Exploring the Binding Site

of E-Selectin

from an Enthalpic or Entropic point of view

Inauguraldissertation

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Lionel Tschopp

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Auf Antrag von:

Prof. Dr. B. Ernst, Institut für Molekulare Pharmazie, Universität Basel,

Prof. Dr. J. Eustache, Ecole Nationale Supérieure de Chimie de Mulhouse

Basel, den 19.09.2006

Prof. Dr. Hans-Peter Hauri

Dekan

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Institute of Molecular Pharmacy
Pharmacenter
University of Basel
Klingelbergstr. 50-70
CH-4056 Basel
Switzerland

Declaration

"I declare, that I wrote this thesis *Exploring the Binding Site of E-Selectin from an Enthalpic or Entropic point of view* with the help indicated and only handed it in to the faculty of science of the University of Basel and to no other faculty and no other university."

Lionel Tschopp Basel, the 19th of September 2006

Acknowledgements

I would like to warmly thank Professor Beat Ernst for welcoming me in his group of the Institute of Molecular Pharmacy and for his support along these three years of doctoral work.

Thank you Professor Eustache, for accepting to be the co-referee of this work, but also for being one of the professors that gave me the will for working in medicinal chemistry.

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I do not want to forget all the other members of the Institute who I met during my PhD, among them Dr Michele Porro, Dr Claudia Riva, Dr Oliver Schwardt and Dr Said Rabbani.

Last but not least, I would like to thank the Swiss National Fund for Research for funding the major part of the present work.

A Yanis, et à sa maman Zineb

A mon père

Abstract

E-selectin is a member of a family of cell-adhesion proteins, which plays a crucial role in many physiological processes and diseases [1], and in particular, in the early phases of the inflammatory response. Its role is to promote the tethering and the rolling of leukocytes along the endothelial surface [2]. These steps are then followed by integrin-mediated firm adhesion and final transendothelial migration. Therefore, control of the leukocyte-endothelial cell adhesion process may be useful in cases, where excessive recruitment of leukocytes can contribute to acute or chronic diseases such as stroke, reperfusion injury, psoriasis or rheumatoid arthritis [3].

In this work, efforts to develop *in silico*-based protocols to study the interaction between E-selectin and its ligands, are presented. Hence, different protocols had to be developed and validated. In particular, a new procedure for the analysis of the conformational preferences of E-selectin antagonists was established and the results compared to those obtained with the MC(JBW)/SD approach, which had already demonstrated its validity in the past [161,168]. Thus, the comparison between the two protocols permitted to recognize a different conformational preference of the two methods for the orientation of the sialic acid moiety of sLe^x (**3**) (torsions Φ_3 and Ψ_3, *Figure* A), which reflects the contrasting opinions existing for the conformation adopted by sLe^x (**3**) in solution [150–168]. A more detailed analysis revealed that probably both approaches deliver only a partially correct view and that in reality, in solution, sLe^x (**3**) exists as a mixture of low energy conformers and not as supposed to date [150–154,161–163] as a population of a single conformer.

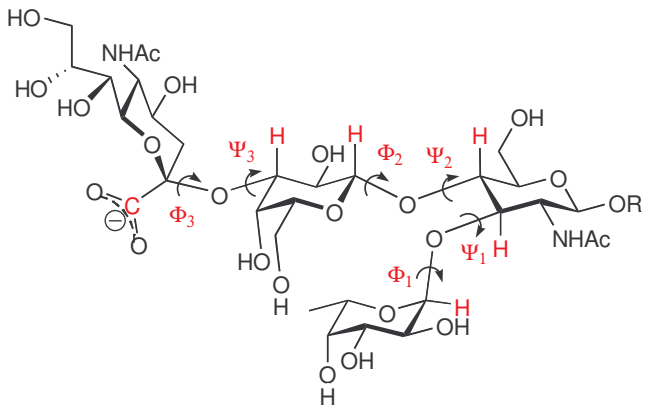

Figure A: sLe^x (**3**) and the Φ, Ψ convention for the definition of the glycosidic torsions.

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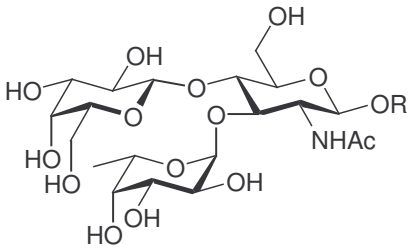
Index of the abbreviations

Ac	acetyl
alox	alumina or aluminium oxide
Arg	arginine
Asc	ascorbate or ascorbic acid
Asn	asparagine
Bn	benzyl
Bu	butyl
Bz	benzoyl
CAN	cerium ammonium nitrate
CD34	cell differentiation antigen 34 or sialomucin
CR	consensus repeat
CRD	carbohydrate recognition domain
dba	dibenzylidene acetone
DCE	1,2-dichloroethane
DCM	dichloromethane
DDQ	2,3-dicyano-5,6-dichloro-parabenzoquinone
DME	1,2-dimethoxy ethane
DMF	dimethyl formamide
DMTST	dimethyl(methylthio)sulfonium triflate
EGF	epidermal growth factor
ELISA	enzyme-linked immunosorbent assay
ESL-1	E-selectin ligand 1
Et	ethyl
Fuc	fucose
FucT III	fucosyl tranferase III
Gal	galactose
GDP	guanosyl diphosphate
GlcNac	*N*-acetyl-glucosamine
Glu	glutamic acid or glutamate
Gly-CAM-1	glycosylated cell-adhesion molecule 1
hIg	human immunoglobuline

HPLC	high pressure liquid chromatography
HUVEC	human umbilical vein endothelial cell
IC_{50}	inhibitory concentration for 50 % inhibition
ICAM-1	intercellular vascular cell-adhesion molecule 1
IgG	immunoglobuline G
Il-1	interleukine 1
i-Pr	isopropyl
IR	infrared spectroscopy
JAM-A	junctional adhesion molecule A
Lac	lactic acid
LC/MS	mass spectrometry coupled with liquid chromatography
Le^a	$Lewis^a$
Lec	lectin domain
Le^x	$Lewis^x$
LPS	lipopolysaccharide
Lys	lysine
MAdCAM-1	mucosal vascular addressin cell adhesion molecule 1
MBP-A	mannose binding protein A
Me	methyl
MMP	matrix metalloproteinases
MS	mass spectrometry
Napht	naphtyl
NeuNAc	*N*-acetyl-neuraminic acid
NMR	nuclear magnetic resonance
nOe	nuclear Overhauser effect
NOESY	nuclear Overhauser effect spectroscopy
OSE	trimethylsilylethyl
PAB	*para*-(*N*-methyl-*N*-phenyl)amino-benzyl
PBB	*para*-bromobenzyl
Pd/C	palladium on charcoal
Ph	phenyl
PMB	*para*-methoxybenzoyle
PMN	polymorphous neutrophils
PSGL-1	P-selectin glycoprotein ligand 1

Quant.	quantitative
r.t.	room temperature
rIC_{50}	inhibitory concentration for 50 % inhibition relatively to sialyl Lewis^x
ROESY	rotating frame Overhauser effect spectroscopy
SAR	structure-activity relationships
Ser	serine
SLe^a	sialyl Lewis^a
SLe^x	sialyl Lewis^x
SN2	nucleophilic substitution type 2
STD	saturation transfer difference
Tal	talose
TEAB	tetraethylammonium bromide
Tf	triflyl or trifluoromethyl sulfonyl
TLC	thin-layer chromatography
TMS	tetramethylsilane
TNF-α	tissue necrosis factor α
Tos	tosyl or toluene sulfonyl
Tyr	tyrosine
Tzl	triazole
VCAM-1	endothelial vascular cell-adhesion molecule 1

Index of the molecules



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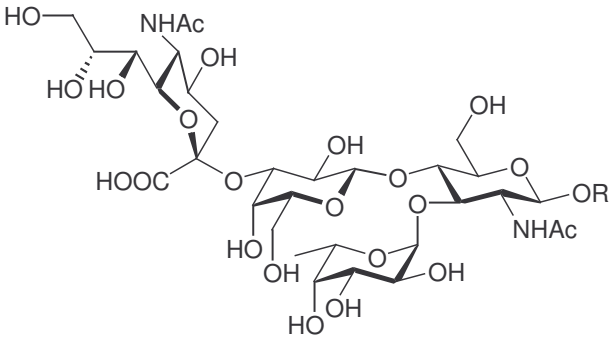

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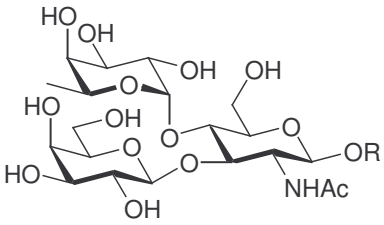

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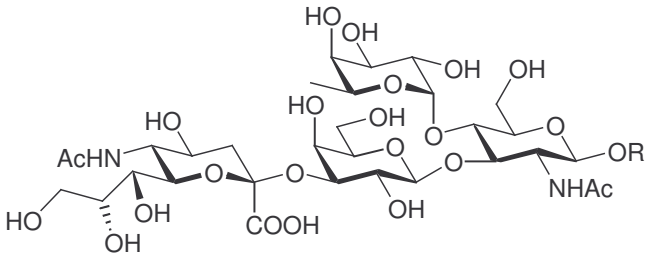

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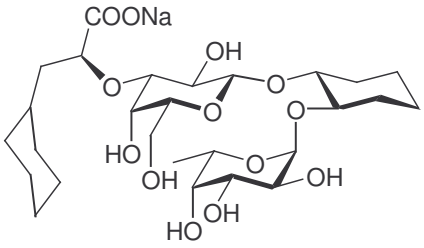

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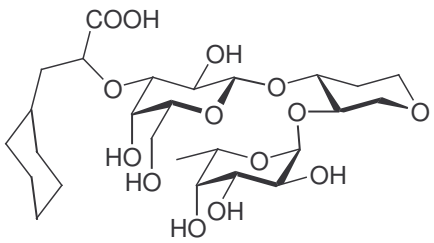

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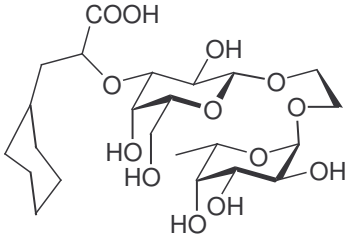

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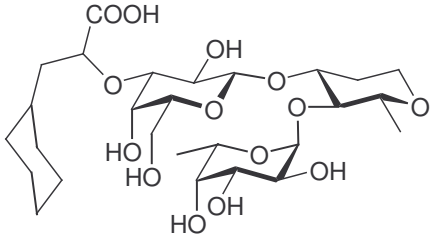

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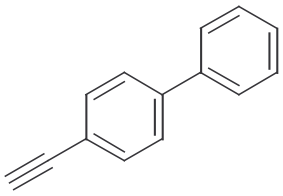

