Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 6, No. 3, pp. 239-244, 1996

0960-894X/96 $15.00 + 0.00

S0960-894X(96)00008-X

Synthesis and Biological Activity of Conformationally Constrained Sialyl Lewis X Analogues with Reduced Carbohydrate Character.

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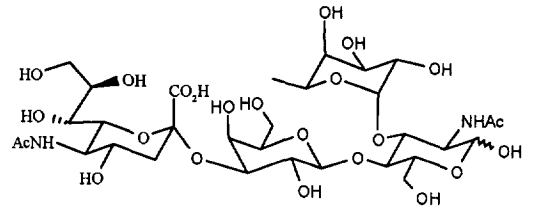
Abstract. Two conformationally constrained analogues of sialyl Lewis X have been synthesised in which the GlcNAc residue has been replaced with cyclohexyl and phenyl rings. The cyclohexyl derived compound was equipotent to sLex and sLea *in vitro*, suggesting the main role of the GlcNAc residue in sLex is conformational, and represents a simplified inhibitor of adhesion.

Cell adhesion processes are integral to a number of therapeutic areas including infection, cancer, and inflammation. In particular, neutrophil-endothelial cell recognition is an initial stage in the inflammatory response and involves the rolling of the neutrophil along the cell surface.[1] This is mediated[2] by the interaction of the terminal tetrasaccharide unit, sialyl Lewis X (sLex) **1**, of a constitutive neutrophil surface glycoprotein with the E-selectin protein on human endothelial cells. *In vivo* experiments suggest that if selectin / sLex interaction is antagonised then inflammation is substantially reduced[3] and indeed, the sLex tetrasaccharide itself has been shown to inhibit the adhesion process *in vivo*.[4] However, the molecule is of high molecular weight, is extensively functionalised with polar groups, and contains three glycosidic bonds potentially susceptible to glycosidase enzymes. In the light of this, the syntheses of a number of analogues have been investigated by several research groups.[5] As a result, it has been shown that both the fucose and sialic acid (Neu5Ac) residues of sLex were required for recognition by the protein,[6] but that the key structural feature of the Neu5Ac is simply the carboxylic acid moiety.[7] Thus, the 3'-carboxymethyl substituted analogue **2** has similar antagonist affinity[8] to sLex. Sialyl Lewis A (sLea) **3**, a naturally occuring regioisomer of sLex, also binds to E-selectin with similar affinity and it has been shown that the *N*-acetylglucosamine unit of sLex can be replaced by glucose to give **4** with no apparent loss of affinity.[9] It is, therefore, apparent that the N-acetylglucosamine unit may be acting primarily to correctly orientate the acid-bearing galactose moiety and the fucose moiety.

As a first step to producing analogues of reduced glycosidic character and molecular weight but retained antagonist activity, and to testing the hypothesis that the GlcNAc acts primarily as an appropriate template, we have recently reported[10] the synthesis of the novel sLex analogue **5** as an example of an unconstrained system. The C_2-symmetric nature of the 2,3-butanediol linker renders **5** a potential mimic for both sLex and sLea.



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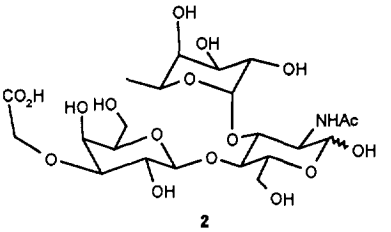

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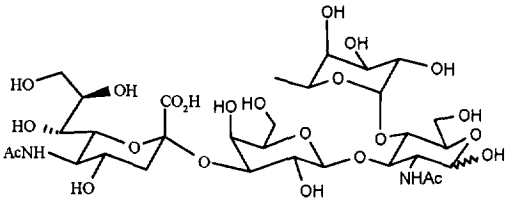

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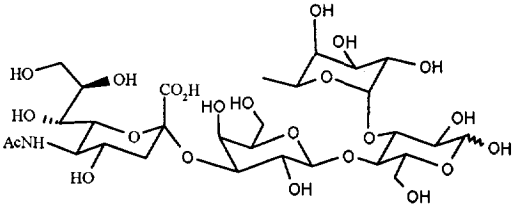

