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Design and synthesis of cyclic sialyl Lewis X mimetics: a remarkable enhancement of inhibition by pre-organizing all essential functional groups†

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Abstract

Two rigid macrocyclic glycopeptides **1** and **2** were designed to mimic the tetrasaccharide SLe^X as inhibitors of P-selectin. While compound **1** was found to be 1000-fold more potent than SLe^X with $IC_{50} = 1$ μM, compound **2** was not soluble in water for evaluation of its activity. © 2000 Elsevier Science Ltd. All rights reserved.

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Sialyl Lewis X (SLe^X) is a tetrasaccharide often found at the non-reducing terminal of certain glycoproteins and glycolipids as a ligand for selectins (Fig. 1(a)).[1–6] The interaction between SLe^X and selectins plays important roles in neutrophil extravasation, lymphocyte recirculation,

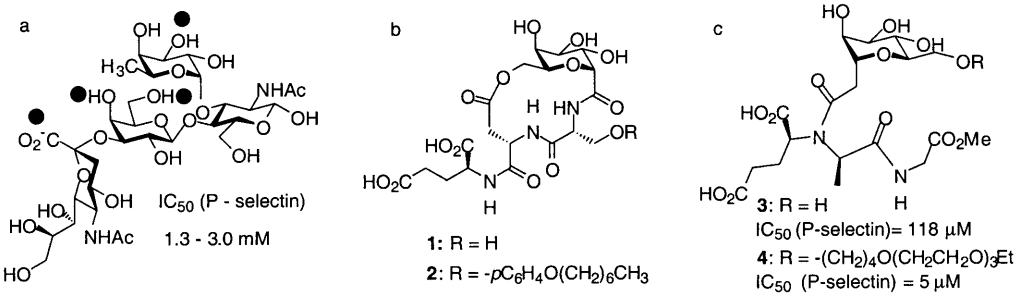

Figure 1. (a) The structure of SLe^X and its crucial functional groups (marked with a ●) for binding with P-selectin. (b) The structures of designed cyclic compounds **1** and **2**. (c) The structures of previously prepared open form SLe^X mimetics **3** and **4**

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† Dedicated to Professor Harry H. Wasserman on the occasion of his 80th birthday.

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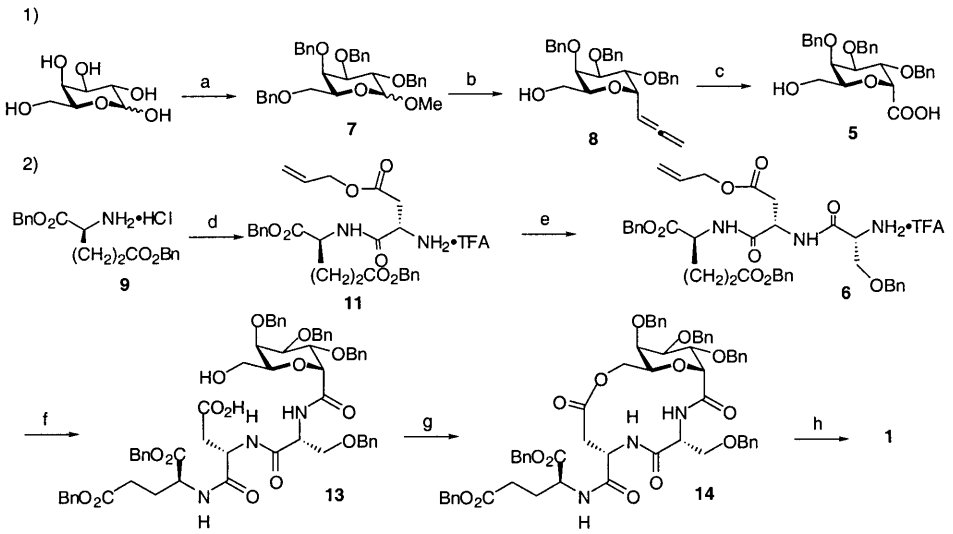
platelet adhesion as well as cancer metastasis. Development of SLe^X mimetics as potential therapeutics has been a subject of current interest.[1] It has been demonstrated that the 3-hydroxyl group of the fucose, the 4- and 6-hydroxyl groups of the galactose, and the negatively charged carboxylate of the sialic acid are the crucial functional groups for the SLe^X epitope binding to P-selectin,[7–9] the first selectin expressed when tissue injury occurs. Due to the weak affinity of SLe^X ($IC_{50} = 1.3–3.0$ mM) for P-selectin,[10] efforts have been directed toward the design of SLe^X mimetics containing minimal functional groups to increase affinity.[8,9,11] One of the approaches is based on combinatorial chemistry using the Ugi reaction, from which compounds **3** and **4** (Fig. 1(c)) were found to be more active than SLe^X.[10] Compound **3** is about 15 times more potent than SLe^X, while introduction of a hydrophobic chain (such as that in compound **4**) has led to a further 20-fold increase in its potency. This result together with those from compounds related to **4** with different hydrophobic groups indicates the possible presence of a hydrophobic pocket in P-selectin near the binding site. In order to further improve the potency of the SLe^X mimics, we have decided to synthesize cyclic SLe^X mimics **1** and **2** (Fig. 1(b)) with all the recognition elements pre-organized in a defined manner to further lower the entropic energy upon binding with selectin.