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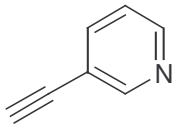

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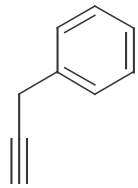

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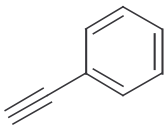

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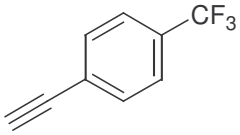

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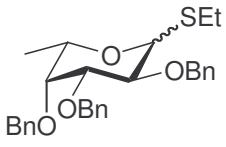

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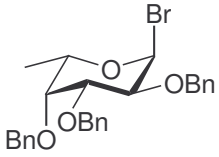

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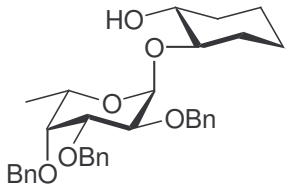

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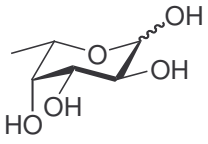

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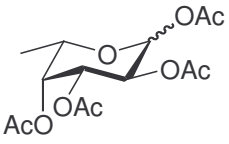

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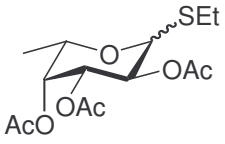

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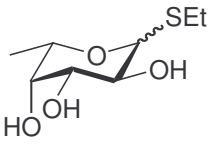

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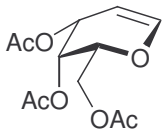

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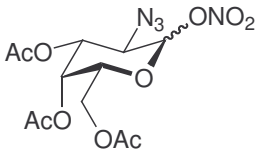

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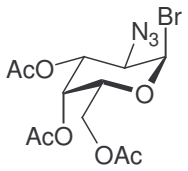

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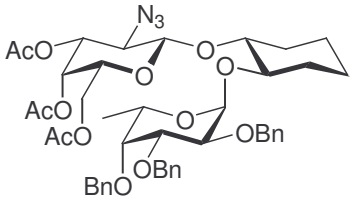

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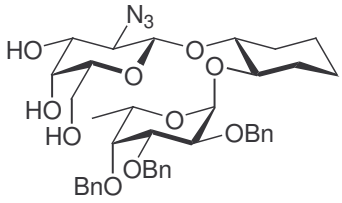

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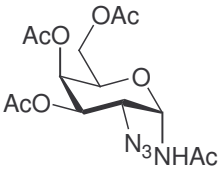

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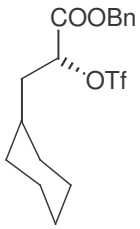

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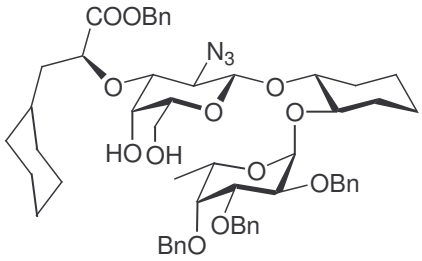

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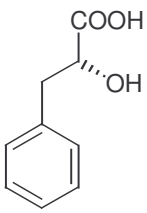

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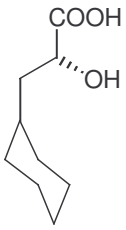

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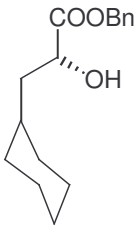

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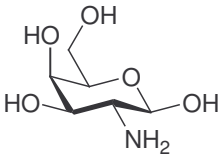

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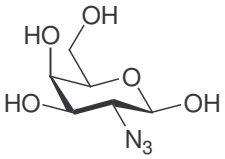

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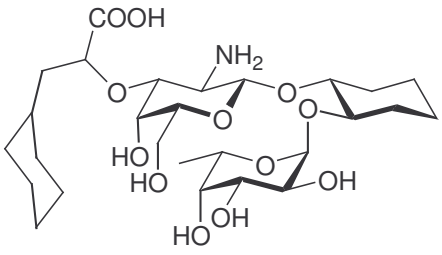

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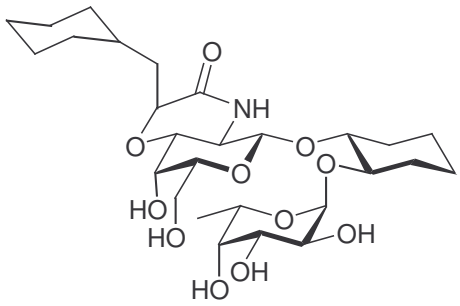

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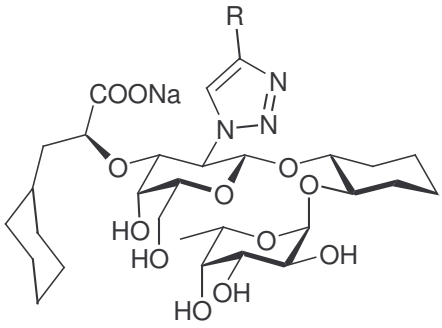

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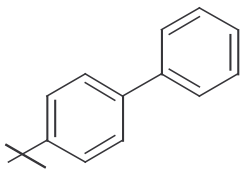

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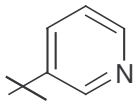

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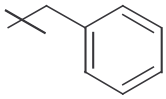

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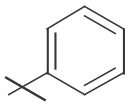

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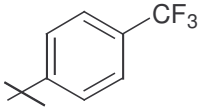

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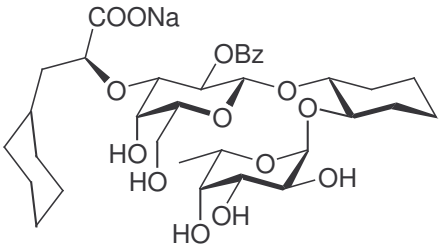

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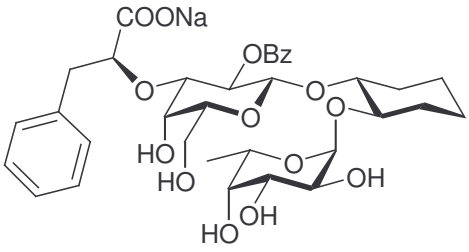

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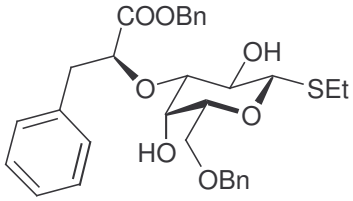

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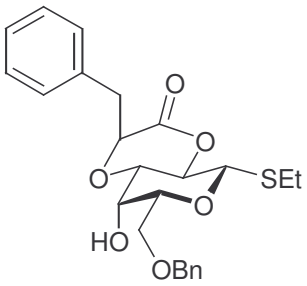

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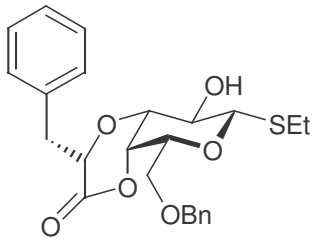

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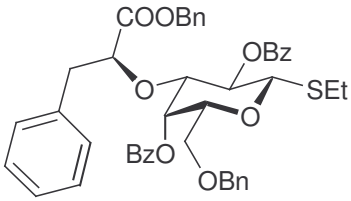

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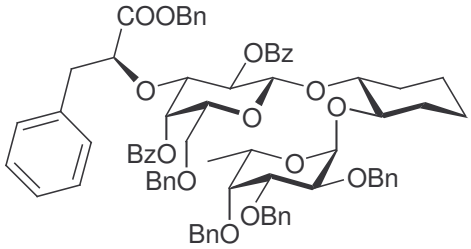

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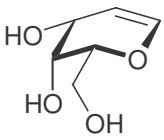

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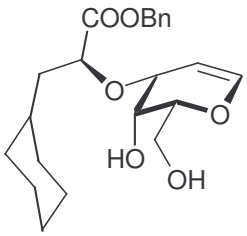

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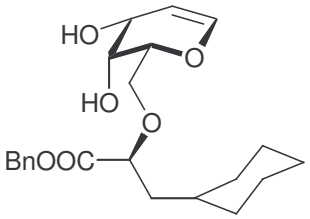

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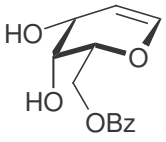

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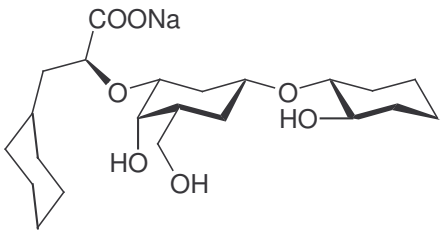

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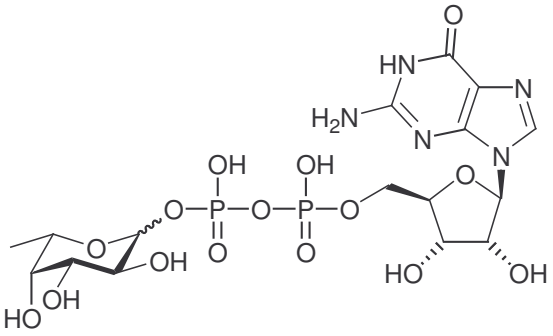

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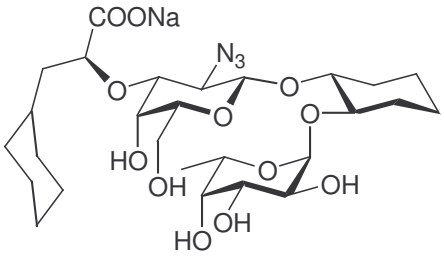

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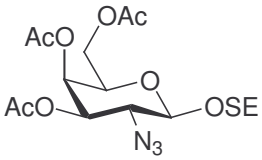

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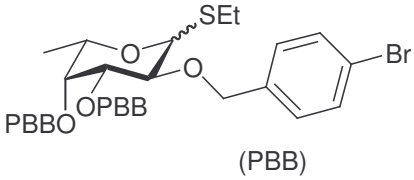

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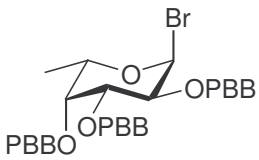

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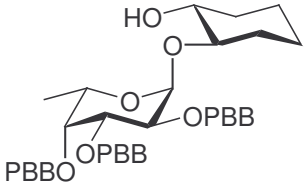

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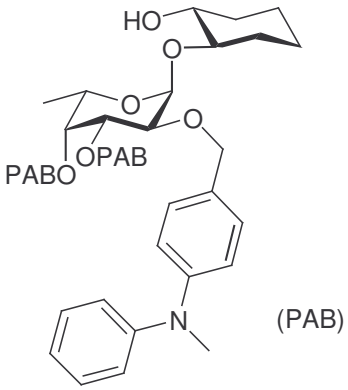

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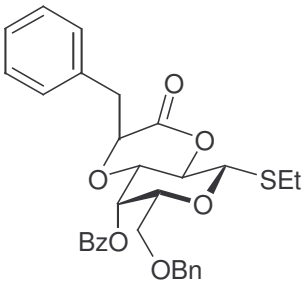

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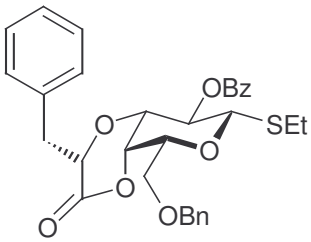

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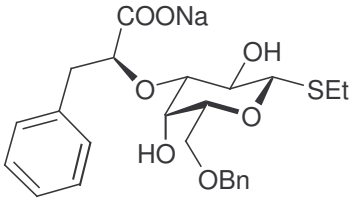

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A. General introduction

Selectins form a family of cell-adhesion proteins. They can be found mainly on the surface of vascular endothelial cells (E- & P-selectin), platelets (P-selectin) and leukocytes (L-selectin). They play a crucial role in many physiological processes, among them inflammation [1].