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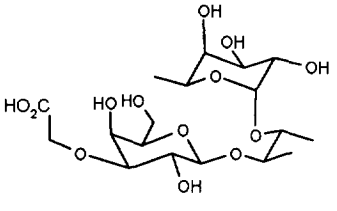
Herein, we report on the replacement of the GlcNAc residue with the conformationally constrained, C_2-symmetric 1,2-cyclohexanediol moiety, to give **6**, and the catechol moiety, to give **7**. The synthesis of the latter involves the use of a base-mediated, phase-transfer method of glycosidation. Both starting diols are commercially available, 1,2-cyclohexanediol as a single enantiomer, and offer the opportunity for subsequent introduction of functionality to probe for additional interactions. Again, the Neu5Ac residue of sLex has been replaced with a simple carboxymethyl substituent. We also report the biological activity of **5**, **6** and **7**.



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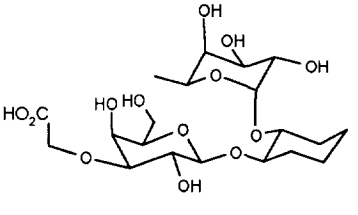

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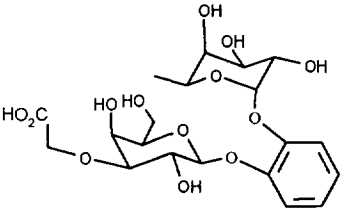
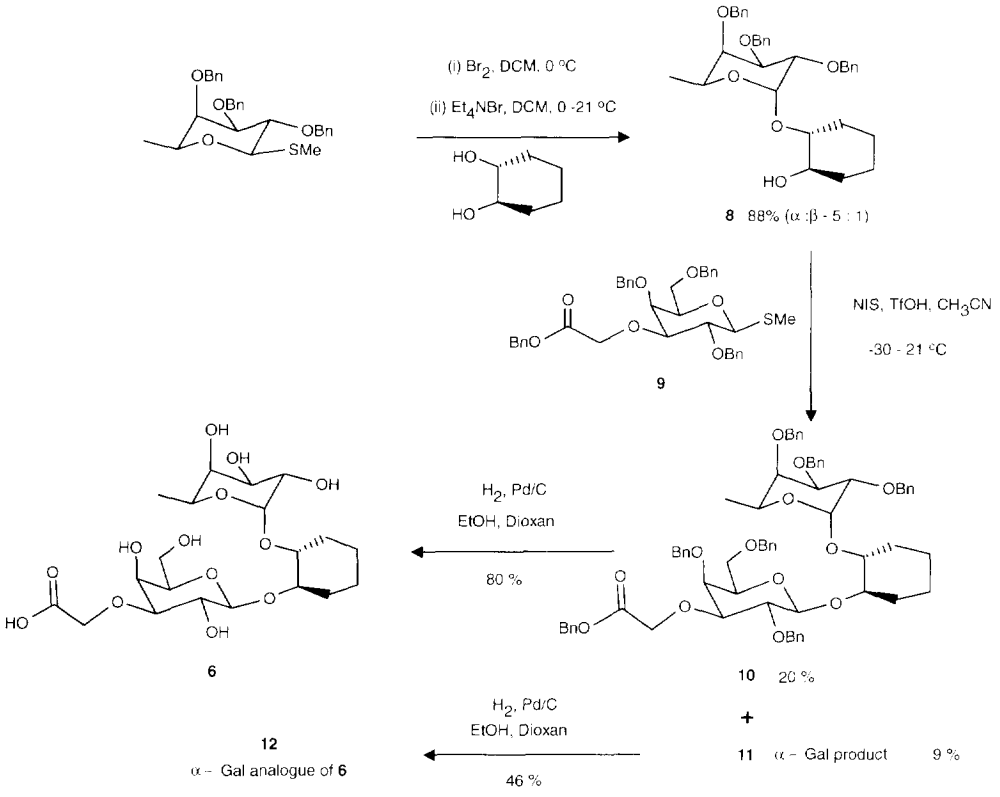