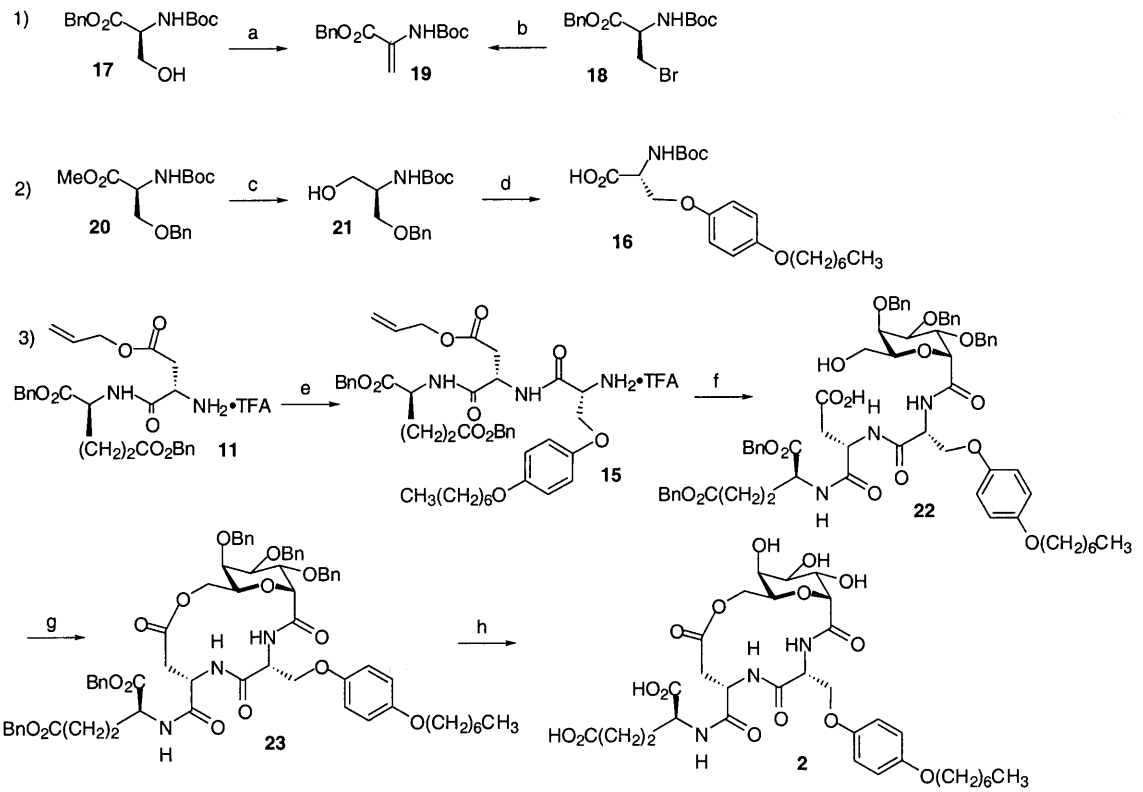
The cyclic glycopeptide **1** was synthesized by coupling the monosaccharide **5** and the tripeptide **6** (Scheme 1). To prepare compound **5**, L-galactose was converted to the fully protected L-galactoside **7** in 55% yield (Scheme 1-1). Upon treatment with 3-TMS 1-propyne and TMSOTf, the anomeric methoxy group of **7** was displaced with allene (compound **8**) in 71% yield with concomitant removal of the benzyl group.[11,12] Subsequent protection of the free hydroxy group of compound **8** with acetic anhydride followed by ozonolysis, Jones oxidation



