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Chemical and Enzymatic Synthesis of High-Affinity Selectin Ligands

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Abstract: Analogs of sialyl Lewis^X have been synthesized chemically using donors of modified sialic acids. The sialic acids were obtained enzymatically by an aldolase reaction. The sLe^x tetrasaccharides modified at C-2 of the GlcNAc moiety and at C-5 of the sialic acid residue were tested as inhibitors for E- and P-selectins. Up to 12-fold higher inhibitory potency was found for the *lyso*-derivative of sLeX compared to the parent compound. © 1997 Elsevier Science Ltd. All rights reserved.

The binding of selectins[1], a group of cell surface lectins, to carbohydrate ligands is mediating the attraction of several groups of leukocytes to areas of inflammation. This has stimulated research to investigate the use of carbohydrates and their mimetics[2] as potential drugs to prevent the adhesion and subsequent migration of leukocytes to the affected tissues in several acute and chronic inflammatory diseases. One of the major ligands of the selectins is believed to be the sialyl Lewis^X tetrasaccharide[3] (sLe^X) determinant found on the termini of glycolipids and glycoproteins. In the last years systematic variations of functional groups of sLe^X have led to a detailed knowledge about structure-activity-relationships of the functional groups involved in binding[4] to selectins (Figure 1). It is known that the acid function in the sialic acid moiety is crucial and can be replaced, i.e. by sulfate groups[5]. Furthermore, the fucose and some of the galactose hydroxyl groups are essential. However, the receptor affinities in cell-based adhesion assays of the low molecular weight sLe^X mimetics reported thus far, could not be significantly improved compared to the natural ligand. Therefore we investigated the introduction, respectively the unmasking of functional groups in the sLe^x tetrasaccharide to increase binding affinity via additional ionic interactions[6]. We focused on the two acetamido groups present and their replacement by hydrogen or amino moieties (Figure 2).

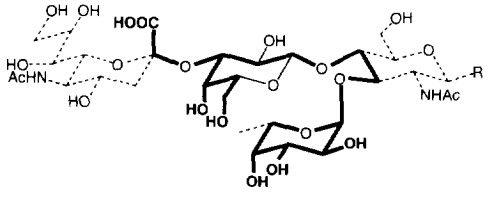
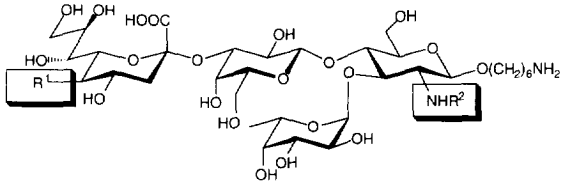


Figure 1: Key polar groups for selectin binding



1a R^1 = NHAc R^2 = Ac
1b R^1 = H R^2 = Ac
1c R^1 = NH_2 R^2 = Ac
1d R^1 = NH_2 R^2 = H

Figure 2: Derivatives of sLe^X tested as ligands for E and P-selectins

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The tetrasaccharides **1a-d** were synthesized from readily available building blocks activated as thioglycosides or trichloroacetimidates. Initially, the Le^X trisaccharide was assembled (Figure 3) followed by sialylation with modified sialic acid donors.

Coupling of thioglycoside **2**[7], available in four steps from glucosamine hydrochloride and thioethylfucoside **3**, gave the disaccharide **4** in 71 % yield. The fucosyl donor **3** can be selectively activated[8] in the presence of a thioethyl moiety in the acceptor reflecting the differences in reactivity proposed by the concept of armed and disarmed donors[9]. Subsequently the benzyloxycarbonyl-hexanolamine spacer was introduced by activating disaccharide **4** with NIS-triflic acid[10]. Regioselective reduction of the benzylidene acetal **5** according to *Garegg*[11] gave the acceptor **6** which was dephthaloylated with ethylene diamine/*n*-butanol[12]. The occurrence of side reactions at the urethane moiety is time dependent. Typically, the deprotection was complete after 4 hrs at 80 °C whereas side products appeared after 8 hrs of reaction time. Chemoselective N-acetylation furnished the acceptor **7** and subsequent elongation with the galactosyl imidate **8**[13] gave the trisaccharide **9**. The three acetyl groups were released by *Zemplén* deprotection and the resulting triol **10** was used for regioselective sialylations at position 3''.

