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Synthesis of Sialyl Lewis X Mimetics: Use of *O*-α-Fucosyl-(1*R*, 2*R*)-2-Aminocyclohexanol As Core Structure

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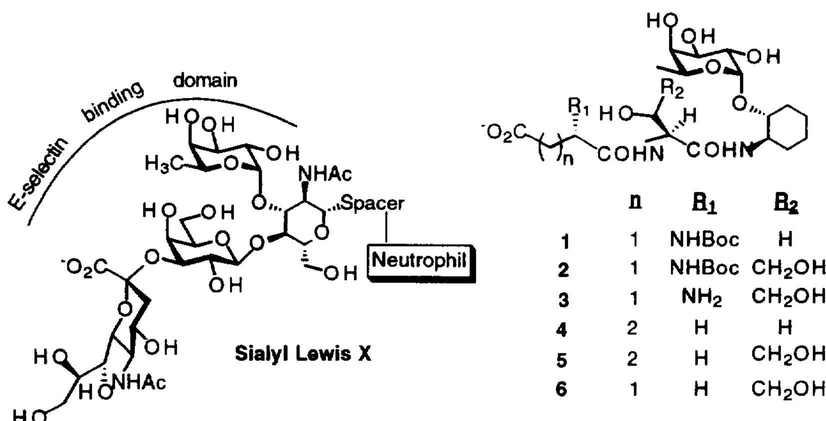
Abstract: Six glycopeptides containing *O*-α-fucosyl-(1*R*, 2*R*)-2-aminocyclohexanol were designed and prepared as sialyl Lewis X mimetics. Compounds **2** and **6** showed better binding affinities than SLe^X (IC_{50} = 0.5 mM) to E-selectin with IC_{50} values of 0.4 and 0.2 mM respectively. Copyright © 1996 Elsevier Science Ltd

Sialyl Lewis X (SLe^x), a terminal tetrasaccharide of cell-surface glycoproteins and glycolipids, has been identified as a ligand for the endothelial leukocyte adhesion molecule-1 (E-selectin), which mediates the early stage of adhesion of leukocytes to activated endothelial cells.[1] Although SLe^x has been considered to be potentially useful as an anti-inflammatory agent and its large scale synthesis has been developed for clinical evaluation,[2] this natural tetrasaccharide can only be used in its injectable form for acute symptoms as it is orally inactive and unstable in the blood stream.[3] The search for novel SLe^x mimetics with simpler structure, higher affinity for the receptor, and better stability against glycosidases, especially fucosidase and sialidase, has been of current interest.[4]

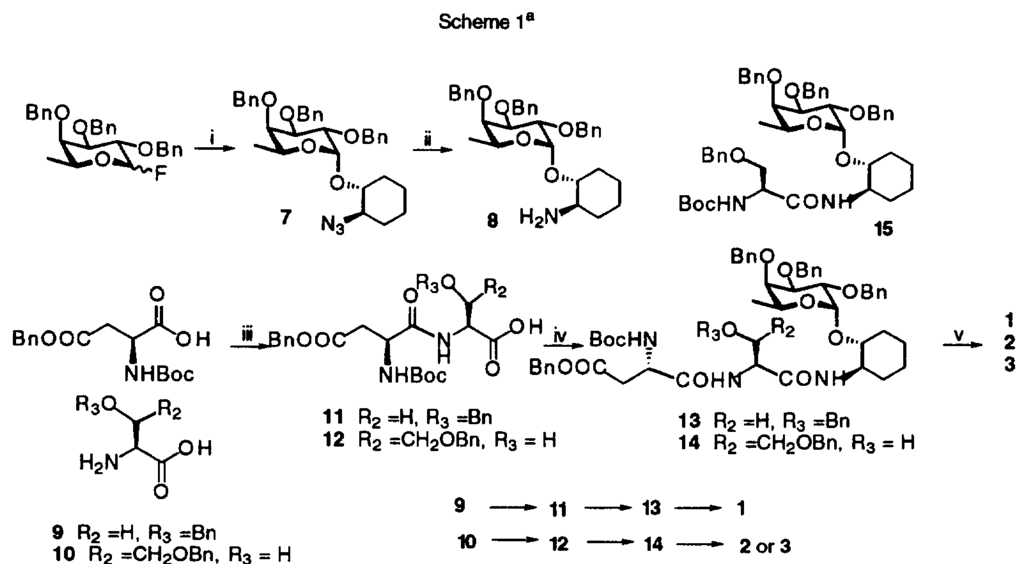


	n	R1	R2
1	1	NHBoc	H
2	1	NHBoc	CH_2OH
3	1	NH_2	CH_2OH
4	2	H	H
5	2	H	CH_2OH
6	1	H	CH_2OH

It has been found that both the free[2] and bound[5] conformations of SLe^x are similar (with the exception that the orientations of $-CO_2^-$ on NeuAc are different), and the six functional groups required for E-selectin binding are the 2-, 3- and 4-OH groups of Fuc, the 4- and 6-OH groups of Gal and the $-CO_2^-$ group of NeuAc.[6] In continuation of our interest in the development of SLe^x mimetics,[7] we report here the design and synthesis of six mimetics (**1-6**), which contain the core structure *O*-α-fucosyl-(1*R*,2*R*)-2-aminocyclohexanol.

