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SYNTHESIS AND INHIBITORY EFFECTS OF BIVALENT SIALYL LEWIS X ANALOGS AT PREVENTING CELL ADHESION

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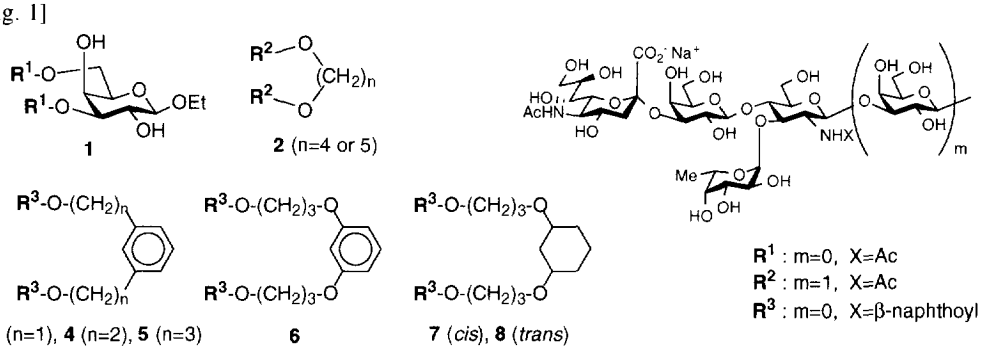
Abstract: Several sialyl Lewis X (SLeX) dimers attached to symmetric linkers, 1,3-di(ω-hydroxyalkyl)-benzene, 1,3-di(ω-hydroxyalkoxy)benzene, and 1,3-di(ω-hydroxyalkoxy)cyclohexane, were synthesized and evaluated for their inhibitory activity against adhesion of HL-60 cells to recombinant soluble E-selectin. The SLeX dimers showed 4 to 6-fold higher inhibitory activity than the corresponding monomers.

In the preceding letter, we reported the synthesis of multivalent polyacrylamide-sialyl Lewis X (SLeX) conjugates and their inhibitory activity against E-selectin-mediated cell adhesion. Bivalent glycoconjugates, as well as multivalent structures, have become recognized for their ability to overcome the low avidity of carbohydrates for proteins.[1] A few observations have been reported on the synthesis and biological activities of SLeX dimers anchored onto a sugar template[2-4] or attached to a 1,ω-alkanediol[2] or a peptide.[5] The most potent reported dimeric structure has been the structure containing bivalent SLeX anchored onto a galactose template (**1**) which exhibited a 5-fold increased inhibitory activity against E-selectin-mediated cell adhesion over the corresponding monovalent form.[2] Alkanediol-linked dimers **2** containing simpler structural features than the sugar-linked dimers only showed the same level of inhibitory activity as the corresponding monomer.[2] These observations suggest that the differences in activity of the dimers are derived from differences in the relative orientation and distances between the SLeX domains. As a part of our program to develop potent

[Fig. 1]



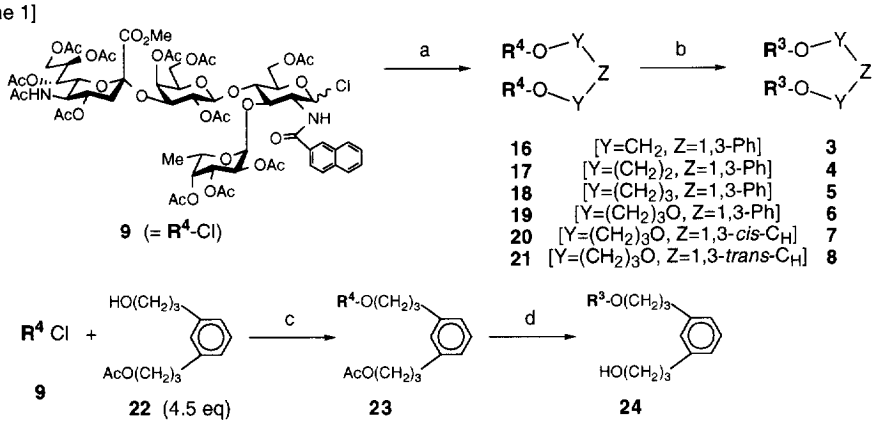
selectin blockers, we have designed and synthesized naphthamido SLeX dimers **3-8** as analogs incorporating rigid symmetrical linkers, and evaluated their inhibitory activity against adhesion of HL-60 human promyelocytic leukemia cells (HL-60) to recombinant human soluble E-selectin.

Synthesis

Each of the diols (0.50 mmol) containing either a phenyl or cyclohexyl ring, 1,3-benzenedimethanol (**10**) and diols **11-15**, were glycosylated by the reaction with chloride **9** (1.00 mmol), as shown in Scheme 1.[6] The obtained products **16-21** were deprotected under basic conditions to afford the bivalent SLeX analogs **3-8**.[7] The monovalent compound **24** was also prepared using a similar route in order to allow the direct comparison of its inhibitory activity with the corresponding bivalent compound **5**.