Inflammation is a key biological process for the defense of the organism. Its regulation is therefore very important, as any malfunction can lead to deleterious effects. In a healthy person, tissue injury, or invasion by pathogens usually triggers the release of inflammatory mediators like chemokines or platelet-activating factors [2,3], initiating a complex cascade of reactions that finally lead to the migration of leukocytes to the inflammatory stimulus, and thereby to the healing of injuries or the elimination of pathogens. In case of excessive leukocyte extravasation, massive cell-death and various disease conditions [4,5], like arthritis or myocardial ischemia-reperfusion injury can take place.

In more recent studies, it has also been suggested [6-8] that certain cancer cells travelling in the blood stream exploit the E-selectin-mediated adhesion process to metastasize, using the same route leukocytes use to cross the endothelial membrane.

The development of drugs mediating the adhesion of leukocytes would therefore be of great interest for controlling inflammatory processes, as well as for cancer therapies.

A.I. Selectins and the mediation of leukocyte adhesion

A.I.1. The selectin family

Lectins are carbohydrate-recognizing proteins that are divided in four families [9], namely :

- C-type lectins (**c**alcium), which binding site contains one or more calcium ions.
- S-type lectins (**s**ulfur) also called galectins, which contain free thiol groups.
- P-type lectins (**p**hosphor), which specifically recognize phosphorylated mannose residues.
- Other lectins, not fitting in the three previous categories.

It is to note that enzymes modifying carbohydrate residues are not called lectins, which only recognize and bind to them.

Selectins are membrane bound proteins belonging to the class of the C-lectins, as they all contain a calcium ion in their binding site. Three different selectins have been described so far : E-, P- and L-selectin, the first letter corresponding to the location where they were first identified [10] : E-selectin can be found on the surface of **e**ndothelial cells, as well as P-selectin. P-selectin originally was identified in α-granules of **p**latelets [11,12], while L-selectin was identified on most types of **l**eukocytes. E-selectin in particular was first described in the late 1980's [13] as an antigen of two cell-adhesion mouse monoclonal antibodies, namely H 18/7 and H 4/18. Cloning and characterization [14] in 1989 revealed a C-type lectin, consisting of 589 amino acids, with a molecular weight of 64 kDa in its mature state.

The structures of all three selectins show a high similarity and consist (see figure 1) of five different domains [15,16]. The extracellular part contains a *carbohydrate recognition domain* (CRD, also called *lectin* domain, Lec) at the N-terminus, which is stabilized by a calcium ion. It is 120-130 amino acids long (depending on the selectin type) and is followed by a 35-40 amino acids domain called *epidermal growth factor-like* (or EGF) domain. Although carbohydrate ligands bind specifically to the CRD, it has been shown that the EGF domain is also mandatory for binding, probably stabilizing the conformation

of the CRD [17,18]. The active part of the protein (CRD-EGF or Lec-EGF domain) is separated from the cellular membrane by several repetitive units, called *complement regulatory-like* or *consensus repeat* (CR) domains, each about 60 amino acids long. L-selectin contains two CRs, while E-selectin contains six, and in P-selectin the number varies from four to nine. It was found in case of P-selectin [19], that although those elements are not required for the binding of selectins to their ligands, they play a role in the leukocyte rolling efficiency. This suggests that the CR domains may act as spacers, keeping the CRD at a distance far enough from the cell surface for optimal action. Finally, the transmembrane domain is followed by a short (17-34 amino acids) cytoplasmic *C*-terminal tail, supposedly involved in signal transduction [20].

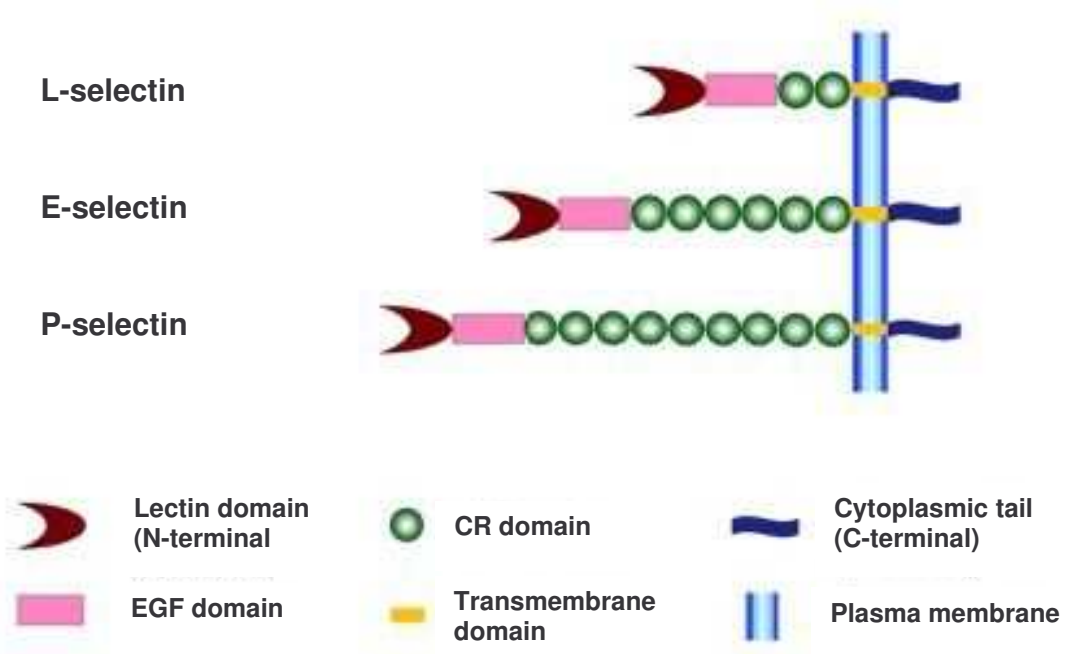


Figure 1 : Schematic representation of the three members of the selectin family.

A.I.2. The inflammatory cascade

In a healthy organism, pathogen invasion or tissue injury usually triggers the release of inflammatory mediators like chemokines or platelet-activating factors [2,3]. These mediators act as a signals initiating and directing the migration of leukocytes through the endothelial membrane of blood or lymphatic vessels to the site of damage. This leukocyte migration is a complex action that can be divided into a series of steps, referred to as the inflammatory cascade [21,22] (see figure 2).

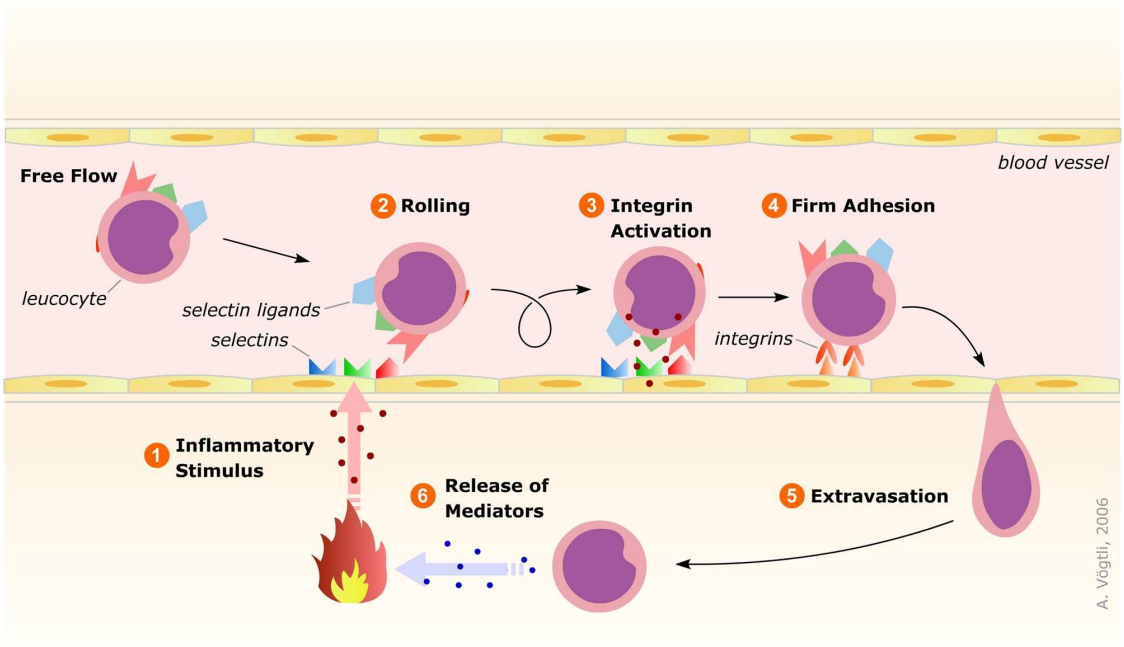


Figure 2 : The inflammatory cascade.

Upon release of the inflammatory mediators (e.g. histamine, thrombin, TNF-α, IL-1 or LPS), the stimulated endothelial cells present E- and P-selectin at their surface. P-selectin, already present in the *Waibel-Palade* bodies of the cells is rapidly transported to the cell surface, and expressed within seconds to minutes after stimulation by thrombin or histamin [23,24] and exposed for 30-60 minutes before subsequent internalization. E-selectin, in contrast, has to be *de novo* synthesized, and its production is induced by TNF-α, IL-1 or LPS [13,25]. Its highest expression level is thus detected after three to four hours. It is then internalized again. Once one or both P- and E-selectin

are present, they can interact with the leukocytes travelling into the lymph or blood flow, initiating the inflammatory cascade.

First step : Tethering and rolling.

Natural ligands of E- and P-selectin (ESL-1, PSGL-1, etc. see part **A.I.3**) are present at the surface of the leukocytes. Their interaction with the selectins is characterized by fast association and dissociation processes [26-28], that aim to slow down the leukocyte flow. Once interacting with the endothelial monolayer (tethering), leukocytes roll on the surface of the blood vessels (see figure 3).

