

## STEREOSELECTIVE SYNTHESIS OF LEWIS-ASSOCIATED TRISACCHARIDES AS E-SELECTIN INHIBITORS

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**Abstract:** Three types of Lewis-associated trisaccharides [the Le<sup>a</sup> analogs, their epimers with respect to the fucose residue (the 1c-epi-Le<sup>a</sup> analogs), and the Le<sup>x</sup> analogs] were synthesized in a stereoselective manner. Not only the Le<sup>a</sup> analogs but also the 1c-epi-Le<sup>a</sup> analogs inhibited E-selectin-mediated neutrophil accumulation into pleural cavity in lipoteichoic acid-treated mice, with the trend being Le<sup>a</sup> > 1c-epi-Le<sup>a</sup> > Le<sup>x</sup>.

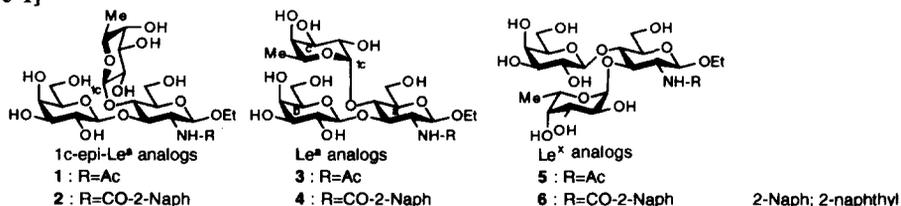
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### Introduction

Selectins are calcium dependent lectins that regulate neutrophil rolling in the early step of the inflammatory response. Sialyl Le<sup>x</sup> (SLe<sup>x</sup>) and Sialyl Le<sup>a</sup> (SLe<sup>a</sup>) represent the sialylated and fucosylated oligosaccharides identified as ligands recognized by the selectins.<sup>1)</sup> Identification of the minimum carbohydrate structure required for selectin binding should therefore provide a means to design more potent inhibitors of selectin mediated-cell adhesion.

Previous structure-functional studies have suggested that both the fucose (Fuc) and sialic acid carboxylate moieties are essential for high binding ability with E-selectin.<sup>2,3)</sup> Replacement of the sialic acid moiety of SLe<sup>x</sup> or SLe<sup>a</sup> with a sulfate group has also provided analogs which inhibit E-selectin-mediated adhesion.<sup>4)</sup> These findings have focused intense attention on the search of SLe<sup>x</sup> mimetics that would maintain high affinity while using a simpler structure.<sup>5)</sup> Moreover, a synthesized SLe<sup>a</sup> tetrasaccharide analog has been reported to show higher inhibitory activity than the reducing tetrasaccharide SLe<sup>x</sup> *in vitro*.<sup>3)</sup> The sulfated Le<sup>a</sup> tetra- and pentasaccharides were also reported to have higher affinity than the corresponding SLe<sup>x</sup> derivatives *in vitro*.<sup>4a)</sup> These results suggest that Le<sup>a</sup> structures may be more potent inhibitors of E-selectin-mediated cell adhesion than Le<sup>x</sup>, although NMR and molecular modeling studies have demonstrated conformational similarities between the two structures.<sup>6)</sup> A more interesting observation was that non-sialylated trisaccharide Le<sup>a</sup> exhibited a slight

[Figure 1]



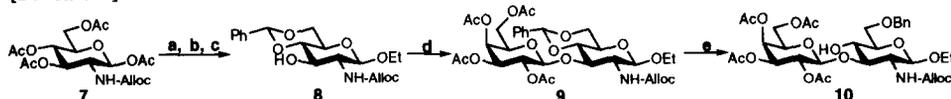
inhibitory effect against E-selectin.<sup>3)</sup> This finding suggests that the Le<sup>a</sup> trisaccharide alone might contain the minimal structural features required for inhibitors of E-selectin. However, there are few reports concerning investigation on structure-activity relationships of Le<sup>a</sup> analogs.<sup>4a)</sup> Therefore, our current interest in this area is to identify the requirement of the following two moieties of Le<sup>a</sup> structure; one is the configuration of the Fuc residue that is revealed to be essential for recognition by E-selectin and the other is the substituent on the glucosamine (GlcN) nitrogen. Transformation of these moieties has not been studied concerning Le<sup>a</sup> structure, although *N*-modification of the GlcN concerning SLe<sup>x</sup> analogs was reported to improve the ability to inhibit E-selectin-mediated adhesion.<sup>7, 8)</sup> Herein, we report the stereoselective preparation of the *N*-modified Le<sup>a</sup> analogs (**3** and **4**) and their epimers with respect to the Fuc residue (the 1*c*-epi-Le<sup>a</sup> analogs, **1** and **2**), and their inhibitory activity both on human E-selectin-mediated cellular adhesion *in vitro*<sup>9)</sup> and on an inflammatory lung injury animal model<sup>10)</sup>.