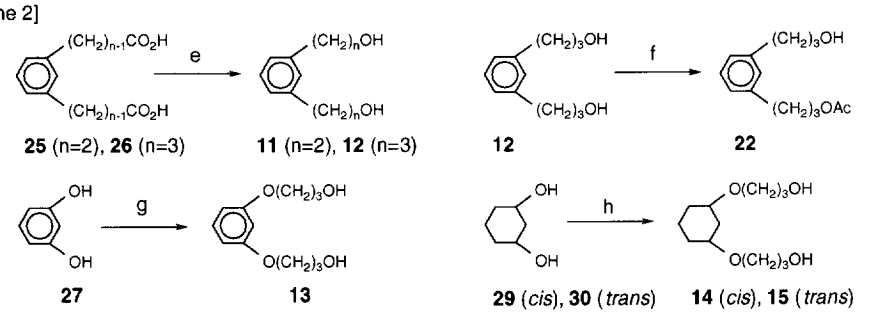
The preparation of diols **11-15** is illustrated in Scheme 2. Diols **11**[8] and **12**,[8] respectively, were synthesized by reducing the corresponding dicarboxylic acids **25** and **26** using borane-methyl sulfide

[Scheme 1]



Reagents and Conditions: (a) diol, $Sn(OTf)_2$, TMU, MS4Å, CH_2Cl_2, r.t. (**16**; 11%, **17**; 49%, **18**; 34%, **19**, 54%, **20**; 44%, **21**; 46%). (b) NaOMe, MeOH, then H_2O, r.t. (**3**; 58%, **4**; 74%, **5**; 61%, **6**; 82%, **7**; 76%, **8**; 68%). (c) $Sn(OTf)_2$, TMU, MS4Å, CH_2Cl_2, r.t. (57%). (d) NaOMe, MeOH, then H_2O, r.t. (55%).

[Scheme 2]



Reagents and Conditions: (e) BH_3•SMe_2, THF, r.t. (**11**; 76%, **12**; 83%). (f) AcOH-H_2SO_4-H_2O, r.t. (28%). (g) i) $THPO(CH_2)_3Br$ (**28**), K_2CO_3, 18-Crown-6, acetone, r.t. ii) PPTS, MeOH, r.t. (78%). (h) i)NaH, $TBDMSO(CH_2)_3OTs$ (**31**), THF, reflux. ii) AcOH-THF-H_2O (3:1:1), r.t. (**14**; 11%, **15**; 9%).

complex.[9] Diol **13**[10] was prepared by direct di-*O*-alkylation of resorcinol (**27**) with the 1,3-propanediol derivative **28** followed by deprotection. Diols **14** and **15** were prepared respectively from 1,3-cyclohexanediols [1,3-$C_H(OH)_2$] (**29** and **30**) by a similar method as described for diol **13**. Since the *trans*-diol **15** was prepared as a racemate, bivalent **8** was obtained as a 1:1 mixture of diastereomers. The monoacetylated diol **22** was prepared from diol **12** using the method reported by Babler and Coghlan.[11]

Biological activity

The synthesized bivalent SLeX analogs **3-8** were evaluated for their inhibitory activity against adhesion of HL-60 cells to recombinant human soluble E-selectin (rhsE) by a method reported previously.[2] The results are summarized in Table 1.

[Table 1] Inhibitory activity of bivalent SLeX analogs against adhesion of HL-60 cells to rhsE.

Compound	Y	Z	type of SLeX	IC_{50} (mM) HL-60/rhsE
bivalents				
3	CH_2	1,3-Ph	R^3	0.030
4	$(CH_2)_2$	1,3-Ph	R^3	0.060
5	$(CH_2)_3$	1,3-Ph	R^3	0.032
6	$(CH_2)_3O$	1,3-Ph	R^3	0.037
7	$(CH_2)_3O$	1,3-C_H(*cis*)	R^3	0.041
8	$(CH_2)_3O$	1,3-C_H(*trans*)	R^3	0.049
monovalents				
24	$(CH_2)_3$	1,3-Ph	R^3	0.20
32*1	OEt		R^1	1.5

*1; SLeX-OEt (X=Ac), see reference 6.

Each inhibitory activity is indicated as a 50% inhibitory concentration (IC_{50}) at mM scale. The inhibitory activity was found to be improved approximately 8-fold after *N*-naphthoylation of the GlcN moiety of SLeX (**24** vs. **32**) and a further 4 to 6-fold after dimerization (**5** vs. **24**). The IC_{50} value improved from 0.20 mM for the monovalent **24** to 0.032 mM for the corresponding divalent structure **6**, greater than a 6-fold increase in potency, even though the absolute SLeX concentration increased only 2-fold. A similar phenomenon, so called a 'cluster effect', has already been reported for the adhesion of synthetic oligosaccharides having Gal-GalNAc residues to the mammalian hepatic lectin.[12]

In conclusion, we have prepared a series of bivalent SLeX analogs and have evaluated for their inhibitory activity against E-selectin-mediated cell adhesion *in vitro*. We have found that simple rigid linkers of SLeX dimers **3-8** could replace the galactose moiety described for the original bivalent SLeX compound **1**. Optimization of ring size, substitution positions onto the ring, and the length of alkylene are currently in progress.