7

The 1,2-cyclohexanediol derived compound **6** was synthesised according to the route shown in Scheme 1. (1R,2R)-*trans*-1,2-cyclohexanediol was coupled with methyl 2,3,4-tri-*O*-benzyl-1-thio-β-L-fucopyranoside[10] using a halide-mediated glycosidation protocol[10,11] to give the required fucose-diol adduct **8** in excellent yield

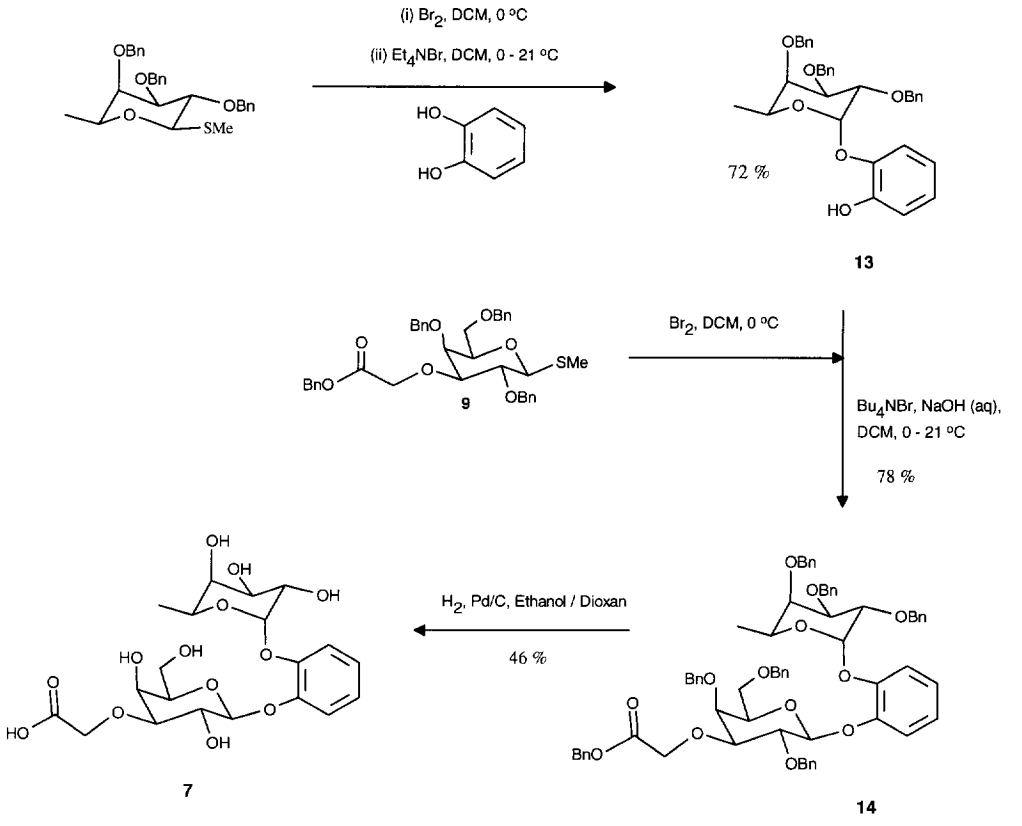
(5:1 mixture of α– and β–anomers, separable by HPLC). Activation of the galactose-acid donor **9**,[10] using *N*-iodosuccinamide (NIS) / triflic acid (TfOH)[12] as promotor in CH_3CN, gave a mixture of the α- and β-galactosides **11** and **10** in 29 % combined yield and a ratio of 1 : 2.3 (unoptimised), which were separated by flash column chromatography. The predominant required β-anomer **10** was hydrogenolysed to give the desired product **6** in good yield. In a similar manner, the Gal α–isomer **12** was also formed.



