Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 6, No. 17, pp. 2043-2048, 1996

0960-894X/96 $15.00 + 0.00

PII: S0960-894X(96)00370-8

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

Naonori Imazaki*, Haruhiko Koike, Hiroshi Miyauchi, and Masaji Hayashi

Research Center, Sumitomo Pharmaceuticals Co., Ltd.,

1-98, Kasugadenaka 3-chome, Konohana-ku, Osaka 554, Japan

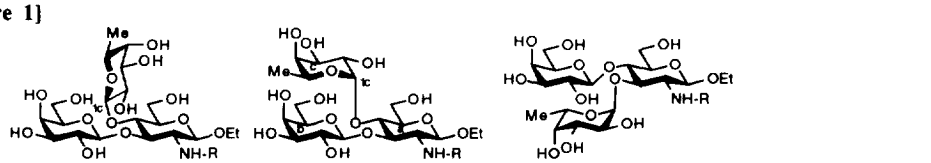
Abstract: Three types of Lewis-associated trisaccharides [the Le^a analogs, their epimers with respect to the fucose residue (the 1c-epi-Le^a analogs), and the Le^x analogs] were synthesized in a stereoselective manner. Not only the Le^a analogs but also the 1c-epi-Le^a analogs inhibited E-selectin-mediated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

Introduction

Selectins are calcium dependent lectins that regulate neutrophil rolling in the early step of the inflammatory response. Sialyl Le^x (SLe^x) and Sialyl Le^a (SLe^a) represent the sialylated and fucosylated oligosaccharides identified as ligands recognized by the selectins.[1] 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.[2,3] Replacement of the sialic acid moiety of SLe^x or SLe^a with a sulfate group has also provided analogs which inhibit E-selectin-mediated adhesion.[4] These findings have focused intense attention on the search of SLe^x mimetics that would maintain high affinity while using a simpler structure.[5] Moreover, a synthesized SLe^a tetrasaccharide analog has been reported to show higher inhibitory activity than the reducing tetrasaccharide SLe^x *in vitro*.[3] The sulfated Le^a tetra- and pentasaccharides were also reported to have higher affinity than the corresponding SLe^x derivatives *in vitro*.[4a] These results suggest that Le^a structures may be more potent inhibitors of E-selectin-mediated cell adhesion than Le^x, although NMR and molecular modeling studies have demonstrated conformational similarities between the two structures.[6] A more interesting observation was that non-sialylated trisaccharide Le^a exhibited a slight

[Figure 1]



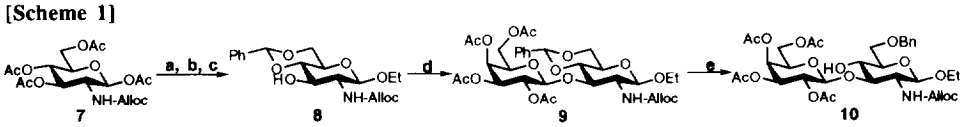
inhibitory effect against E-selectin.[3] This finding suggests that the Le^a trisaccharide alone might contain the minimal structural features required for inhibitors of E-selectin. However, there are few reports concerning investigation on structure-activity relationships of Le^a analogs.[4a] Therefore, our current interest in this area is to identify the requirement of the following two moieties of Le^a 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^a structure, although *N*-modification of the GlcN concerning SLe^x analogs was reported to improve the ability to inhibit E-selectin-mediated adhesion.[7, 8] Herein, we report the stereoselective preparation of the *N*-modified Le^a analogs (**3** and **4**) and their epimers with respect to the Fuc residue (the 1c-epi-Le^a analogs, **1** and **2**), and their inhibitory activity both on human E-selectin-mediated cellular adhesion *in vitro* [9] and on an inflammatory lung injury animal model[10].

