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Spiroketal Glycomimetics: The Synthesis of a Conformationally Restrained Sialyl Lewis X Mimic.

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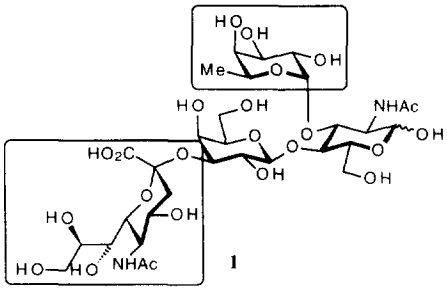
Abstract

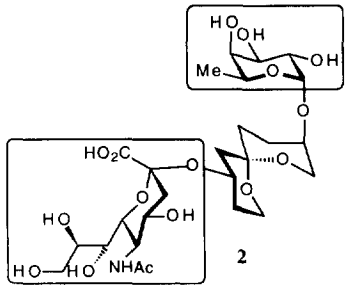
The synthesis of a spiroketal scaffold and its specific elaboration with N-acetyl neuraminic acid and fucose monosaccharides to yield the conformationally restrained Sialyl Lewis X mimic **2** is described.

Introduction

The Sialyl Lewis X (sLe^x) tetrasaccharide **1** is a carbohydrate ligand found on neutrophils that will support binding to the E-selectin glycoprotein which is inducibly expressed by endothelial cells, after stimulation, in areas of inflammation.[1] This sLe^x-E-selectin interaction results in the neutrophils rolling along the endothelium. Subsequent higher affinity protein-protein interactions mediated by the integrins and members of the immunoglobulin family, fix the neutrophils and allow extravasation into the site of tissue injury.[2] This extravasation process is normally strictly controlled as the migration of an excessive number of leukocytes can lead to host tissue damage which typifies many disease states such as ARDS and reperfusion injury.[3]



1



2

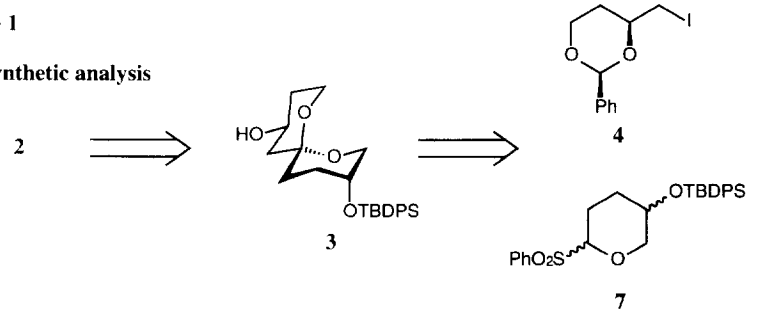
The therapeutic potential of blocking the sLe^x-selectin interaction has been amply demonstrated *in vivo* in animal models,[4] although, the expense of the sLe^x

tetrasaccharide **1**, its potential susceptibility to enzymatic modification *in vivo* and the low affinity for E-selectin (K_d 10^{-4}M *in vitro*) hamper this therapeutic potential.[5]

Not surprisingly intense research effort has focused on the search for sLe^x mimics with higher affinity and reduced oligosaccharide character. Thorough structure-activity studies indicate the necessity of the sialyl carboxylic acid, the fucose residue and to a lesser extent the 4- and 6- hydroxyl functionalities of the galactose.[6] The N-acetyl glucosamine residue appears to be acting as a scaffold to position the fucose relative to the galactose because modifications such as substitution by glucose and reductive ring cleavage of the N-acetyl glucosamine residue have little effect on activity *in vitro*.[7] These studies have stimulated the synthesis of several sLe^x mimics featuring replacement of the carbohydrate functionality. To date these have incorporated the essential carboxylic acid and fucose functionalities on a relatively unfunctionalized flexible linker and these have shown disappointing biological activity.[8] The degree of flexibilty or rigidity displayed by an oligosaccharide in solution and whether those conformations are similar to the conformation of the oligosaccharide bound to a carbohydrate binding protein is subject of much speculation, although it is clear that oligosaccharides are not completely rigid.[9] Detailed NMR studies on the sLe^x tetrasaccharide indicate the predominance of one conformer in aqueous solution and recent studies comparing the interaction of sLe^x with E-selectin have shown that the bound conformation of sLe^x differs only at the sialic acid residue to the predominant solution conformation.[10]