Scheme 1. Reagents and conditions: (a) (i) IRA-120, MeOH, 12 h; (ii) NaH (5 equiv.), BnBr (6 equiv.), Bu_4NI (0.2 equiv.), 4 h, 55%; (b) TMSOTf (0.5 equiv.), 3-TMS-1-propyne (2 equiv.), CH_3CN, 12 h, 71%; (c) (i) Ac_2O, pyridine, 2 h; (ii) O_3, then Me_2S, 8 h; (iii) Jones' oxidation, 0.5 h; (iv) cat. NaOMe, MeOH, 1 h, 65%; (d) (i) *N*-Boc-β-allyl-L-aspartic acid **10** (1.1 equiv.), EDC (1.1 equiv.), HOBt (1.1 equiv.), NMM (2.0 equiv.), 2 h; (ii) TFA, 1 h, 90%; (e) (i) *N*-Boc-*O*-benzyl-L-serine **12** (1.1 equiv.), EDC (1.1 equiv.), HOBt (1.1 equiv.), NMM (2 equiv.); (ii) TFA, 1 h, 90%; (f) (i) **2** (1.1 equiv.), EDC (1.1 equiv.), HOBt (1.1 equiv.), NMM (2 equiv.), 2 h; (ii) $Rh(PPh_3)_3Cl$ (0.1 equiv.), EtOH, H_2O, 2 h, 70%; (g) PPh_3 (1.3 equiv.), DEAD (1.3 equiv.), THF, 12 h, 27%; (h) Pd/C, H_2, AcOH/THF/H_2O, 12 h, 83%

and deacetylation gave the monosaccharide **5** in 65% yield in four steps. The tripeptide **6** was prepared from the benzyl protected glutamic acid **9** with the allyl protected aspartic acid **10** and benzyl protected serine **12** (Scheme 1-2). Coupling of the tripeptide **6** and monosaccharide **5** with EDC followed by removal of the allyl protective group gave the open form glycopeptide carboxylic acid **13** in 70% yield. Macrolactonization of **13** was accomplished in 27% yield under Mitsunobu conditions to give the fully protected cyclic glycopeptide **14**. Removal of all benzyl groups by catalytic hydrogenation yielded the desired cyclic glycopeptide **1**.[13]

Compound **2** was synthesized by coupling monosaccharide **5** with tripeptide **15**, which was in turn prepared from dipeptide **11** and the serine derivative **16**. Attempts to directly synthesize **16** from serine esters failed as either Mitsunobu reaction on serine **17** or displacement of the bromide from **18** led to the formation of dehydroserine **19**, presumably through α-deprotonation (Scheme 2-1). The serine derivative **16** was successfully synthesized in 45% yield by first reducing D-serine **20** to serinol **21** followed by Mitsunobu reaction to introduce the hydrophobic chain (Scheme 2-2). Coupling of dipeptide **11** with serine derivative **16** and monosaccharide **5** followed by deprotection gave the open form glycopeptide carboxylic acid **22** (Scheme 2-3) in 68% yield in four steps. The macrolactonization of **22** was achieved using EDC, DMAP and DMAP·HCl[14]