Synthesis

The crucial step in the synthesis of the 1c-epi-Le^a and Le^a 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**)[11,12]. 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**[13] 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 $Hg(CN)_2$ afforded disaccharide **9** in 61% yield. Selective opening of the benzylidene acetal of **9** produced the desired glycosyl acceptor **10** in 73% yield.[14]

[Scheme 1]

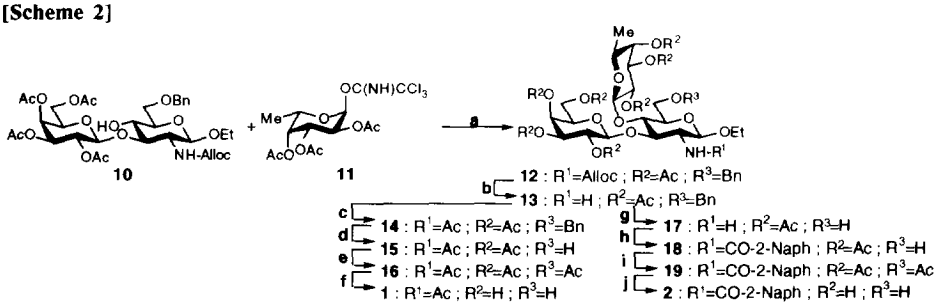


Reagents and conditions:
a) EtOH, TMSOTf (cat.), MS4A, $ClCH_2CH_2Cl$ (100%); b) NaOMe, MeOH (98%); c) $PhCH(OMe)_2$, *p*-TosOH, MeCN (100%);
d) tetra-*O*-acetyl-α-D-galactosyl bromide, $Hg(CN)_2$, $HgBr_2$, MS4A, $ClCH_2CH_2Cl$ (61%); e) $NaBH_3CN$, TMSCl, THF, 0°C→rt (73%).

For preparation of the 1c-epi-Le^a analogs (**1** and **2**), stereoselective introduction of the Fuc residue onto the hydroxyl group of **10** was achieved by applying the Schmidt's procedure[12] with tri-*O*-acetyl fucose α-trichloroacetimidate (**11**)[11] in the presence of TMSOTf, as shown in Scheme 2. This fucosylation provided the expected β-glycosylated trisaccharide **12** in 76% yield accompanying with 10% of the corresponding α-isomer. The alloc group on **12** was removed by treatment with $Pd(PPh_3)_4$ 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**.[15] 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 $NaHCO_3$.

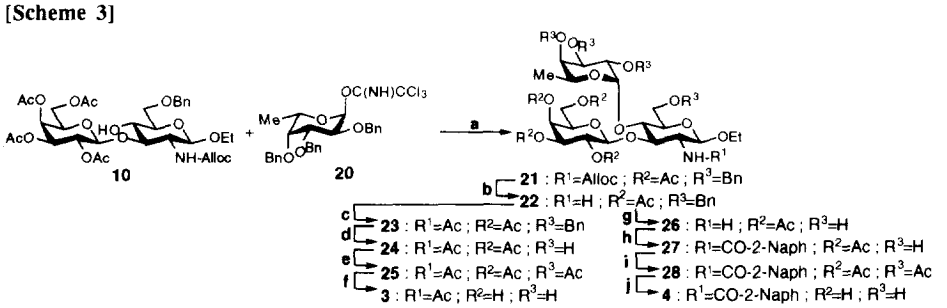
[Scheme 2]



Reagents and conditions:
a) TMSOTf (cat.), Et_2O-THF (**12**; 76%, α-isomer; 10%); b) $Pd(PPh_3)_4$, PMHS, THF (90%); c) Ac_2O, DMAP (cat.), pyridine (95%); d) 10%Pd-C, HCO_2NH_4, EtOH, reflux; e) Ac_2O, DMAP (cat.), pyridine (89% yield from **14**); f) NaOMe, MeOH (95%); g) 10%Pd-C, HCO_2NH_4, EtOH, reflux; h) 2-naphthoyl chloride, $NaHCO_3$, CH_2Cl_2; i) Ac_2O, DMAP (cat.), pyridine (75% yield from **13**); j) NaOMe, MeOH (93%).