Scheme 1

Retrosynthetic analysis



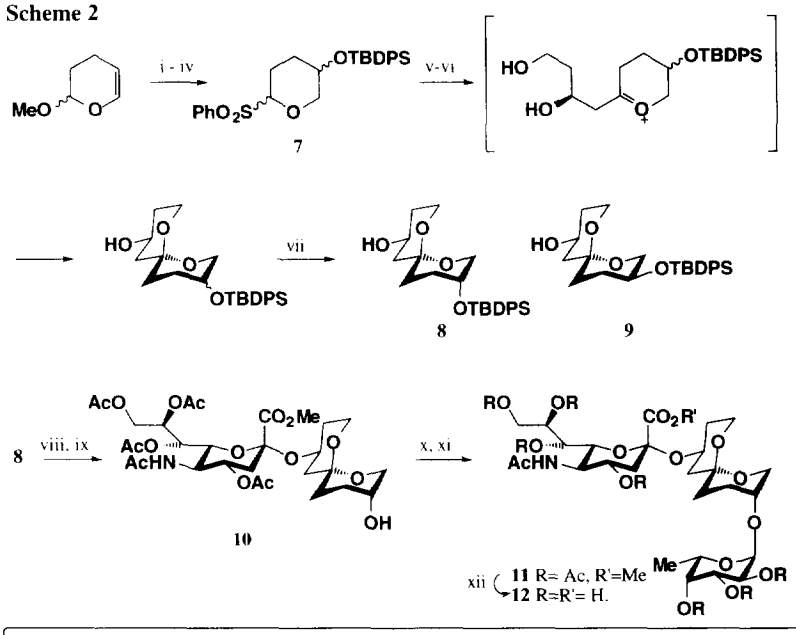
In a departure from these flexible sLe^x mimetics we have investigated the replacement of the N-acetyl glucosamine-galactose disaccharide with a rigid scaffold which would maintain the exact spacial orientation of the sialic acid and the fucose residues relative to sLe^x. Our first synthetic target was the minimally functionalised mimic **2**. Computer modelling suggested that the spiroketal **2** may be a good mimic of the tetrasaccharide **1**; excellent overlap of the fucose and the carboxylic acid residues in **2** and sLe^x was observed.[11] Additionally the sLe^x/E-selectin interaction is calcium

dependent and we envisaged that the oxygen lone pairs present on the spiroketal nucleus might contribute in the binding of calcium ions.

Results and Discussion

Initially the spiroketal alcohol **3** (Scheme 1) was chosen as our key intermediate as we wished to introduce the sialic acid moiety prior to the more labile α-fucosyl linkage.[12] Utilizing the 2-benzenesulfonyltetrahydropyran methodology developed within our group for the synthesis of spiroketals, alkylation of the anion generated from the racemic sulfone **7** (Scheme 2) with the known chiral iodide **4** and spontaneous elimination of benzene sulfininic acid gave an intermediate enol ether.[13] Subsequent acid-catalysed benzylidene acetal cleavage and concommitant spiroketalisation furnished the desired spiroketal scaffold as a mixture of diastereoisomers in 82% overall yield. Conversion of the alcohols **8** and **9** into their benzoate esters facilitated their separation by column chromatography and provided crystalline material which was amenable to X-ray analyis which confirmed their structural assignments.[14]

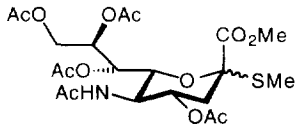
Scheme 2



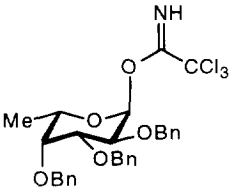
Reagents & Conditions

i) BH_3.DMS, NaOH/H_2O_2,80%, 2:1 *trans:cis*; Reference 17c ii) TBDPS-Cl, imidazole, DMF, 60 °C, 96%; iii) PhSH, cat. CSA, $CHCl_3$, reflux, 95%; iv) *m*CPBA, NaOAc, DCM, 97%; v) *n*BuLi, DMPU/THF, -78 °C, then Iodide **4**; vi) CSA, MeOH/Et_2O, 82% over two steps; vii) BzCl, DCM, pyr. rt, Then separation and NaOMe, Et_2O, 82% overall. viii) 2.5 eq donor **5**, NIS, cat. TMSOTf, MeCN, -40 °C, 78%, αβ, 3:1; ix) HF.pyr., pyr., THF, rt, 85% combined; x) 1.5 eq donor **6**, Et_2O, cat. TMSOTf, 95%, αβ, 4:1; then HPLC separation; xi) H_2, $Pd(OH)_2$, MeOH, rt, then Ac_2O, pyr., rt, 85%; xii) NaOMe, MeOH, H_2O, then Dowex H^+ resin, 85%.