As part of the design, fucose was retained as the only carbohydrate moiety, and (1*R*,2*R*)-2-aminocyclohexanol was chosen to replace GlcNAc as the *trans*-hydroxyamine moiety is equivalent to the configuration of the *trans*-diol moiety in GlcNAc. The terminal carboxylic acid group is kept a certain distance from fucose by proper spacers, which contain 1 (**1** and **4**) or 2 (**2**, **3**, **5** and **6**) hydroxyl groups to substitute for the 4- and 6-OH of Gal. Molecular modeling showed that replacement of GlcNAc with (1*R*,2*R*)-2-aminocyclohexanol provides a good scaffold for positioning the carboxylate and the hydroxyl groups in a similar orientation to that of the natural ligand.

Scheme 1[a]



[a](i) (1*R*,2*R*)-2-azidocyclohexanol, $SnCl_2$, $AgClO_4$, CH_2Cl_2, 4 Å MS (α form, 60%); (ii)PPh_3 (59%) or LAH (100%); (iii) *N*-hydroxysuccinimide, EDAC (35%); **9** or **10**, Et_3N (**9** -> **11**, 51%; **10** -> **12**, 76%); (iv) EDAC, HOBT, **8** (**11** -> **13**, 43%; **12** -> **14**, 45%); (v) $Pd(OH)_2/C$, H_2 (**13** -> **1**, 59%; **14** -> **2** (6 hours), 53%; **14** -> **3** (18 hours), 49%).

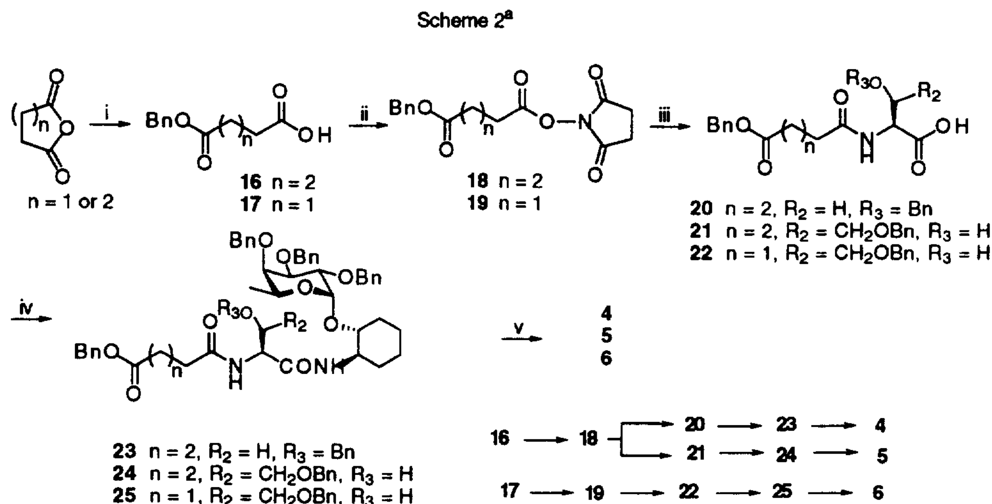
The syntheses of **1**-**3** are shown in Scheme 1. (1*R*,2*R*)-2-azidocyclohexanol obtained by lipase resolution,[8] was coupled with 2,3,4-tri-*O*-benzyl-L-fucopyranosyl fluoride[9] to give compound **7** in good yield. Both triphenylphosphine and LAH reduced the azido group of **7** to give amine **8** but the latter process gave a much higher yield. *O*-Benzyl-*N*-Boc-L-aspartic acid was coupled with *O*-benzyl-L-serine to generate acid **11** which was coupled with **8** to give **13**. Hydrogenolysis of **13** afforded target **1**. Since **15** is sensitive to TFA druing the removal of the Boc group, it was not used in the peptide extension.

Compound **2** and **3** were obtained in a similar synthetic strategy using compound **10**[10] (Scheme 1), and purified by silica gel and Bio Gel P2 column chromatography.