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

[Scheme 3]

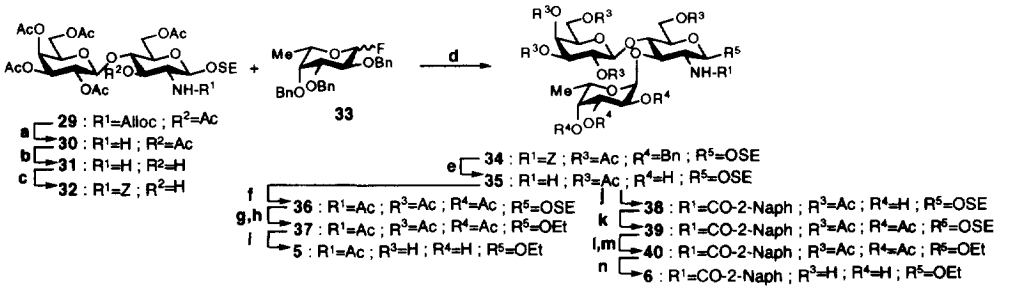


Reagents and conditions:
a) TMSOTf (cat.), Et_2O-THF (**21**; 80%, β-isomer; 7%); b) $Pd(PPh_3)_4$, PMHS, THF (94%); c) Ac_2O, DMAP (cat.), pyridine (87%); d) 10%Pd-C, HCO_2NH_4, EtOH, reflux; e) Ac_2O, DMAP (cat.), pyridine (74% yield from **23**); f) NaOMe, MeOH (100%); g) 10%Pd-C, HCO_2NH_4, EtOH, reflux; h) 2-naphthoyl chloride, $NaHCO_3$, CH_2Cl_2; i) Ac_2O, DMAP (cat.), pyridine (44% yield from **22**); j) NaOMe, MeOH (92%).

The Le^x analogs (**5** and **6**) were prepared from compound **29**,[8] 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 benzyloxycarbonyl 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.[8]

[Scheme 4]



Reagents and conditions:
a) $Pd(PPh_3)_4$, PMHS, THF; b) MeOH; c) Z-Cl, $NaHCO_3$, CH_2Cl_2 (75% yield from **29**);
d) $AgClO_4$, $SnCl_2$, TMU, MS4A, $ClCH_2CH_2Cl$ (78%); e) 10%Pd-C, HCO_2NH_4, EtOH, reflux (90%);
f) Ac_2O, DMAP (cat.), pyridine (74%); g) 1,1-dichloromethyl methyl ether, $ZnCl_2$, CH_3Cl;
h) EtOH, $Sn(OTf)_2$, TMU, MS4A, CH_2Cl_2 (35% yield from **36**); i) NaOMe, MeOH (96%);
j) 2-naphthoyl chloride, $NaHCO_3$, CH_2Cl_2; k) Ac_2O, DMAP (cat.), pyridine (92% yield from **35**);
l) 1,1-dichloromethyl methyl ether, $ZnCl_2$, CH_3Cl; m) EtOH, $Sn(OTf)_2$, TMU, MS4A, CH_2Cl_2 (55% yield from **39**);
n) NaOMe, MeOH then H_2O (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*[9], 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_{50} 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_{50} value of approximately 1 mM[8] under the same conditions.[9] 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.[7,8] 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.[10] 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^x 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^a analogs are the most potent inhibitors, Le^a > 1c-epi-Le^a > Le^x. In addition, the inhibitory potency of the Le^a analogs was approximately equal to that of a SLe^x analog which showed 52% inhibition in this model.[10)]

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

Compound No.	Structure	*in vitro* a) IC50 (mM)	*in vivo* b) inhibition (%)
1	1c-epi-Le^a (acetamide)	>6.6[c)]	37
3	Le^a (acetamide)	1.4	51
5	Le^x (acetamide)	>6.6[c)]	31
2	1c-epi-Le^a (naphthamide)	>6.6[c)]	49
4	Le^a (naphthamide)	2.0	62
6	Le^x (naphthamide)	>6.6[c)]	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^a trisaccharides were found to be potent E-selectin inhibitors *in vitro* and *in vivo*. And moreover, the 1c-epi-Le^a trisaccharides were found to show inhibitory activities *in vivo*. This finding suggests that E-selectin binding requirements for the Le^a 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.

Acknowledgment: We appreciate Dr. S. A. DeFrees in Cytel Corporation (La Jolla, CA) for his helpful discussion. We thank K. Yokokawa for her experimental contribution to a part of this work. We also appreciate N. Kawamura for performing the *in vitro* assay and K. Yamada and S. Ishibashi for performing the *in vivo* assay.

