33 wt%, and the degree of substitution (ds) of SLe^X was 0.17 per glucosamine residue of CMCht.[18]

To synthesize the SLe^X-CMPul conjugate (**2**), treatment of **9** with LiN_3 afforded **10** (90%), which was coupled with imidate **11** ($BF_3{\cdot}OEt_2$ / CH_2Cl_2) to give **16** (52%). Hydrogenation of **16** with 10% Pd-C in THF-HCl, gave amine **17** (89%).

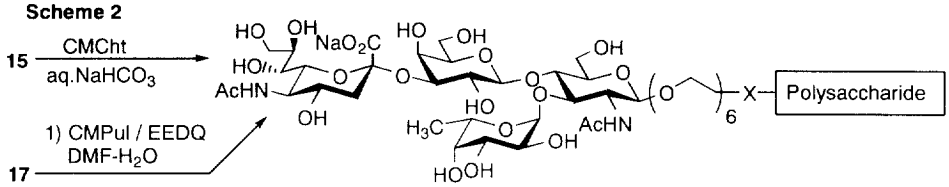


Scheme 1

a) 1) $Me_2SSMe{\cdot}OTf$ / CH_2Cl_2 / 0°C (95%, α:β =15:1) 2) Et_3SiH / TfOH / CH_2Cl_2 / MS4A / -78°C (87%)
b) LiN_3 / DMF (90%) c) $BF_3{\cdot}OEt_2$ / CH_2Cl_2 / MS4A / 0°C (**12**: 61%, **16**: 52%)
d) Pd-C / H_2 / MeOH / rt (77%) e) 1) NaOMe / MeOH / rt, 2) aq.NaOH / rt (**15**:93%, **5**: quant.)
f) Pd-C / H_2 / THF-HCl / rt (89%) g) 1) NaOMe / MeOH / rt, 2) aq.NaOH / rt,
3) Pd-C / H_2 / MeOH / pTsOH (93%) h) $EtCO_2Su$ / MeOH / NMM (90%)

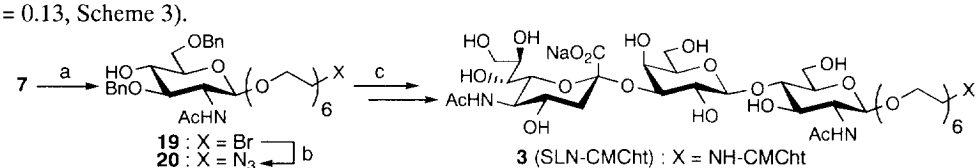
Condensation of amine **17** with CMPul[9] (molecular weight: about 190kDa, ds of carboxymethyl group was 0.6 per glucose residue.) using 1-ethoxy-carbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) in DMF-H_2O at 40°C, and subsequent deprotection with 1 N aq. NaOH, gave a SLe^X-CMPul conjugate (**2**), which was determined to contain 31 wt% SLe^X by the same method as **1**.[18] (Ds of SLe^X was 0.13 per glucose residue of CMPul.)

Scheme 2



1 (SLe^X-CMCht) : X = NH, Polysaccharide = CMCht
2 (SLe^X-CMPul) : X = NHCO, Polysaccharide = CMPul

A SLN-CMCht conjugate (**3**) and a SLN-CMPul conjugate (**4**) were synthesized similarly. (**3**, ds = 0.17, **4**, ds = 0.13, Scheme 3).



Scheme 3

a) 1) BnBr / BaO / $Ba(OH)_2$•$8H_2O$ / DMF / rt (85%) 2) Et_3SiH / TfOH / CH_2Cl_2 / MS4A / -78°C (87%)
b) LiN_3 / DMF (90%) c) same procedure as **1** and **2**

The ability to actively target the inflammatory lesion *in vivo* was evaluated using the model of arachidonic acid-induced ear edema in mice.[19] Drastic enhancement of accumulation in the targeted lesion was observed. AUC (Area Under the blood concentration-time Curve) 0-24 hr of macromolecules **1** and **2** at the inflammatory lesion was about 60-fold and 300-fold higher than that of the monovalent SLe^X derivative (**6**)[20], respectively. Less accumulation of CMCht conjugate **1** compared to **2** was due to the degradation of CMCht by lysozyme *in vivo*. Moreover, **1** showed 2-fold higher accumulation in the inflammatory lesion than **3**, and **2** showed 2.5-fold higher accumulation than **4**. We confirmed drug arrival at the E-selectin expressed at the inflammatory lesion by examining the inhibition of leukocyte rolling[21]in the inflammatory model using IL-1β activated rat mesentery venules. (*In vivo*) The multivalent SLe^X conjugate **2** was shown to have 6-fold higher inhibitory activity than the SLe^X analogue (**5**). (IC_{50} value; **1**: 0.57 μM, **2**: 0.40 μM, **5**: 2.63 μM)

In summary, SLe^X-polysaccharide conjugates were shown to be a useful novel system for active-targeting DDS to inflammatory lesions. We are presently trying to introduce anti-inflammatory agents to SLe^X-polysaccharide conjugates and evaluate their increased therapeutic effects.

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19) This evaluation was performed using radiolabelled **1**-**4**. **1** and **3** were radiolabeled using *N*-succinimidyl [2,3-^{3}H]-propionate. **2** and **4** were radiolabeled using [^{3}H]-glycine and EEDQ. (Radioactivity: **1** and **3**, 5 μCi / mg, **2** and **4**, 15 μCi / mg)

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