L-selectin also plays an important role in this first step. Indeed, it is expressed by leukocytes and able to interact in particular with E-selectin [29], but also with PSGL-1 ligands present on the already tethered leukocytes, increasing the number of leukocytes attracted to the inflammation site.

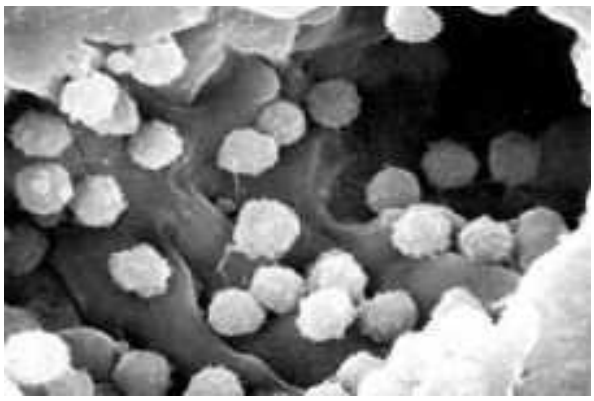


Figure 3 : Leukocytes rolling on the surface of a vessel [30].

Second step : Integrin activation.

The close interaction of leukocytes with the endothelial surface leads to a contact with cytokines released by the inflammation process. This triggers the activation of leukocytes integrins [26,31]. Integrins are important players in the adhesion process and therefore also play an essential role in the inflammatory cascade.

Third step : Firm adhesion.

Activated integrins on the surface of leukocytes can now play their role. They interact with their endothelial ligands (namely the immunoglobulines VCAM-1 and ICAM-1, and others like MAdCAM-1 [26,31], cf. figure 6) stronger than selectin mediated interactions, thus stopping the leukocytes' rolling.

Fourth step : Extravasation or transendothelial migration.

When leukocytes finally firmly adhere to the endothelial membrane, they can cross it, to the site of inflammation (figure 4). This process in itself is complex and not completely understood. It has already been shown that leukocytes may use both the paracellular and transcellular routes [32]. Transmigration is probably facilitated by extracellular proteases, such as matrix metalloproteinases (MMPs) as well as by some junctional adhesion molecules (JAM-A, -B or -C) [33].

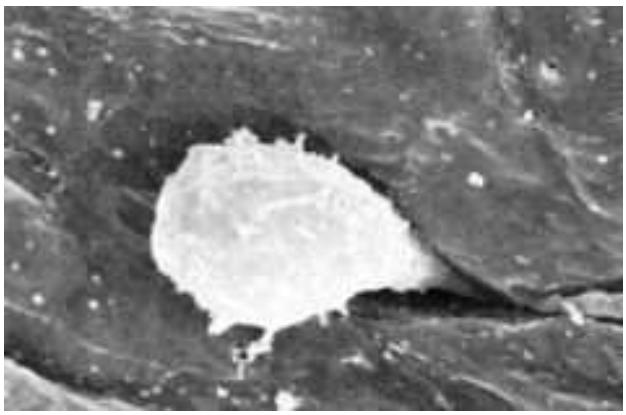


Figure 4 : A leukocyte transmigrating to the site of an inflammation [30].

A.I.3. The natural ligands of the selectins

As selectins are carbohydrate binding proteins, their natural ligands are glycolipids or glycoproteins. More precisely, it is generally accepted that ligands containing the trisaccharides Lewis^x (Le^x, **1**), Lewis^a (Le^a, **3**) or the sialylated tetrasaccharides sialyl Lewis^x (sLe^x, **2**) and sialyl Lewis^a (sLe^a, **4**) shown in figure 5 would bind the selectins [34-36].

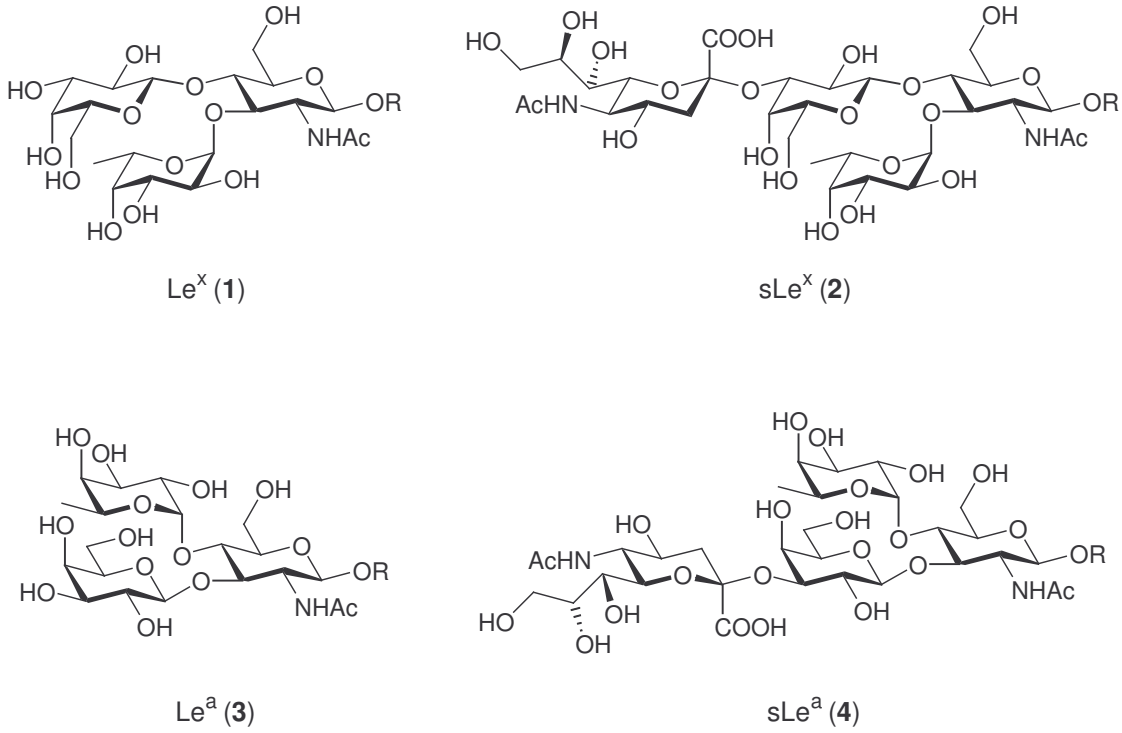


Figure 5 : The natural selectin ligands carbohydrate epitopes.

Soluble recombinant forms of the selectins as well as selectin-IgG fusion proteins have been used as affinity probes to isolate and identify some of their natural glycoprotein ligands :

- L-selectin is binding to five endothelial glycoproteins called MAdCAM-1 [37,38], Gly-CAM-1 [39], CD34 [40], Spg200 [41] and the podocalyxin-like protein [42]. The sialomucin Gly-CAM-1 is of particular interest in this case, as its posttranslational modifications have been intensively studied, showing that sialylation, fucosylation and sulfation are essential to the binding to L-selectin

[39,43,44].

- The natural ligand to P-selectin is PSGL-1, a homodimeric protein linked by two disulfide bridges [45], which is also a sialomucin. Further studies showed that sialylation and fucosylation were required for the binding [46-50] of this glycoprotein, as well as sulfation of two of the three N-terminal tyrosines it contains (Tyr^{46} and one between Tyr^{48} and Tyr^{51}). This makes it a potential ligand for L-selectin as well [51-54].
- Screening for E-selectin ligands revealed a glycoprotein, subsequently named ESL-1. In contrast to the ligands presented above, ESL-1 requires *N*-linked glycan modifications for binding E-selectin. It is also noticeable that ESL-1 only binds E-selectin, and not P-selectin [55]. Three other glycans were also identified as E-Selectin ligands [56], each containing a sialyl di-Le^x motif. In addition, E-selectin recognizes PSGL-1, but with the difference to P-selectin that the sulfation of the tyrosine residues is not mandatory [48,57].

Interactions between the three selectins and some of their ligands are summarized in figure 6 below. In the following we will focus more on E-selectin.

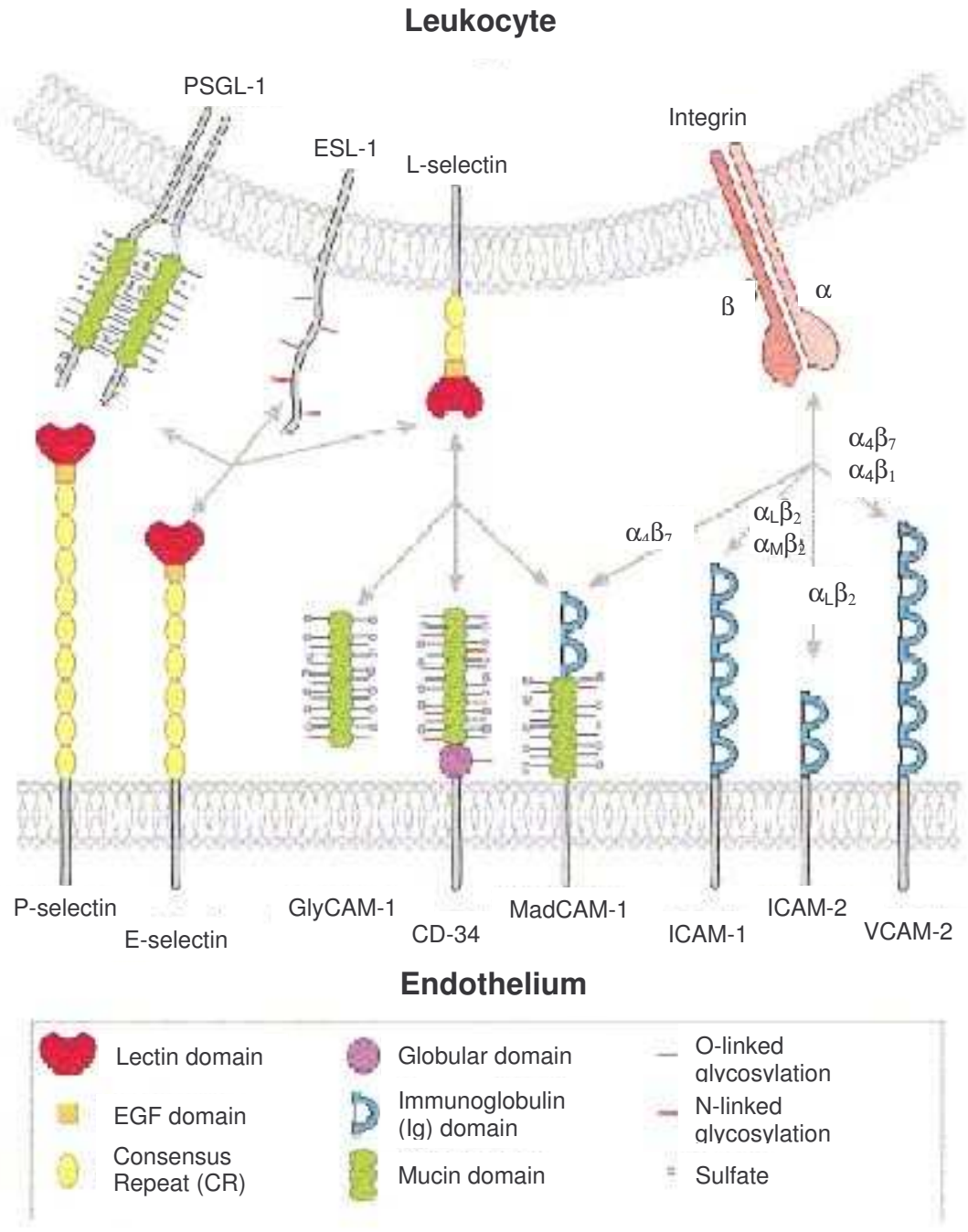


Figure 6 : Selectins, integrins and their binding partners. The depicted selectin ligands have been identified by affinity isolation with the respective selectins as affinity probes [2].