### Synthesis

The crucial step in the synthesis of the 1*c*-epi-Le<sup>a</sup> and Le<sup>a</sup> trisaccharide analogs (**1**, **2**, **3**, and **4**) is stereoselective introduction of the Fuc residue to disaccharide intermediate **10** by using two differentially protected trichloroacetimidates (**11** and **20**)<sup>11,12)</sup>. An allyloxycarbonyl group (alloc) on **10** was used to protect the GlcN nitrogen and allow for the later introduction of alternate acyl groups.

Preparation of the common intermediate **10** is shown in Scheme 1. Compound **8** was prepared in 98% overall yield from tetraacetate **7**<sup>13)</sup> by glycosylation with EtOH in the presence of TMSOTf followed by hydrolysis with NaOMe and treatment with benzaldehyde dimethyl acetal. The condensation of the resulting 4,6-*O*-benzylidene acetal **8** with protected galactosyl bromide in the presence of Hg(CN)<sub>2</sub> afforded disaccharide **9** in 61% yield. Selective opening of the benzylidene acetal of **9** produced the desired glycosyl acceptor **10** in 73% yield.<sup>14)</sup>

[Scheme 1]



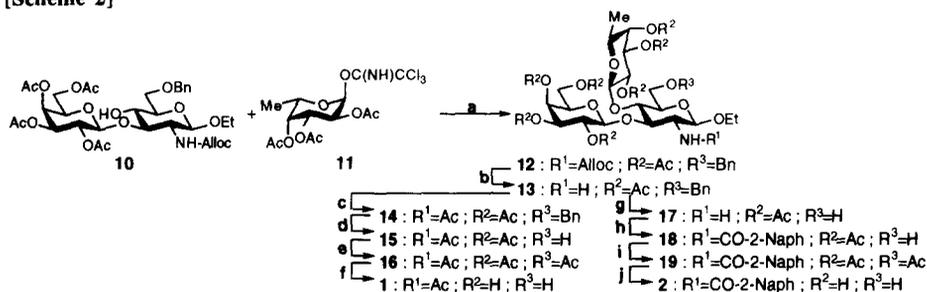
**Reagents and conditions:**

- a) EtOH, TMSOTf (cat.), MS4A, ClCH<sub>2</sub>CH<sub>2</sub>Cl (100%); b) NaOMe, MeOH (98%); c) PhCH(OMe)<sub>2</sub>, *p*-TosOH, MeCN (100%); d) tetra-*O*-acetyl- $\alpha$ -D-galactosyl bromide, Hg(CN)<sub>2</sub>, HgBr<sub>2</sub>, MS4A, ClCH<sub>2</sub>CH<sub>2</sub>Cl (61%); e) NaBH<sub>3</sub>CN, TMSCl, THF, 0°C→rt (73%).

For preparation of the 1*c*-epi-Le<sup>a</sup> analogs (**1** and **2**), stereoselective introduction of the Fuc residue onto the hydroxyl group of **10** was achieved by applying the Schmidt's procedure<sup>12)</sup> with tri-*O*-acetyl fucose  $\alpha$ -trichloroacetimidate (**11**)<sup>11)</sup> in the presence of TMSOTf, as shown in Scheme 2. This fucosylation provided the expected  $\beta$ -glycosylated trisaccharide **12** in 76% yield accompanying with 10% of the corresponding  $\alpha$ -isomer. The alloc group on **12** was removed by treatment with Pd(PPh<sub>3</sub>)<sub>4</sub> in the presence of polymethylhydrosiloxane (PMHS) to afford amine **13** in 90% yield. *N*-Acetylation of **13** afforded **14**, which was then deprotected utilizing benzylic hydrogenation to afford **15**. After peracetylation of compound **15** for purification, compound