Scheme 1

The modified route shown in Scheme 2 was used to introduce an aromatic constrained GlcNAc replacement to give **7**. The usual halide mediated glycosidation procedure gave the required fucose-diol adduct **13** in good yield as a single anomer. Use of the same conditions as in Scheme 1 for the reaction of this with **9** in the formation of **14** proved unsuccessful and so an alternative strategy was utilised[13] in which the anion of **13** was the nucleophile, generated under phase transfer conditions. Generally glycosidation with phenols to give β-anomeric products has been achieved with directing groups at position 2.[14] With non-participating groups at position 2, non-phase-transfer conditions give the predominance of α-products[14] due to the anomeric effect. However, it has been shown[13] that use of the phase transfer conditions can give β-products, although no clear reasons were given.[13] Therefore, the thiogalactose intermediate **9** was converted to the bromide *in situ*, and subsequently reacted under phase-transfer conditions with the catechol anion to afford the coupled β-

galactoside **14** in excellent yield as a single anomer. The glycosyl bromide, generated *in situ* for both steps, exists in solution as an equilibrium mixture of the two anomers, in which the α-anomer is favoured due to the anomeric effect. Thus, the first catechol hydroxyl reacts preferentially with the more reactive β-glycosyl bromide presumably under thermodynamic control, whilst the more reactive anion of **13** is able to react with the α-glycosyl bromide, to give the kinetic β-glycoside product **14**, and could explain the observed stereochemical control. Catalytic hydrogenation of **14** to remove the benzyl protecting groups gave the desired **7** in 46 % yield.



Scheme 2

Compounds **6**, **7** and **12**, as well as compound **5**,[10] were assayed[15] for their ability to inhibit the binding to E-selectin immobilised on SPA beads of radiolabelled HL60 cell membrane containing sLex. Results are shown in the Table.

Table: Inhibition of HL60 cell, sLex mediated adhesion to E-selectin.

Compound	IC50 (mM)
sLex	0.3
5	2.1
6	0.5
7	>10
12	>10

SLex and the significantly simplified analogue **6** can be considered to be equiactive. Compound **5** is less active than compound **6** thus demonstrating the advantage of the more highly constrained system. The somewhat lower activity of an sLex analogue with an unconstrained GlcNAc replacement (ethylene glycol) has recently been similarly noted.[5] Since the cyclohexyl template may be considered merely an underivatised form of the GlcNAc template with approximately similar conformational constraint, it is apparent that the role of the GlcNAc residue in sLex and sLea is largely conformational. The catechol derivative **7** was found to be inactive (IC_{50} >10 mM) in this assay, and the importance of the conformational effect of the cyclohexyl template is therefore supported. Compound **12** was inactive, consistent with our hypothesis regarding appropriate relative positioning of the Gal-acid and Fuc residues.

Compound **6** represents a highly interesting advance over sLex in furnishing a compound of lower molecular weight, reduced synthetic complexity, modified physical properties, and of reduced carbohydrate character. We are currently investigating whether introduction of functionality on this template will afford additional interactions not present in sLex / sLea.

Acknowledgements. The authors wish to express their sincere gratitude to Miss E. Draeger for technical support in the synthesis of intermediates, and Dr. R. Upton for NMR measurements and analyses.

All new compounds tested were characterised based on their ^1H NMR, MS, and elemental analysis.

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15. E-Selectin-dependent adhesion was determined in a Scintillation Proximity Assay (SPA). The characteristics of adhesion in this assay are similar to those reported for a conventional cell-based adhesion assay (Priest, R., Nawaz, S., Green, P. M. and Bird, M. I. *Biochem. Soc. Trans.* **1995**, *23*, 162S). In brief, recombinant human E-selectin-ZZ is immobilised on SPA beads (Amersham, UK) *via* an IgG linking. Cell surface glycoproteins from HL60 cells (a human myelocytic cell line) are radiolabelled by incubation of cells with [^{3}H]-methionine for 16 h. Radiolabelled cell membranes are then prepared and incubated with the E-selectin-coated beads in a Ca^{2+}-containing buffer at pH 7.2 for 30 min at room temperature. The amount of bound ligand is determined by scintillation counting. In this assay, the binding of membranes is inhibited by EDTA and antibodies to E-selectin, and by pre-treatment of the membranes with neuraminidase.

(*Received in Belgium* 4 *August* 1995; *accepted* 18 *December* 1995)