The benzoylation, separation and cleavage procedure provided the diastereomerically pure alcohols **8** and **9** in 82% combined overall yield. Sialylation of the alcohol **8** with the known sialyl donor **5** at low temperature gave the sialosides as an inseparable mixture (α:β, 3:1) in good yield.[15]



5



6

Silyl ether cleavage was achieved using hydrofluoric acid pyridine complex buffered with pyridine to give a good yield of the alcohol **10** and the β anomer which were separable by column chromatography. Fucosylation with the trichloroacetimidate fucosyl donor **6**[16] and catalytic trimethylsilyl trifluoromethane sulfonate as activator gave the fucosides in excellent yield as an inseparable mixture of anomers. The desired α,α glycoside was purified by HPLC and deprotected in a two-step procedure. Firstly benzyl ether hydrogenolysis followed by acetylation gave the *per*-acetate methyl ester **11**[17] in 85% yield, then deacetylation followed by methyl ester hydrolysis and neutralisation with acidic resin went in 85% yield to give the target sLe^x glycomimetic **12**.

The sLe^x mimic **2** showed a low level inhibition of neutrophil/E-selectin binding (35% inhibition at 10mM, when compared with sLe^x IC_{50} 0.3mM).[18] Having established efficient methodology for the synthesis of glycomimetics built on a spiroketal core, work is underway to develop a second generation of mimetics which incorporate additional functionality to pick up additional binding sites.

Acknowledgements

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11. By displaying only the two hydroxyl substituents carrying the monosaccharides in fixed trajectories the spiroketal (2) is designed to be a minimum conformationally restrained sLe^x mimetic. The additional functionality not bonded directly to the sialic acid or the fucose although present in the Gal-GlcNAc disaccharide (in particular the galactose 4- and 6-hydroxyl) is not present in the mimetic (2). We treat the spiroketal motif as a scaffold which may be further elaborated with functionality displaying fixed orientation. We wish to thank Dr S. Lister and Dr N. Taylor for their valuable contribution in this area.
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14. The X-ray coordinates for compounds **8** and **9** are available from the Cambridge Crystallographic Data Centre.
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17. Data for compound **11**. The following abreviations are used, neu for N-acetyl neuraminic acid and fuc for fucose. All assignments were confirmed by COSY, NOESY, TOCSY and HMQC experiments.
δ_H(500MHz, $CDCl_3$) 5.39 (1H, dd, *J* 10.8, 3.3, H-3 fuc); 5.33-5.30 (3H, m, H-4 fuc, H-8 neu, H-7 neu); 5.21 (1H, d, *J* 9.4, NH); 5.17 (1H, d, *J* 3.8, H-1 α-fuc); 5.06 (1H, dd, *J* 10.8, 3.3, H-2 fuc); 4.89 (1H, m, H-4 neu); 4.41-4.34 (3H, m, H-5 fuc, H-9 neu, H-10ax); 4.12 (1H, dd, *J* 12.8, 4.1, H-9 neu); 3.95-4.05 (2H, m, H-5 neu and H-6 neu); 3.77 (3H, s, OMe); 3.69 (2H, bs, H-2ax, H-2eq); 3.66 (1H, dd, *J* 11.6, 4.4, H-8ax); 3.60 (1H, bd, *J* 11.6, H-8eq); 3.57 (1H, bs, H-3eq); 2.54 (1H, dd, *J* 12.8, 4.7, H-3eq neu); 1.87, 1.95, 2.01, 2.02, 2.03, 2.11, 2.14 (each 3H, s, Ac); 1.95-2.05 (3H, m, H-11eq, H-4 or H-5 and H-3ax neu); 1.85-1.70 (2H, m, H-9eq, H-4 or H-5); 1.60 (1H, bd, J 13); 1.52 (1H, dq, *J* 12.5, H-9ax); 1.45 (1, bd, J 13); 1.39 (1H. t, *J* 12, H-11ax); 1.14 (3H, d, *J* 6.5, fuc-Me). δ_C (100MHz, $CDCl_3$) 171.05, 170.71, 170.51, 170.29, 169.96, 168.85 (q, CO); 98.45, 97.15 (q, C-6 and C-2 neu); 95.10 (CH, C-1 fuc); 72.58, 71.27, 70.91, 69.19, 68.56, 68.14, 67.57, 67.38, 64.51, (CH); 62.99 (C-9, neu); 61.99, 59.15 (CH_2); 52.85 (CO_2Me); 52.80, (CH); 49.55 (C-5 neu); 43.23 (CH_2); 37.28 (CH_2, C-3 neu); 33.00, 29.72 (CH_2); 23.25 ($COCH_3$); 22.39 (CH_2); 21.09, 20.77 ($COCH_3$); 15.97 (CH_3, fuc). $[\alpha]_D^{29}$ = -116.3, (c= 0.18, $CHCl_3$), m/z (*M* $^+NH_4$ electrospray) 950.9, Found C, 52.95; H, 6.45; N, 1.47%. $C_{41}H_{58}NO_{23}$ requires C, 52.79; H, 6.27; N, 1.50%.
18. We thank R. Priest for performing this assay. The sLe^x analogue **2** was assayed for its ability to inhibit the binding to E-selectin immobilised on SPA beads of radiolabelled HL-60 cell membrane containing sLe^x.[19]
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