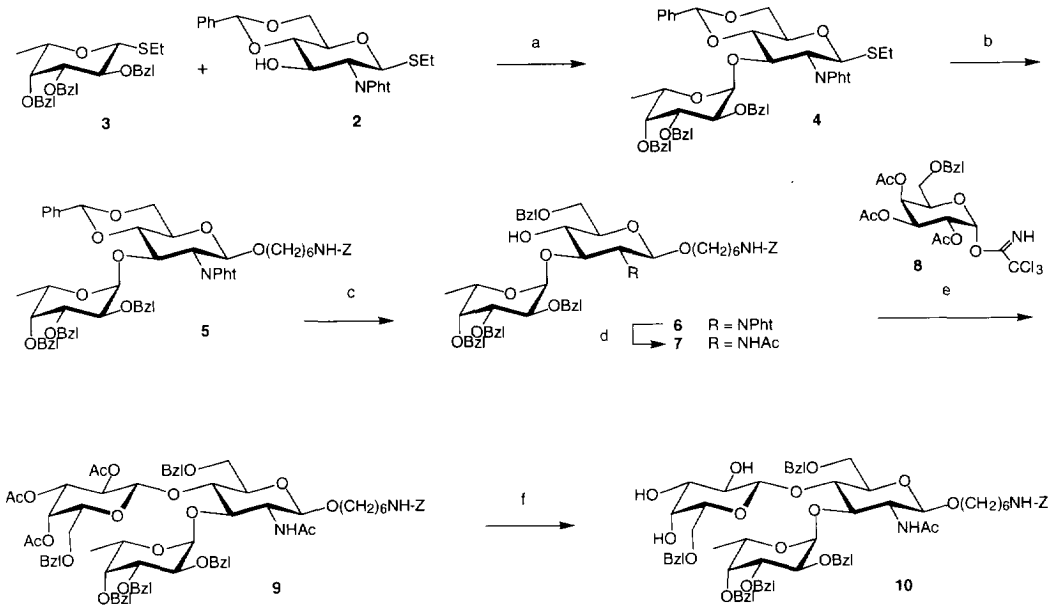

Figure 3: a) $CuBr_2$, Bu_4NBr, DMF-CH_2Cl_2 (71 %); b) NIS, triflic acid, Z-aminohexanol, CH_3CN (89 %); c) $NaCNBH_3$, HCl-Et_2O, THF (75 %); d) 1. ethylene diamine, n-butanol 80 °C, 4h; 2. Ac_2O, methanol, ethyl acetate (98 %); e) BF_3-OEt_2, molecular sieves 4 Å, CH_2Cl_2; f) NaOMe, MeOH (e-f: 47 %); Z = benzyloxycarbonyl-.

The modified sialic acids **12b**[14a] and **12c**[14b] were obtained enzymatically from 2-deoxy mannose **11b** or 2-azido mannose **11c** and pyruvate using sialic acid aldolase. Conversion to the thiomethyl donors **13a-c** was performed in a three step prodedure[15]: acid catalyzed esterification in methanol, followed by acetylation and thiomethylation with TMS-SMe/ TMS-triflate. This reaction sequence afforded the sialyl donors **13a-c** in good yields (Figure 4).

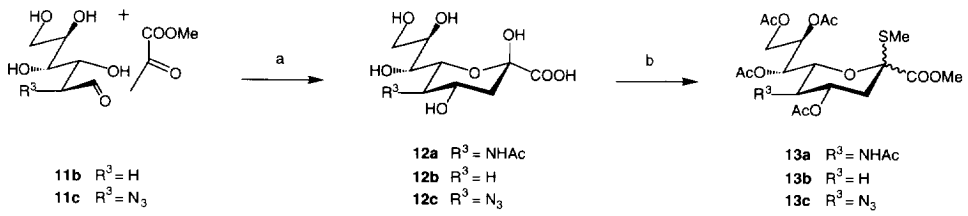

Figure 4: a) sialic acid aldolase b) 1. MeOH, H^+; 2. Ac_2O-pyridine, 3. TMS-SMe, TMS-OTf; **13a** (70 %), **13b** (79 %), **13c** (73 %).

Regioselective α-(2→3)-sialylation of the Le^x trisaccharide **10** was conducted under the conditions described[16] and afforded the tetrasaccharide **14 a** in 54 % yield for the N-acetyl neuraminyl donor **13a**. For the modified donors **13b** and **13c**, however, the yields were significantly lower.