A.II. Structural determination of the selectins

A good knowledge of selectins' biology requires a comprehensive understanding of the 3D-structure of these proteins, of their carbohydrate ligands, and complexes thereof. In the case of E-selectin, the ligand of reference is sLe^x (**2**, figure 5). For the purpose of uncovering the structural specificities of E-selectin, and in particular of its binding site, different methods have been used : (i) *Structure-activity relationship* (SAR) studies, measuring the affinity to E-selectin of sLe^x and derivatives thereof in order to determine the pharmacophores. (ii) NMR studies (e.g. using nOe, STD or multidimensional methods) performed with sLe^x (**2**) in solution alone (determining solution conformation) or in combination with the protein (giving hints about the bioactive conformation). (iii) Theoretical binding models have been developed with help of molecular modeling. (iv) X-ray crystal structure of selectins co-crystallized with sLe^x.

A.II.1. SAR studies to the determination of the pharmacophore

The first chemical synthesis of sLe^x (**2**) has been reported in 1991 [58]. However, despite the availability of very efficient glycosylation methods, the chemical syntheses of polysaccharides requires selectively protected sugars, greatly increasing the number of steps and hence the length and cost of production, especially in large scale. In order to simplify the synthesis, chemo-enzymatic [59] approaches have been developped.

In extensive studies, all functional groups of sLe^x have been chemically modified in a systematic process to identify the groups being crucial for binding to E-selectin. Those groups are named pharmacophores.

- The role of the different hydroxyl groups of fucose (Fuc) was determined by Gaeta *et al.* [60], and Hasegawa *et al.* [61]. They replaced them individually or collectively by hydrogen atoms, obtaining deoxy-derivatives that were found to be completely inactive against E-selectin. In analogy to the mannose-binding protein (MBP-A), which CRD domain shows 80% sequence homology to the one of E-selectin [62], it was assumed that the fucose hydroxyl groups are

binding to the calcium ion of the protein. It is, however, noticeable that only the Fuc-3 hydroxyl was found to be critical for binding to P-selectin.

- Gaeta *et al.* [60] also tried to substitute the fucose moiety by an arabinose (formally replacing the 5-methyl group by a hydrogen) in order to check the influence of the methyl. The compound obtained was found to be five-fold less active than the original one. This shows that the methyl is important for activity, although not crucial.
- The role of the galactose (Gal) hydroxyl groups was first determined by Kunz *et al.* [63], who produced deoxy- and fluoro-derivatives of sLe^x (**2**). Thanks to systematic replacements at each position, they noted that the affinity of the molecule was significantly reduced in the absence of the Gal-4 hydroxyl and disappeared when the Gal-6 one was replaced, stressing the importance of these groups for binding.
- The contribution of the various functional groups of the *N*-acetyl-neuraminic acid (Neu*N*Ac, or sialic acid) moiety, has also been examined in details [61,64,65]. Among them (glycerol side chain, amide, 3-OH, carboxylic acid), only the carboxylic acid was found of particular importance for the binding.
- Finally, several studies were directed at the *N*-acetyl-glucosamine (Glc*N*Ac) moiety [66-68]. No direct contribution to the binding was detected. It was therefore suggested that this part of the molecule acted mostly as a spacer, maintaining the critical functional groups of both sides of the molecule in the required spatial orientation.

All these informations allow for the representation of the pharmacophore, based on the structure of sLe^x (**2**, figure 7).

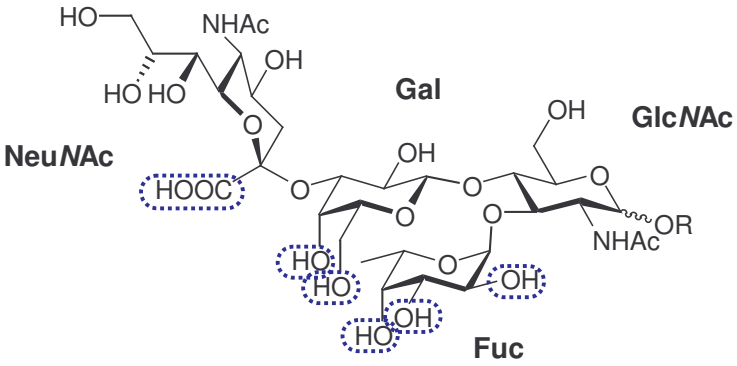


Figure 7 : Pharmacophore elements of the sLe^x (**2**) molecule.

A.II.2. Solution conformation and bioactive conformation of sLe^x determined by NMR

Early work towards the structural determination of selectins was devoted to the study of the conformation of sLe^x (**2**) in solution. The method of choice for these studies was NMR, using labeled or unlabeled molecules.

Initially, three different studies [69] agreed in finding only one single stable conformation of the molecule in water. ROESY and NOESY NMR spectroscopy [70] revealed significant interglycosidic nuclear Overhauser effect (nOe) between the following sLe^x protons (illustrated in figure 8) :

- H-3 of Glc*N*Ac and H-1 of Fuc ;
- H-4 of Glc*N*Ac and H-1 of Gal ;
- H-2 of Gal and H-5 and methyl of Fuc ;
- H-3 of Gal and H-3(axial) of Neu*N*Ac.

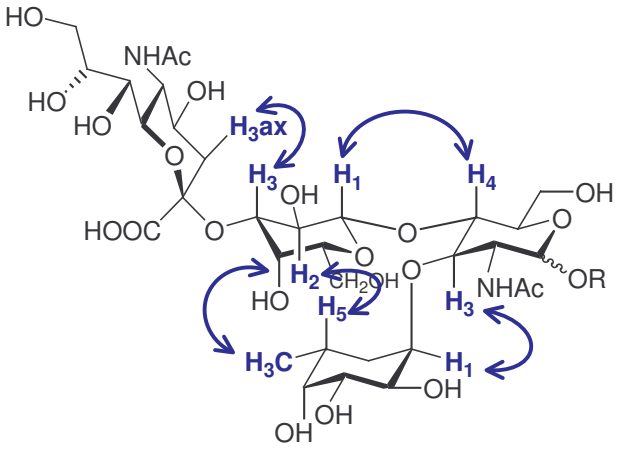


Figure 8 : nOe recorded between various protons of sLe^x (**2**) in solution.

Further ROESY experiments combined with molecular modeling [71] led to distinctive values for the various dihedral angles Φ and Ψ (as defined in figure 9), at least for the ones between Fuc-Glc*N*Ac and Glc*N*Ac-Gal. Subsequent studies, however, focusing on the Gal-Neu*N*Ac linkage [71-74] showed much higher flexibility of this part of the molecule and the energy minima evaluated by the different teams are not in agreement, suggesting that sLe^x (**2**) in solution may exist as an ensemble of conformations, although the Le^x (**1**) core of the molecule may be relatively rigid. Some of the angles values are listed in table 1.

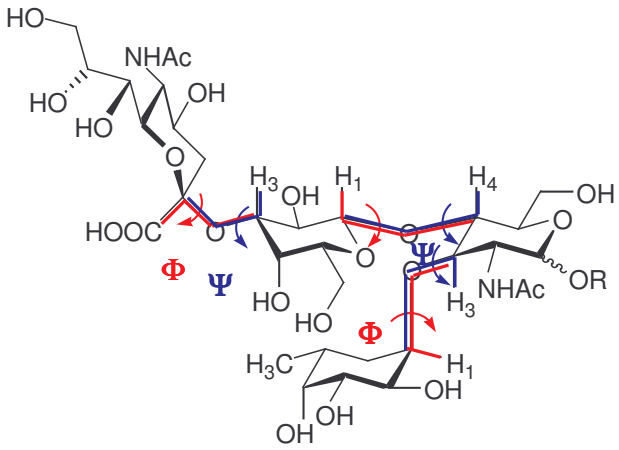


Figure 9 : Interglycosidic dihedral angles as they are defined for sLe^x (**2**).

The bioactive conformation was first investigated by Peters *et al.* [75-77] and Cooke *et al.* by transfer-nOe (trNOE) spectroscopy [74]. Significant changes were observed in the bound state in comparison to the free (in solution) state. Indeed, the significant nOe between H-3 of Gal and H-3(axial) of Neu*N*Ac completely disappeared in the bound state, whereas a new interaction appeared between H-3 of Gal and H-8 of Neu*N*Ac. New dihedral angles were then deduced, corresponding to the bioactive conformation [76,77]. They were comparable to the angles found in one of the solution conformations suggested by Breg *et al.* [73] (see table 1 for the values).

Combining these findings suggests that sLe^x (**2**) in solution easily flips between three to four different minimal energy conformations, one of them being very close to the expected bioactive (bound to E-selectin) conformation. The results also show the pre-organization in solution of the Le^x (**1**) core close to the bioactive conformation. One decisive element of this pre-organization, as shown in the model from Peters *et al.* [75,78], is the stacking of the Gal and Fuc moieties above one another, with Glc*N*ac playing the role of a spacer. This spatial arrangement is stabilized by an interaction of the hydrophobic faces of the two monosaccharide units. This also explains the loss of activity observed after replacing the methyl group of fucose by other substituents with a different size or polarity. Noteworthy is also the position of the pharmacophore. In the bioactive conformation, all of its parts would be in line along one side of the tetrasaccharide (figure 7).

	Ref.	Neu*N*Ac-Gal		Gal-Glc*N*Ac		Glc*N*Ac-Fuc	
		Φ	Ψ	Φ	Ψ	Φ	Ψ
Solution conformations	[69,70]	163° -170° -79° 68°	-57° -8° 7° -20°	48°	15°	22°	30°
	[72,73]	-95° -70° -160°	-45° 5° -20°	50°	15°	48°	22°
	[72]	n.e.	n.e.	46°	18°	48° -23°	24° 15°
	[79]	-95° -70° -160°	-60° 0° -25°	65°	15°	65°	40°
Bioactive conformations	[72]	-58°	-20°	25°	33°	70°	14°
	[79]	-70°	8°	52°	22°	20°	34°
	[75,76]	-76°	6°	39°	12°	38°	26°
	[80]	-65°	-12°	34°	16°	41°	22°
	[81]	-43°	-12°	45°	19°	29°	41°

Table 1 : Interglycosidic dihedral angles values, as evaluated by different groups.