The synthesis of **4**-**6** are shown in Scheme 2. Compound **16**, prepared from glutaric anhydride and benzyl alcohol, was coupled with *O*-benzyl-L-serine to give **20**. Acid **20** was subsequently reacted with the free amine **8** to yield **23**, which afforded **4** after hydrogenolysis. Compound **5** was prepared in a similar manner

using **10**, and purified by silica gel and Bio Gel P2 columns. It should be mentioned that methanol was not a suitable solvent for the last deprotection step, as the methyl ester of **5** was obtained when the hydrogenolysis was carried out in methanol. To circumvent this problem, a mixture of ethyl acetate and water was used. The same conditions were used in the preparation of compound **6** with succinic anhydride as the starting material instead of glutaric anhydride.

Scheme 2[a]



[a] (i) BnOH, pyr, DMAP, 50°C (**16**, 63%; **17**, 49%); (ii) *N*-hydroxysuccinimide, CH_2Cl_2 (**16** -> **18**, 46%; **17** -> **19**, 34%); (iii) **9**, DMF/H_2O (5:1), Et_3N (**18** -> **20**, 73%); **10**, DMF/H_2O (5:1), Et_3N (**18** -> **21**, 90%; **19** -> **22**, 87%); (iv) EDAC, HOBT, CH_2Cl_2, **8** (**20** -> **23**, 49%; **21** -> **24**, 55%; **22** -> **25**, 52%); (v) $Pd(OH)_2$/C, H_2, MeOH (**23** -> **4**, 59%); $Pd(OH)_2$/C, H_2, EtOAc/H_2O, 1:1 (**24** -> **5**, 52%; **25** -> **6**, 65%).

The activities of **1-6** were evaluated as inhibitors of SLe^x binding to E-selectin (IC_{50} = 0.5 mM) in a cell free assay[11]. Compounds **3**, **4** and **5** showed moderate binding affinities towards E-selectin with IC_{50} of 10 mM, 6 mM and 7 mM, which were about 10 times less effective than the natural ligand. Both **2** and **6**[12], however, showed very good binding affinities towards the protein with IC_{50} of 0.4 mM and 0.2 mM respectively. Compound **1** was inactive, suggesting the importance of the primary OH group. A diastereomer of **6** with inversion of the β carbon of the hydroxy threonine moiety exhibited an IC_{50} of >5mM, indicating the importance for the orientation of the primary OH group. It appears that compounds with a proper distance between the fucose and the corresponding spacer distance in SLe^x gave better activities.

In addition, compounds **1-6** were not substrates for α-fucosidase. They were stable for at least three days at room temperature in the presence of α-fucosidase at pH 5.5. On the contrary, SLe^x was easily hydrolyzed by fucosidase, galactosidase and sialidase.

In summary, this study[13] provides further demonstration that simple and stable low molecular weight fucosylglycopeptides can be used as SLe^x mimetics.

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12. Compound **2**: ^1H NMR (500 MHz, D_2O) δ 1.12 (d, 3H, J = 6.5 Hz, H-6), 1.08-1.26 (m, 4H), 1.39 (s, 9H), 1.65-1.80 (m, 3H), 2.14 (m, 1H), 2.74 (m, 2H), 3.39-3.88 (m, 9H), 4.37 (m, 2H), 4.98 (d, 1H, J = 3.5 Hz, H-1). ^{13}C NMR (125 MHz, D_2O) δ 15.82, 23.70, 24.58, 28.03, 29.30, 31.78, 37.32, 52.05, 53.40, 55.55, 62.93, 67.14, 68.32, 69.95, 71.99, 72.25, 76.23, 82.21, 94.06, 157.59, 170.80, 173.42, 175.77. HRMS calcd for $C_{25}H_{43}N_3O_{13}Cs$ ($M+Cs^+$) 726.1850, found 726.1832. Compound **6**: ^1H NMR (500 MHz, D_2O) δ 1.09 (d, 3H, J = 6.5 Hz, H-6), 1.25 (m, 3H), 1.45 (m, 1H), 1.65 (m, 3H), 2.18 (m, 1H), 2.57 (m, 2H), 2.63 (m, 2H), 3.40 (m, 1H), 3.47 (d, 2H, J = 6.5 Hz), 3.72 (m, 5H, H-2, H-3, H-4 and H-5), 4.22 (ddd, 1H, J = 2.0, 6.5, 6.5 Hz), 4.49 (d, 1H, J = 2.0 Hz), 5.03 (d, 1H, J = 3.0 Hz, H-1) ^{13}C NMR (125 MHz, D_2O) δ 15.83, 23.61, 24.66, 28.72, 30.19, 30.89, 31.37, 53.57, 54.96, 62.66, 67.23, 68.29, 69.95, 71.32, 72.22, 75.24, 93.03, 172.22, 175.67, 178.00. HRMS calcd for $C_{20}H_{34}N_2O_{11}Cs$ ($M+Cs^+$) 611.1217, found 611.1241.

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