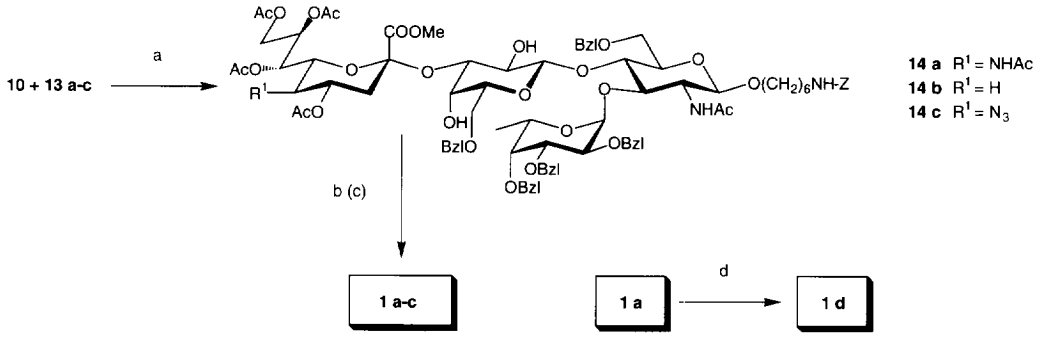

Figure 5: a) NIS, triflic acid, CH_3CN, -40 °C (**14a**: 54 %, **14b**: 21 %, **14c**: 26 %,); b) 1. NaOMe-MeOH 2. H_2-Pd-MeOH, HCOOH 3. NaOH-H_2O (**1a**: 70 %; **1b**: 72%); c) 1. K_2CO_3-MeOH-H_2O; 2. $HCOONH_4$-Pd/C(10%)-MeOH; (**1b**: 81%); d) Bu_4NOH-H_20, 95 °C, 3d (53 %).

Deprotection of **14b** can be performed under the conditions described for the deblocking of **14a**[17] using a three step procedure with formic acid as the hydrogen donor in the catalytic hydrogenation. The final basic treatment is needed to open the lactone ring that is formed in the deacetylation step. Despite the increased acid sensitivity of the 5-deoxy sialoside **1b** compared to the N-acetyl derivative **1a**, the acidic conditions are well tolerated. However, the neuraminyl compound **1c** decomposes in the presence of formic acid. We found a suitable method to deprotect the highly acid sensitive sialoside **1c** in a mild catalytic transfer hydrogenation with ammonium formate as the hydrogen source[18]. In contrast, the base stability of the tetrasaccharide **1a** is very high. Basic hydrolysis[19] of the two acetamides at pH 13 furnishes the *lyso*-sLe^X tetrasaccharide **1d** in 53 % yield after purification[20] by Sephadex chromatography. The four tetrasaccharides **1a-d** were then examined for their inhibitory potency[21] towards E- and P-selectin (Table 1).

	1a	**1b**	**1c**	**1d**
E-selectin IC_{50}	1000 µM	700 µM	900 µM	270 µM
P-selectin IC_{50}	2000 µM	700 µM	1000 µM	160 µM

Table 1: Inhibition of HL60 cell adhesion to recombinant E- and P-selectin-IgG fusion proteins by synthetic sLe^X tetrasaccharides **1a-d**. IC_{50} values are concentrations of inhibitors required to block adhesion of 50 % of the cells compared to the negative control[21].

Upon removal of the acetamido or acetyl group in the sialic acid moiety of **1a**, the inhibitory potency of the resulting derivatives **1b** and **1c** was slightly improved. Surprisingly, the binding affinity of the fully deacetylated *lyso*-tetrasaccharide **1d** to P-selectin was significantly enhanced by a factor of 12.5. In contrast, a recent study[22] reported that the blocking of immobilized P-selectin-Ig by oligosaccharides related to sLe^X and sLe^A was not enhanced when the GlcNAc residues carried an azido or amino function at C-2. For E-selectin the study found a 6 fold enhanced inhibitory potency of the $sLe^X(GlcNH_2)$ compared to sLe^X in the E-selectin-Ig competitive binding assay, albeit on much lower concentration levels in the cell-free systems used (IC_{50}s of 77 µM and 380 µM, respectively). These data are in agreement with the improvement of the IC_{50} of **1d** over the reference compound **1a**. Taken together, the data obtained strongly support our view of a synergic effect caused by the two amino groups of the *lyso*-tetrasaccharide **1d** resulting in an improved binding to E- and P-Selectin.

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