Scheme 2. Reagents and conditions: (a) PPh_3 (1.3 equiv.), DEAD (1.3 equiv.), 4-(*n*-heptyloxy)-phenol (1 equiv.), 12 h; (b) sodium 4-(*n*-heptyloxy)-phenoxide (1 equiv.), DMF, 12 h; (c) $LiBH_4$ (1 equiv.), THF, 1 h, 97%; (d) (i) PPh_3 (1.3 equiv.), DEAD (1.3 equiv.), 4-(*n*-heptyloxy)-phenol (1 equiv.); (ii) Pd/C, CH_2Cl_2; (iii) PDC, DMF, 48%; (e) (i) **16** (1.0 equiv.), EDC (1.1 equiv.), HOBt (1.1 equiv.), NMM (2.0 equiv.), 2 h; (ii) TFA, 1 h, 90%; (f) **5** (1.1 equiv.), EDC (1.1 equiv.), HOBt (1.1 equiv.), NMM (2.0 equiv.), 2 h; (ii) $Pd(PPh_3)_4$ (0.1 equiv.), morpholine (1.2 equiv.), 1 h, 75%; (g) EDC (3.0 equiv.), DMAP (1.0 equiv.), DMAP·HCl (1.0 equiv.), 0°C, 40–50%; (h) Pd/C, H_2, AcOH/THF/H_2O

at 0°C in 40–50% yield. Using other coupling reagents such as DEAD/PPh_3, 2,4,6-trichlorobenzoyl chloride,[15,16] and 2,2′-dipyridyl disulfide[17,18] either led to a much lower yield or completely failed. Unfortunately, subsequent deprotection of all the benzyl groups of macrolactone **23**[19] under catalytic hydrogenation conditions gave a white solid product insoluble in the solvent system such as water, DMSO, methanol, methanol/CH_2Cl_2/H_2O, acetic acid, THF or acetonitrile, although mass spectra indicated a complete removal of all benzyl groups; this is most likely due to the amphiphilic nature of compound **2**. No racemization during peptide coupling was observed in the preparation of **1** and **2**.

Glycopeptide **1** was assayed[11] and its IC_{50} value in inhibiting P-selectin was determined to be 1 μM as compared to 118 μM for compound **3**.[10] Therefore, pre-organization of the necessary point of contacts by introducing a macrolactone ring indeed greatly increased the potency of the mimics, to an extent similar to the hydrophobic effect exhibited in **4**. Although compound **2** is insoluble, changing the structure of the hydrophobic group may increase its solubility.

Acknowledgements

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13. **1**: ^{1}H NMR (600 MHz, D_2O) δ 4.55 (d, J=6.6 Hz, 1H), 4.51–4.54 (m, 2H), 4.43 (dd, J=1.8, 11.9 Hz, 1H), 4.30 (dd, J=4.9, 9.2 Hz, 1H), 4.11 (dd, J=3.5, 10.1 Hz, 1H), 3.99 (dd, J=9.7, 11.9 Hz, 1H), 3.91 (d, J=3.5 Hz, 1H), 3.90 (dd, J=6.6, 10.1 Hz, 1H), 3.81 (dd, J=5.7, 11.8 Hz, 1H), 3.75 (dd, J=4.4, 11.8 Hz, 1H), 3.66 (dd, J=1.8, 9.7 Hz, 1H), 2.87 (dd, J=4.4, 15.8 Hz, 1H), 2.81 (dd, J=4.9, 15.8 Hz, 1H), 2.30–2.35 (m, 2H), 2.06–2.11 (m, 1H), 1.83–1.88 (m, 1H); ^{13}C NMR (150 MHz, D_2O) δ 177.86, 175.53, 172.53, 171.94, 171.48, 171.39, 75.66, 75.50, 70.79, 69.84, 67.96, 66.60, 61.48, 55.32, 52.96, 51.26, 36.38, 30.66, 26.70; HRMS calcd for $C_{19}H_{27}N_3NaO_{14}$ (FAB, M+Na): 544.1391; found: 544.1382.
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