References and notes

1) Boschelli, D., H., *Drugs Fut.*, **1995**, *20*, 805.

2) a) Rmphal, j., Y.; Zheng, Z.-L.; Perez, C.; Waiker, L., E.; DeFrees, S, A.; Gaeta, F., C., A., *J. Med. Chem.*, **1994**, *37*, 3459. b) Brandkley, B., K.; Kiso, M.; Abbas, S.; Nikrad, P.; Srivasatava, S.; Foxall, C.; Oda, Y.; Hasegawa, A., *Glycobiology*, **1993**, *3*, 633.

3) Nelson, R. M.; Dolich, S.; Arffo, A.; Cecconi, O.; Bevilacqua, M. P., *J. Clin. Invest.*, **1993**, *91*, 1157.

4) a) Yuen, C.-T.; Bezouska, K.; O'Brien, J.; Stoll, M.; Lemoine, R.; Lubineau, A.; Kiso, M.; Hasegawa, A.; Bockovich, N., J.; Nicolaou, K. C.; Feizi, T., *J. Biol. Chem.*, **1994**, *269*, 1595. b) Lubineau, A.; Gallic, J. L.; Lemoine, R., *J. Chem. Soc., Chem. Commun.*, **1993**, 1419.

5) For recent studies on SLe^X mimetics, see; Birkbeck, A. A.; Ley, S. V.; Prodger, J. C., *BioMed. Chem. Lett.*, **1995**, *5*, 2637.

6) a) Tyrrell, D; James, P.; Rao, C.; Abbas, S.; Dasgupta, F.; Nashed, M.; Hasegawa, A.; Kiso, M.; Asa, D.; Kidd, J.; Brandley, B., K., *Proc. Natl. Acad. Sci. USA*, **1991**, *88*, 10372. b) Berg, E. L.; Robinson, M. K.; Mansson, O.; Butcher, E. C.; Magnani, J. L., *J. Biol. Chem.*, **1991**, *266*, 14869.

7) Ramphal, J. Y.; Hiroshige, M.; Lou, B.; Guadino, J.; Hayashi, M.; Chen, S. M.; Chiang, L. C.; Gaeta, F. C. A.; DeFrees, S. A., *J. Med. Chem.*, **1996**, *39*, 1357.

8) Hayashi, M.; Tanaka, M.; Itoh, M.; Miyauchi, H., *J. Org. Chem.*, **1996**, *61*, 2938.

9) DeFrees, S. A.; Kosch, W.; Way, W.; Paulson, J.C.; Sabesan, S.; Halcomb, R. L.;Huang, D.-H.; Ichikawa, Y.; Wong, C.-H., *J. Am. Chem. Soc.*, **1995**, *117*, 66.

10) Tojo, S.; Koike, H.; Yokota, S.; Hamazume, Y.; Natsume, Y.; Phillips, L.; Paulson, J.; Hayashi, M. and Morooka, S., submitted.

11) a)Schmidt, R., R.; Wegmann, B.; Jung, K.-H., *Liebigs Ann. Chem.*, **1991**, 121. b)Schmidt, R. R., *Angew. Chem. Int. Ed. Engl.*, **1986**, *25*, 212.

12) Schmidt, R. R.; Toepfer, A., *Tetrahedron Lett*, **1991**, *32*, 3353.

13) Boullanger, P.; Banoub, J.; Descotes, G., *Can. J. Chem.*, **1987**, *259*, 1343.

14) Garegg, P. J.; Hultberg, H.; Wallin, S., *Carbohydr. Res.*, **1982**, *108*, 97.

15) Selected ^{1}H-NMR data for structural determination of **16** (270MHz,$CDCl_3$); δ5.89 (1H, d, J=8.6 Hz, NHAc), 4.96 (1H, d, J=7.9 Hz, H-1b or H-1c), 4.80 (1H, d, J=7.3 Hz, H-1b or H-1c), and 4.65 (1H, d, J=5.3 Hz, H-1a).

(*Received in Japan* 14 *June* 1996; *accepted* 22 *July* 1996)