A.II.3. Binding models design with help of modeling and X-ray structural information

The E-selectin binding site has also been studied more closely by mutagenesis [16], revealing that a relatively small and shallow patch of the lectin domain (CRD) was responsible for the direct binding to sLe^x, involving the amino acids Arg^{97}, Lys^{111}, Lys^{113}, Ser^{47} and Tyr^{48}. Based on these results, and those about the bioactive conformation of sLe^x, different models were suggested for the binding mode of the tetrasaccharide. Many different models have been developed, that all showed partial agreement with the finally published crystal structure of sLe^x co-crystallized with Lec-EGF [82]. Three of them are discussed below.

- The first relevant model was developed by Graves *et al.* [83] who solved the X-ray structure of the Lec-EGF domain (no ligand). More details about the binding mode of sLe^x (**2**) were uncovered at the same time after co-crystallization of the tetrasaccharide with a selectin-like mutant of MBP-A [83] (mannose-binding protein, see part **A.II.1**). The model confirmed the binding of Fuc-2 and Fuc-3 hydroxyl groups with the calcium ion. Unexpectedly however, the carboxylic acid of Neu*N*Ac was not shown to interact with the protein despite the findings of earlier SAR studies (see part **A.II.1**).
- Kogan *et al.* [84] as well as Ernst *et al.* [78] independently proposed models based on molecular modeling docking studies. Both agree in the principal interactions important for binding, namely the interaction of Fuc-2 and Fuc-3 hydroxyls with the calcium ion embedded in the protein, the binding of Gal-6 hydroxyl to the Tyr^{94} residue in the CRD, and the contact of the carboxylic acid of NeuNAc with Arg^{97}. Slight differences are also noticeable, like the contact of the Gal-4 hydroxyl with Asn^{105}, not predicted by Kogan *et al.*, the coordination of the calcium ion by the protein and the fucose hydroxyl assumed to bind to Asn^{82}. Both models are illustrated on figure 10 and figure 11.

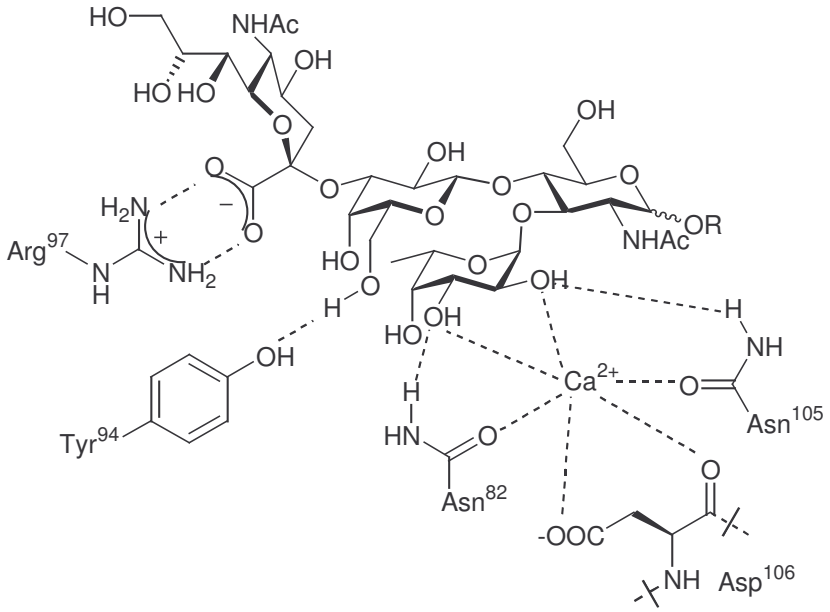


Figure 10 : Binding mode of sLe^x, as suggested by Kogan *et al.* [84].

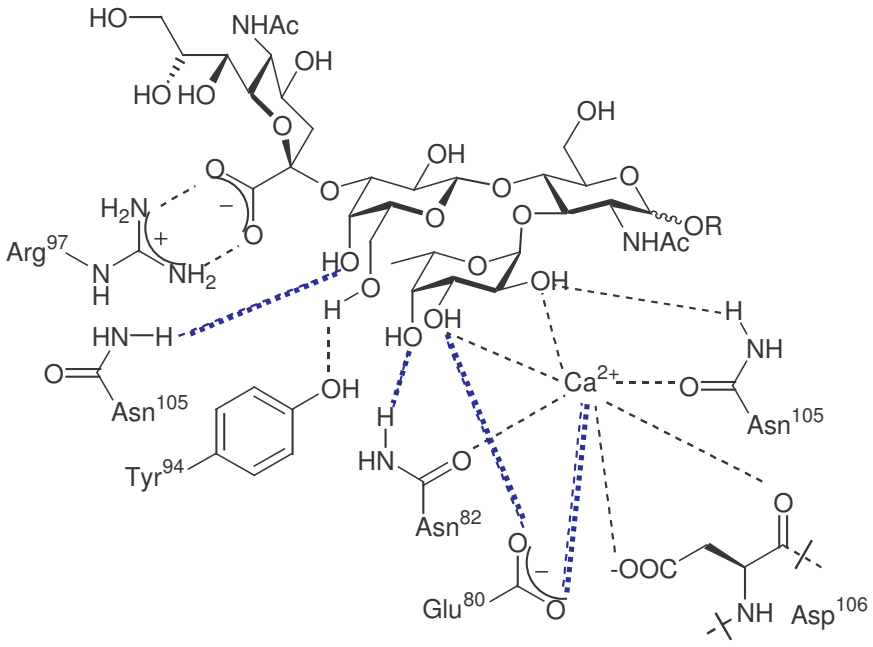


Figure 11 : Binding mode of sLe^x, as suggested by Ernst *et al.* [78]. Differences with the previous model (figure 10) are highlighted in blue.

Definitive answers were finally found after the publication by Somers *et al.* [82] of the crystal structure of sLe^x bound to the Lec-EGF domains of both E- and P-selectin. They devised a new binding model (figure 12) that provided some surprises. In particular, the selectin-bound calcium ion appeared to be complexed by the Fuc-3 and the Fuc-4 hydroxyl groups instead of the previously suggested Fuc-2 and Fuc-3, the Fuc-4 hydroxyl being also involved in hydrogen bonds with Asn^{82} and Glu^{80}, and the Fuc-3 hydroxyl with Asn^{105}. Fuc-2 hydroxyl further interacts with Asn^{83} and Glu^{107} through water-mediated hydrogen bonds. On behalf of the Gal moiety, contacts between Gal-4 hydroxyl with Tyr^{94} and Gal-6 hydroxyl with Glu^{92} were detected. Finally the carboxylic acid of Neu*N*Ac interacts with the amino acid residues Arg^{97} and Tyr^{48}. It occurs that the conformation of sLe^x (**2**) deduced from this crystal structure is very similar to the bioactive conformation suggested by Peters *et al.* Based on NMR measurements [76,77].

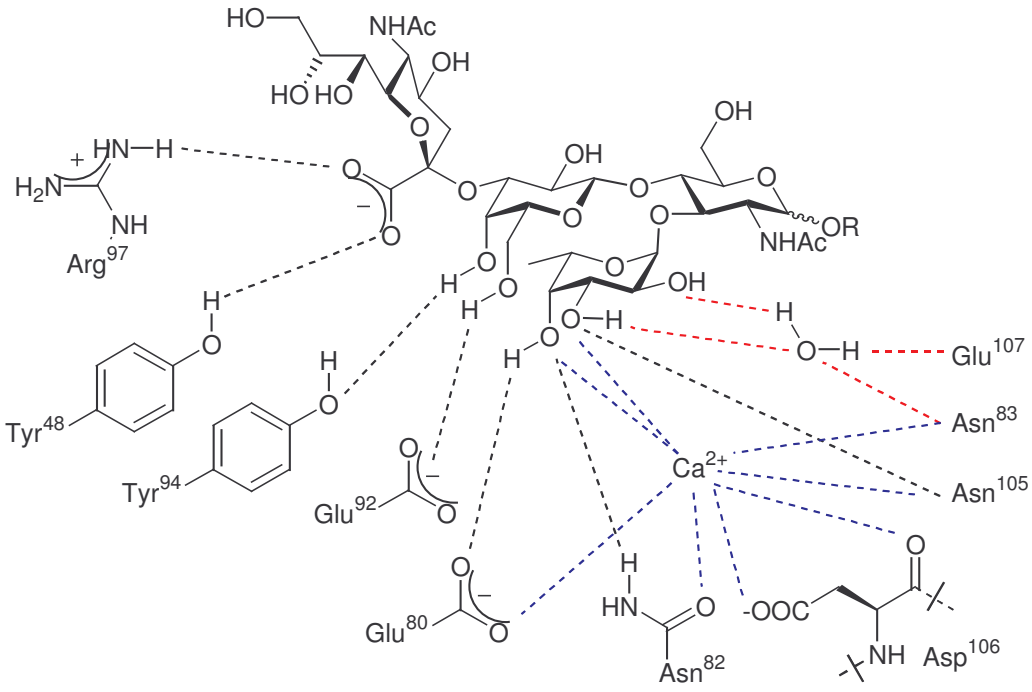


Figure 12 : Binding mode of sLe^x, as proposed by Somers *et al.* [82], based on the crystal structure.

A.III. Early developments in the design of E-selectin antagonists

Since it has been demonstrated that selectins play a key role in the regulation of the inflammatory cascade, they have become targets of choice for the development of new anti-inflammatory agents. Due to the extensive studies that were carried out towards the structure and binding mode of sLe^x (**2**), the molecule was consequently used as a lead.

Mimics thereof were therefore designed and synthesized [85], mainly following two goals :

- **Simplification of the structure** : Smaller molecules, ideally bearing the minimal pharmacophoric groups in the correct spatial orientation and easier to synthesize. They will also be more suited to library design.
- **Overcoming pharmacokinetic and pharmacodynamic hurdles** : sLe^x is a carbohydrate molecule with all of its disadvantages, namely hydrolytical instability, high polarity leading to low bioavailability and fast renal excretion.