**16** was provided in overall yield of 89% from **14**.<sup>15)</sup> Compound **16** was deprotected under basic conditions to provide the desired acetamide analog **1** in 95% yield. In the case of naphthamide analog **2**, the amino group of **17** was selectively acylated with 2-naphthoyl chloride in the presence of  $\text{NaHCO}_3$ .

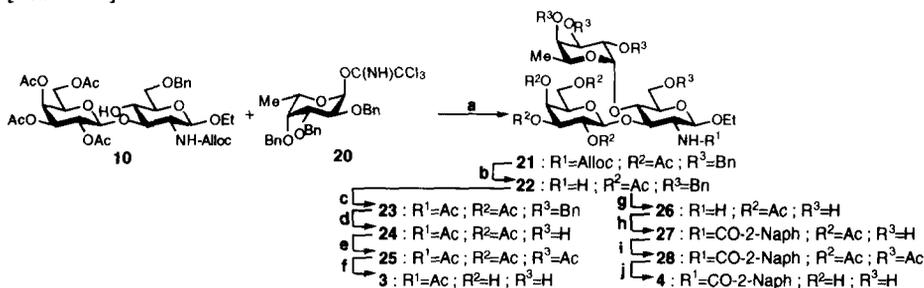
[Scheme 2]

**Reagents and conditions:**

- a) TMSOTf (cat.),  $\text{Et}_2\text{O}$ -THF (**12**; 76%,  $\alpha$ -isomer; 10%); b)  $\text{Pd}(\text{PPh}_3)_4$ , PMHS, THF (90%); c)  $\text{Ac}_2\text{O}$ , DMAP (cat.), pyridine (95%); d) 10%Pd-C,  $\text{HCO}_2\text{NH}_4$ , EtOH, reflux; e)  $\text{Ac}_2\text{O}$ , DMAP (cat.), pyridine (89% yield from **14**); f) NaOMe, MeOH (95%); g) 10%Pd-C,  $\text{HCO}_2\text{NH}_4$ , EtOH, reflux; h) 2-naphthoyl chloride,  $\text{NaHCO}_3$ ,  $\text{CH}_2\text{Cl}_2$ ; i)  $\text{Ac}_2\text{O}$ , DMAP (cat.), pyridine (75% yield from **13**); j) NaOMe, MeOH (93%).

Preparation of the *N*-acylated  $\text{Le}^a$  analogs (**3** and **4**) is shown in Scheme 3. Reaction of tri-*O*-benzyl fucose  $\alpha$ -trichloroacetimidate (**20**)<sup>12)</sup> with acceptor **10** afforded the desired  $\alpha$ -glycosylated trisaccharide **21** in 80% yield accompanying with 7% of the corresponding  $\beta$ -anomer. These results are consistent with Schmidt et al., who did not mention about formation of  $\beta$ -fucoside product during fucosylation of lactose derivative.<sup>12)</sup> The desired products **3** and **4** were synthesized from **21** using the similar conditions as described above.

[Scheme 3]