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References

1) Miyauchi, H.; Tanaka, M.; Koike, H.; Kawamura, N.; Hayashi, M., the preceding paper and references sited therein.

2) (a) DeFrees, S. A.; Gaeta, F. C. A.; Lin, Y.-C.; Ichikawa, Y.; Wong, C.-H. *J. Am. Chem. Soc.* **1993**, *115*, 7549. (b) 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.

3) Lin, C.-H.; Shimazaki, M.; Wong, C.-H.; KoKetsu, M.; Juneja, L. R.; Kim, M. *Bioorg. Med. Chem.* **1995**, *3*, 1625.

4) (a) Turunen, J. P.; Majuri, M.-L.; Tiisala, S.; Paavonen, T.; Miyasaka, M.; Lemström, K.; Seppo, A.; Penttilä, L.; Renkonen, O.; Renkonen, R. *J. Exp. Med.* **1995**, *182*, 1133. (b) Seppo, A.; Turunen, J. P.; Penttilä, L.; Keane, A.; Renkonen, O.; Renkonen, R. *Glycobiology* **1996**, *6*, 65.

5) Baisch, G.; Öhrlein, R. *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 1812.

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

7) ^{1}H-NMR data (270 MHz, D_2O): **3**; δ1.11 (6H, d, *J*=6.3 Hz), 1.76 (2H, t, *J*=12.3 Hz), 1.99 (6H, s), 2.72 (2H, m), 5.06 (2H, m), 6.37 (1H, t, *J*=7.2 Hz), 6.63 (2H, d, *J*=7.2 Hz), 6.82 (1H, s), 7.33-7.51 (6H, m), 7.58-7.73 (6H, m), and 7.86 (2H, s). **4**; δ1.11 (6H, d, *J*=6.3 Hz), 1.76 (2H, t, *J*=11.5 Hz), 1.98 (6H, s), 2.15 (4H, m), 2.71 (2H, m), 5.05 (2H, d, *J*=3.6 Hz), 6.00 (3H, m), 6.42 (1H, m), 7.18 (4H, m), 7.38-7.61 (8H, m), and 7.85 (2H, s). **5**; δ1.13 (6H, d, *J*=6.6 Hz), 1.30 (4H, m), 1.77 (2H, t, *J*=11.5 Hz), 1.98 (6H, s), 2.72 (2H, m), 5.17 (2H, d, *J*=3.6 Hz), 5.81 (1H, s), 6.27 (2H, d, *J*=7.6 Hz), 6.41 (1H, t, *J*=7.6 Hz), 7.27-7.41 (4H, m), 7.54-7.62 (8H, m), and 8.09 (2H, s). **6**; δ1.09 (6H, d, *J*=6.6 Hz), 1.50-1.85 (6H, m), 1.97 (6H, s), 2.71 (2H, m), 4.50 (2H, d, *J*=7.6 Hz), 5.05 (2H, m), 5.28-5.32 (3H, m), 5.84 (1H, br.), 7.15-7.53 (12H, m), and 7.99 (2H, s). **7**; δ0.02-0.28 (3H, m), 0.70 (1H, m), 0.88 (1H, m), 1.03-1.23 (3H, m), 1.12 (6H, d, *J*=6.3 Hz), 1.41-1.62 (6H, m), 1.75 (2H, t, *J*=11.5 Hz), 1.98 (6H, s), 2.72 (2H, m), 2.77-2.89 (3H, m), 3.08 (1H, m), 5.14 (2H, m), 7.49 (4H, m), 7.72-7.93 (8H, m), and 8.26 (2H, s). **8**; δ0.32 (1H, m), 0.53-0.76 (6H, m), 1.12 (6H, d, *J*=6.3 Hz), 1.12 (1H, m), 1.53 (4H, m), 1.73 (2H, t, *J*=12.0 Hz), 1.98 (6H, s), 2.58 (2H, m), 2.73 (2H, m), 2.90 (4H, m), 5.14 (2H, m), 7.42 (4H, m), 7.67-7.83 (8H, m), and 8.23 (2H, s).

8) Ruzicka, L.; Buijs, J. B.; Stoll, M. *Helv. Chim. Acta* **1932**, *15*, 1220.

9) Lane, C. F. *Aldrichimica Acta* **1975**, *8*, 20.

10) Rindfusz, R. E.; Ginnings, P. M.; Harnack, V. L. *J. Am. Chem. Soc.* **1920**, *42*, 157.

11) Babler, J. H.; Coghlan, M. J. *Tetrahedron Lett.* **1974**, 1971.

12) Lee, Y. C.; Townsend, R. R.; Hardy, M. R.; Lonngren, J.; Arnarp, J.; Haraldsson, M.; Lönn, H. *J. Biol. Chem.* **1983**, *258*, 199.

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