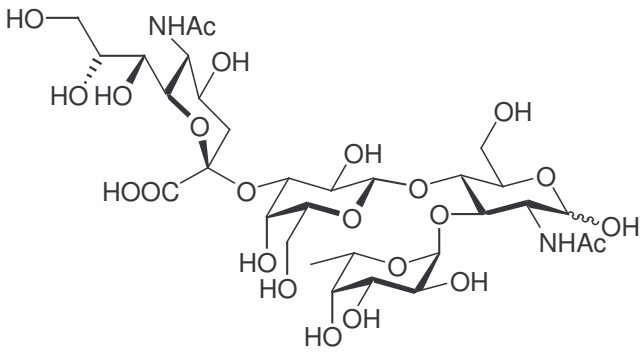
In order to present the different contributions in a comprehensive way, they will be sorted after the numbers of sugar units that have been replaced in comparison with the structure of sLe^x (**2**). Moreover, as affinity values are evaluated in different assay formats in the various research teams publishing on the subject, all IC_{50} values in the following will be given relative to sLe^x, which IC_{50} has been normalized to 1.0 mM ($rIC_{50} = 1$).

A.III.1. Replacement of one sugar unit

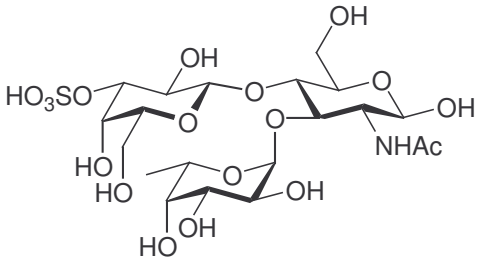
A.III.1.1 Replacement of Neu*N*Ac

On the *N*-acetyl-neuraminic acid (Neu*N*Ac) unit, it has been shown that only the carboxylic acid had significant importance for the binding affinity [61,64,65]. In addition, Neu*N*Ac is the most expensive building block in the synthesis of sLe^x, and its replacement by a negatively charged group at the 3-position of the galactose thus presents many advantages.

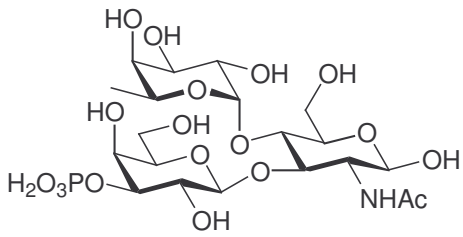
Substitution of Neu*N*Ac by sulfate groups led to the known natural ligands sulfo-Le^x and sulfo-Le^a or related derivatives [61,86,87]. Affinities reported were similar to the one of sLe^x. Phosphate groups were also used for the same purpose [61,88,89], with similar results (figure 13).



sLe^x (**2**, 1.0) [61]



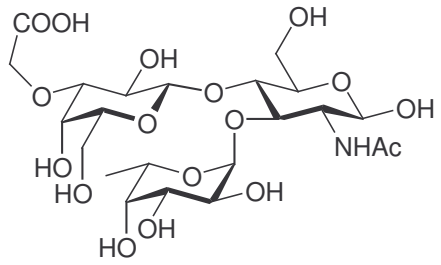
sulfo-Le^x (**5**, 0.3) [88]



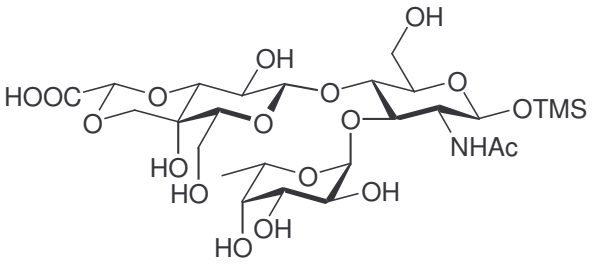
phospho-Le^a (**6**, 0.1) [89]

Figure 13 : sLe^x mimics containing a sulfo or phospho group replacing Neu*N*Ac. rIC$_{50}$ values are shown in brackets.

The most common structural simplification is the simple alkylation of the Gal-3 hydroxyl, introducing a carboxymethyl group. The product obtained (**7**) showed similar activity to sLe^x [90]. In order to gain more knowledge into the binding conditions, Duthaler *et al.* [91] also fixed the carboxymethyl group by embedding it into a cyclic six-membered acetal (**8**). The compound, however, showed no activity against E-selectin as the carboxylic acid conformation obtained was different from the bioactive one (figure 14).



7 (0.8) [90]



8 (inactive) [91]

Figure 14 : Mimics containing a carboxymethyl group replacing Neu*N*Ac. rIC_{50} values are shown in brackets.

The most successful replacement of Neu*N*Ac was obtained using L(-)-cyclohexyllactic acid ((*2S*)-3-cyclohexyl-2-hydroxy-propionic acid). However, as will be discussed later, it was only studied in combination with other replacements.

A.III.1.2 Replacement of Glc*N*Ac

The second sugar unit which was replaced is the Glc*N*Ac moiety. Indeed, it has been shown that its role is to act as a spacer, maintaining ideal stacking between the fucose and galactose units [66-69,92,93], and the crystal structure confirmed that it has no interaction with the protein [82]. The synthesized compounds therefore aim to keep the core conformation as close as possible to the one of sLe^x, but using less synthetically demanding building blocks. One compound (**9**, figure 15) replacing Glc*N*Ac with an indolizidinone type heterocycle proved unactive against E-selectin, but showed the same activity to P-selectin than sLe^x [94]. Compound **10** however, with a quinic acid derivative was equally active as sLe^x in an E-selectin assay [95]. One of the best spacers to date is a (*1R*,*2R*)-cyclohexan-1,2-diol unit and has been introduced by Töpfer *et al.* [96]. It not only dramatically simplifies the synthesis conditions, but compound **11** also showed a 3-fold better activity against E-selectin than the lead.

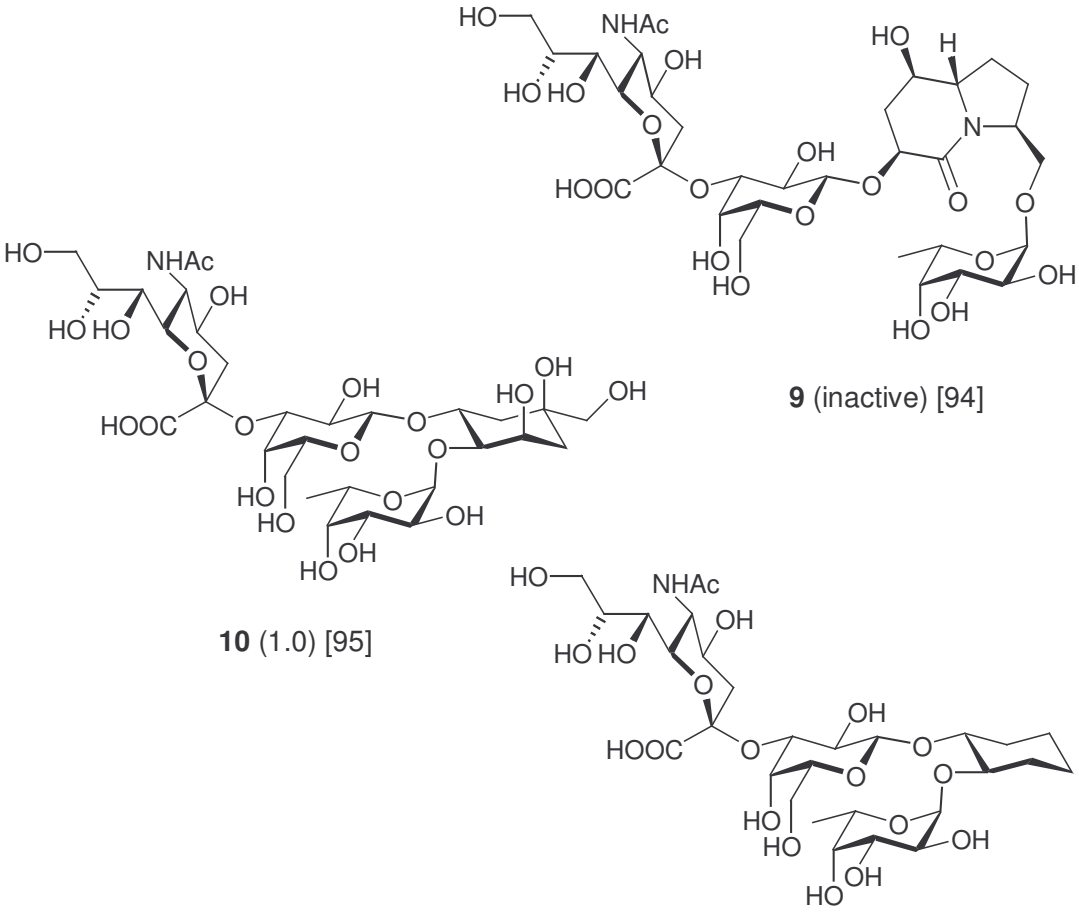


Figure 15 : Mimics containing different spacers in replacement to Glc*N*Ac. rIC_{50} values are shown in brackets.

Up to now, no mimics replacing only the galactose or the fucose units were disclosed.

A.III.2. Replacement of two sugar units

A.III.2.1 Replacement of Neu*N*Ac and Glc*N*Ac together

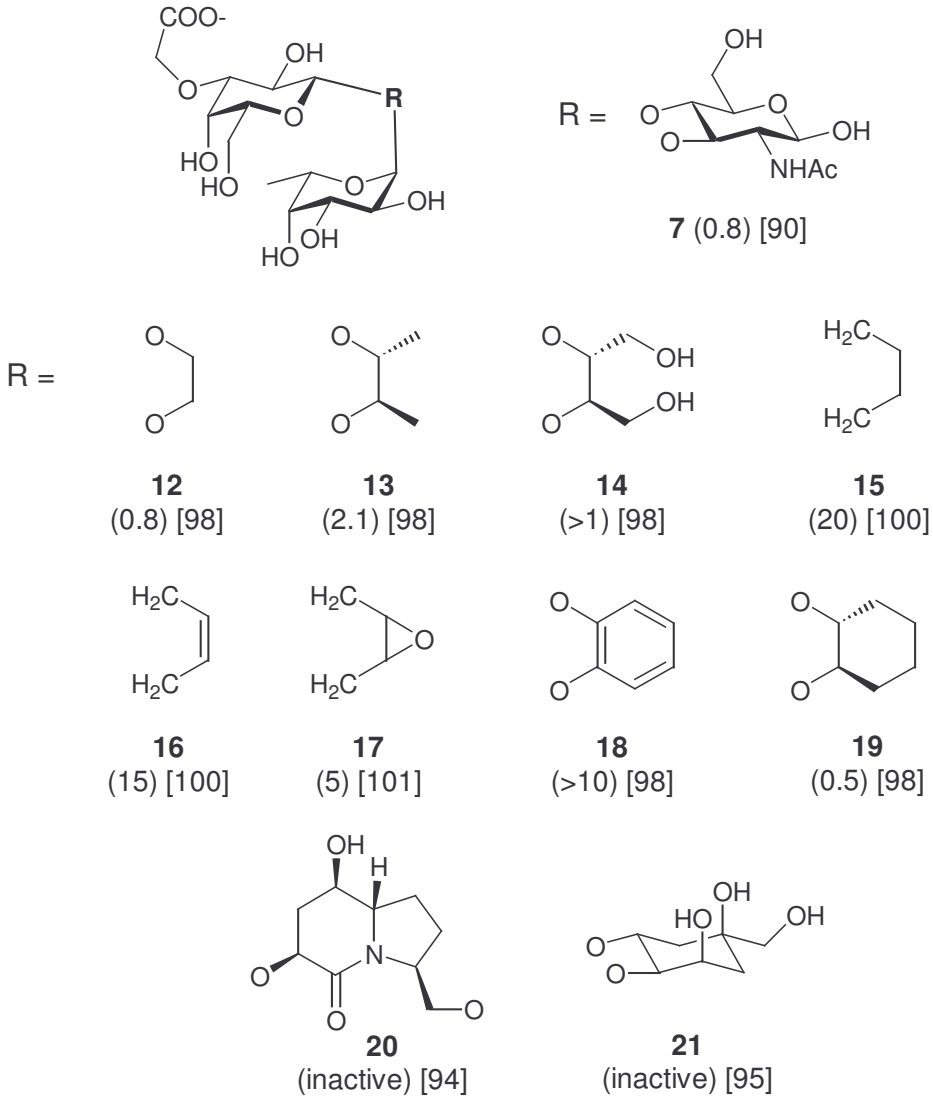


Figure 16 : Mimics used for the testing of Glc*N*Ac replacing spacers in the presence of a carboxymethyl group as Neu*N*Ac replacement. rIC_{50} values are disclosed in brackets.