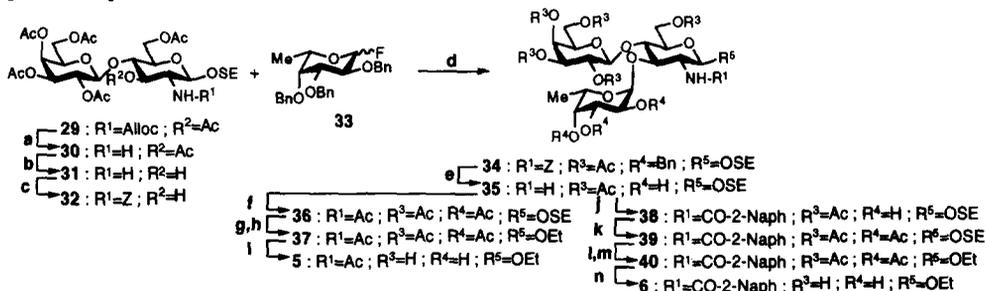
**Reagents and conditions:**

- a) TMSOTf (cat.),  $\text{Et}_2\text{O}$ -THF (**21**; 80%,  $\beta$ -isomer; 7%); b)  $\text{Pd}(\text{PPh}_3)_4$ , PMHS, THF (94%); c)  $\text{Ac}_2\text{O}$ , DMAP (cat.), pyridine (87%); d) 10%Pd-C,  $\text{HCO}_2\text{NH}_4$ , EtOH, reflux; e)  $\text{Ac}_2\text{O}$ , DMAP (cat.), pyridine (74% yield from **23**); f) NaOMe, MeOH (100%); g) 10%Pd-C,  $\text{HCO}_2\text{NH}_4$ , EtOH, reflux; h) 2-naphthoyl chloride,  $\text{NaHCO}_3$ ,  $\text{CH}_2\text{Cl}_2$ ; i)  $\text{Ac}_2\text{O}$ , DMAP (cat.), pyridine (44% yield from **22**); j) NaOMe, MeOH (92%).

The  $\text{Le}^x$  analogs (**5** and **6**) were prepared from compound **29**,<sup>8)</sup> as shown in Scheme 4. Removal of the alloc group from **29**, regioselective deprotection of the 3-*O*-acetyl group and regioselective reprotection of the amino group by benzoyloxycarbonyl chloride (*Z*-Cl) afforded **32** in 75% overall yield. Stereoselective

fucosylation using fucosyl fluoride **33** provided trisaccharide **34** in 78% yield. Trisaccharide **34** was converted into the desired  $Le^x$  analogs **5** and **6** by *N*-modification followed by transformation of 2-(trimethylsilyl)ethyl (SE) glycoside into ethyl glycoside and deprotection by the similar method as we previously reported for synthesis of  $SLe^x$  analogs.<sup>8)</sup>

[Scheme 4]

**Reagents and conditions:**

- a) Pd(PPh<sub>3</sub>)<sub>4</sub>, PMHS, THF; b) MeOH; c) Z-Cl, NaHCO<sub>3</sub>, CH<sub>2</sub>Cl<sub>2</sub> (75% yield from **29**);  
 d) AgClO<sub>4</sub>, SnCl<sub>2</sub>, TMU, MS4A, ClCH<sub>2</sub>CH<sub>2</sub>Cl (78%); e) 10%Pd-C, HCO<sub>2</sub>NH<sub>4</sub>, EtOH, reflux (90%);  
 f) Ac<sub>2</sub>O, DMAP (cat.), pyridine (74%); g) 1,1-dichloromethyl methyl ether, ZnCl<sub>2</sub>, CH<sub>2</sub>Cl<sub>2</sub>;  
 h) EtOH, Sn(OTf)<sub>2</sub>, TMU, MS4A, CH<sub>2</sub>Cl<sub>2</sub> (35% yield from **36**); i) NaOMe, MeOH (96%);  
 j) 2-naphthoyl chloride, NaHCO<sub>3</sub>, CH<sub>2</sub>Cl<sub>2</sub>; k) Ac<sub>2</sub>O, DMAP (cat.), pyridine (92% yield from **35**);  
 l) 1,1-dichloromethyl methyl ether, ZnCl<sub>2</sub>, CH<sub>2</sub>Cl<sub>2</sub>; m) EtOH, Sn(OTf)<sub>2</sub>, TMU, MS4A, CH<sub>2</sub>Cl<sub>2</sub> (55% yield from **39**);  
 n) NaOMe, MeOH then H<sub>2</sub>O (93%).

**Biological Activity**

These trisaccharides **1** - **6** were evaluated for their ability to inhibit the adhesion of HL-60 cells to purified recombinant human E-selectin *in vitro*<sup>9)</sup>, as shown in Table 1. Among the six compounds, only compounds **3** and **4**, the  $Le^a$  analogs, were found to inhibit E-selectin-mediated adhesion, with IC<sub>50</sub> values of 1.4 mM and 2.0 mM, respectively. However, the 1c-epi- $Le^a$  and  $Le^x$  analogs (**1**, **2**, **5**, and **6**) failed to inhibit the cell adhesion at concentrations up to 6.6 mM. In comparison, a  $SLe^x$  analog was reported to inhibit the cell adhesion with the IC<sub>50</sub> value of approximately 1 mM<sup>8)</sup> under the same conditions.<sup>9)</sup> These results indicate that the inhibitory potency of the  $Le^a$  analogs (**3** and **4**) was roughly equivalent to that of the  $SLe^x$ , *in vitro*. Considering the *N*-substituent in the GlcN moiety, naphthamide analog **4** demonstrated approximately the same activity as the corresponding acetamide analog **3**, although previous results have indicated that naphthamide substitution on  $SLe^x$  increased the inhibitory potency in this assay as much as ten fold.<sup>7,8)</sup> To clarify the observed differences, we are now investigating further structure-activity relationships on *N*-substituents and conformational analysis.

We examined the *in vivo* effects of these trisaccharides **1** - **6** on lipoteichoic acid (LTA)-induced murine pleurisy model, in which E-selectin has been demonstrated as playing a significant role.<sup>10)</sup> Each compound was administered intravenously at a dose of 30 mg/kg. The inhibitory effects of these compounds were shown in Table 1. Among the acetamide analogs, compound **3**, the  $Le^a$  analog, and compound **1**, the 1c-epi- $Le^a$  analog, were the most potent inhibiting LTA-induced neutrophil accumulation by 51% and 37%, respectively. Compound **5**, the  $Le^x$  analog, also inhibited by 31%, but not as strongly as the  $Le^a$  analog **3**. A similar inhibitory trend was also observed in the series of naphthamide analogs **2**, **4**, and **6**. Namely, compound **4**, the  $Le^a$  analog, and compound **2**, the 1c-epi- $Le^a$  analog, inhibited the neutrophil accumulation by 62% and 49%,

respectively. However, compound **6**, the Le<sup>X</sup> analog, did not have any effects at a dose of 30 mg/kg. From these results, we can conclude that the *in vivo* results are consistent with the *in vitro* results following a trend in which the Le<sup>a</sup> analogs are the most potent inhibitors, Le<sup>a</sup> > 1c-epi-Le<sup>a</sup> > Le<sup>X</sup>. In addition, the inhibitory potency of the Le<sup>a</sup> analogs was approximately equal to that of a SLe<sup>X</sup> analog which showed 52% inhibition in this model.<sup>10)</sup>

[Table 1] The *in vitro* and *in vivo* ability of the synthesized trisaccharides as E-selectin inhibitors.

Compound No.	Structure	<i>in vitro</i> a) IC50 (mM)	<i>in vivo</i> b) inhibition (%)
1	1c-epi-Le <sup>a</sup> (acetamide)	>6.6 <sup>c)</sup>	37
3	Le <sup>a</sup> (acetamide)	1.4	51
5	Le <sup>X</sup> (acetamide)	>6.6 <sup>c)</sup>	31
2	1c-epi-Le <sup>a</sup> (naphthamide)	>6.6 <sup>c)</sup>	49
4	Le <sup>a</sup> (naphthamide)	2.0	62
6	Le <sup>X</sup> (naphthamide)	>6.6 <sup>c)</sup>	3

a) The IC50 value means the 50% inhibitory concentration for cell adhesion of HL-60 to purified recombinant human E-selectin. For details, see reference 7.

b) Each compound was administered intravenously at a dose of 30 mg/kg. Each data represents the mean of 6-8 determinations. For details, see reference 9.

c) Maximum concentration in use.

In conclusion, the Le<sup>a</sup> trisaccharides were found to be potent E-selectin inhibitors *in vitro* and *in vivo*. And moreover, the 1c-epi-Le<sup>a</sup> trisaccharides were found to show inhibitory activities *in vivo*. This finding suggests that E-selectin binding requirements for the Le<sup>a</sup> structure is not so rigid for the Fuc moiety which may allow for the design of more potent selectin inhibitors. Our synthetic approach provides a facile access to these analogs allowing both modifications on the Fuc or the GlcN moieties. Further investigation on these and other modifications are currently on going.

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