The next step in the simplification process of sLe^x was to combine the effects of Neu*N*Ac and Glc*N*Ac replacements. Starting from the common mimic **7**, which has equal activity

than sLe^x, a variety of spacers have been tested, with the goal to improve the pharmacokinetic properties without loosing activity (figure 16).

Of particular interest is the substitution of the Glc*N*Ac unit with (*1R*,*2R*)-cyclohexan-1,2-diol. Wong *et al.* [97] first suggested that this replacement, in parallel with the substitution of Neu*N*Ac with a carboxymethyl group should be energetically neutral, based on sLe^x. Molecules containing this spacer (**19**) and many others (**12**-**18**) have been synthesized and tested [98-102] by different teams. Not surprisingly, too flexible spacers (**13**-**15**) led to a loss of activity of the resulting molecules. This may be explained by entropic penalties, as the molecules are flexible, and therefore are not pre-organized into the bioactive conformation. It is noticeable too, that compounds containing indolizidinone (**20**) or quinic acid (**21**) proved inactive. Among this series of compounds the only surprise came from the ethyleneglycol spacer (**12**), which affinity towards E-selectin was found equivalent to the one of sLe^x, despite the relative flexibility of this linker. Since similar molecules (e.g. **27**, figure 18) tested in other laboratories showed no activity [103], the activity of **12** should not be over-interpreted.

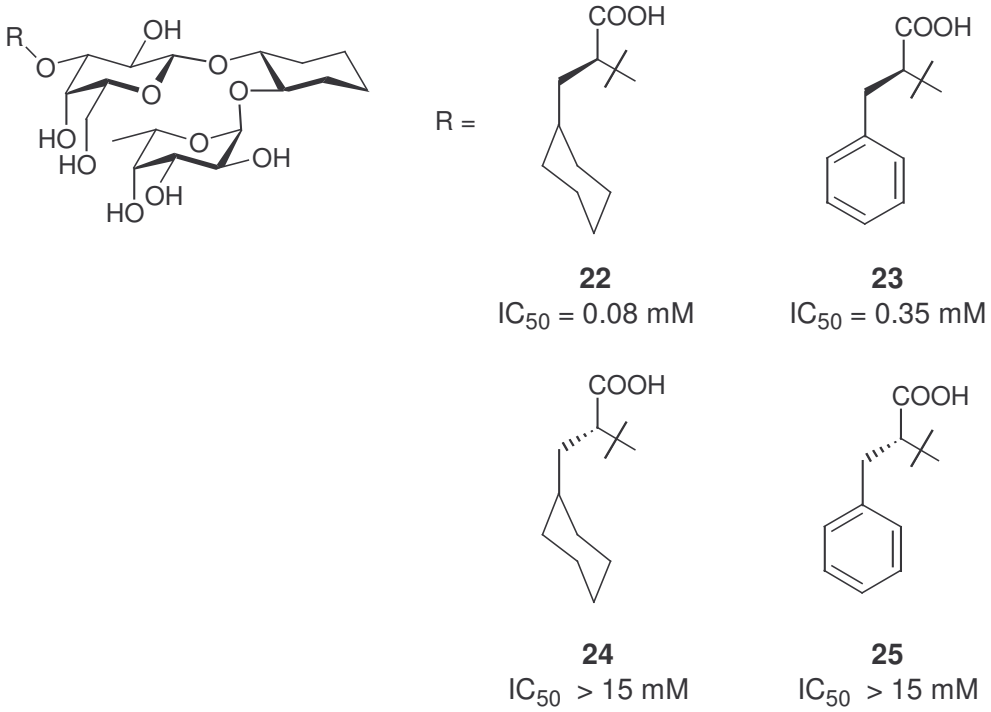
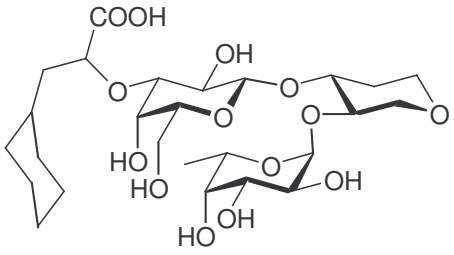
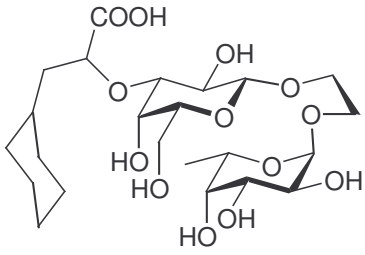


Figure 17 : Mimics developed at Novartis AG, Basel [78,104] : Replacement of the Neu*N*Ac unit in the presence of a cyclohexanediol unit replacing Glc*N*Ac.

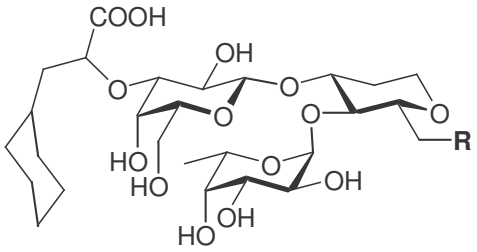
With these results in mind, Ernst *et al.* [103] designed new mimics replacing the carboxymethyl group at Gal-3 with the more sterically constraining (*2S*)-cyclohexyl- or (*2S*)-phenyl-lactic acid (figure 17). In their approach to develop pre-organized mimics adopting a solution conformation as close as possible to the bioactive conformation, they developed a modeling tool [104,105] that is quickly presented in part **B.III.1**. Using this new tool, they could show that the introduction of sterically demanding groups next to the acid function replacing the Neu*N*Ac moiety should force the carboxylate to adopt the bioactive conformation observed in sLe^x. They also confirmed the suitability of cyclohexanediol in replacement to the Glc*N*Ac unit and suggested further modifications of this unit that fulfilled the conformational requirement even better (molecules **26**-**32**, figure 18). Compound **22** (figure 17), also called CGP69669 in the following, is of particular interest to this thesis as it will be our main reference compound after sLe^x (**2**).



26
(0.08)



27
(>10)

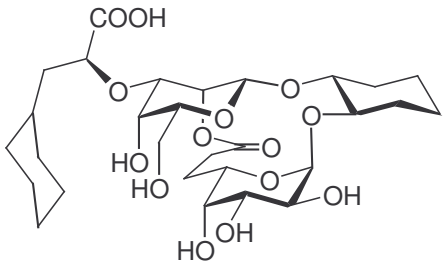


28 : R = OH (0.036)
29 : R = NHC(O)OBn (0.033)
30 : R = NHC(O)Ph (0.037)
31 : R = NHTos (0.042)
32 : R = H (0.039)

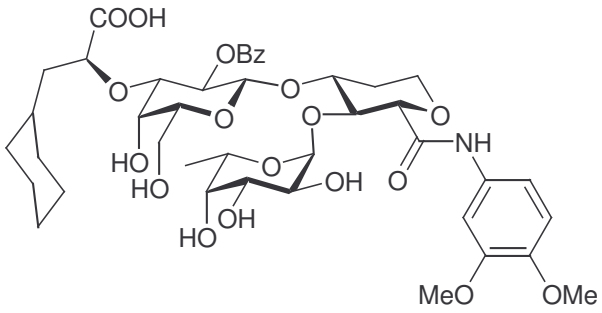
Figure 18 : Mimics developped at Novartis AG, Basel [103]: Further improvements of the diol unit. rIC_{50} values are displayed in brackets.

In additional attempts to improve affinity, various modifications at the Gal-6 position of mimic **22** were investigated, that led, however, to inactive compounds [106]. Increased rigidification of compound **22** was also reached by building macrocyclic mimic **33** [107] (figure 20), that unfortunately turned out to be even less active than sLe^x. Finally, further

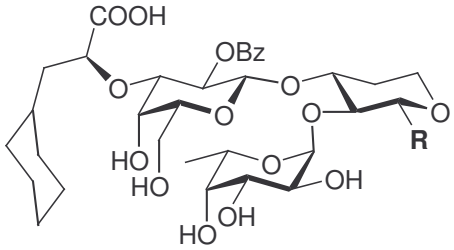
results published by Thoma *et al.* [108] indicated that the presence of a benzoic group at Gal-2 position (resulting from incomplete deprotection) gave compounds (**33**-**35**, figure 19) that were up to three times more active than their Gal-2 deprotected counterparts (**25** and **27**, figure 18). This result will be developed further as part of the present thesis work (see part **B.III**). It is also noteworthy that molecule **34** is probably the best E-selectin inhibitor published to date.



33
(3.0)



34
(0.01)



35 : R = CH_2OH (0.01)
36 : R = H (0.14)

Figure 19 : Mimics developed at Novartis AG, Basel [107,108] : Additional attempts towards better activities. rIC_{50} values are displayed in brackets.

A.III.2.2 Replacement of both Glc*N*Ac and Gal

Another explored approach is the replacement of the central *N*-acetyl-lactosamine part of sLe^x, consisting of Gal and Glc*N*Ac. Neu*N*Ac and Fuc units are kept untouched. In the first approach [96], disappointing affinities were found, pointing to two prerequisites of the linker that have to be fulfilled : (i) The linker should bear groups mimicking the Gal-4 and Gal-6 hyroxyls, which have been proved mandatory for binding [63] ; (ii) Poor activity is expected by the introduction of a too flexible linker, because of high entropic costs upon binding (**37**-**39**, figure 20).

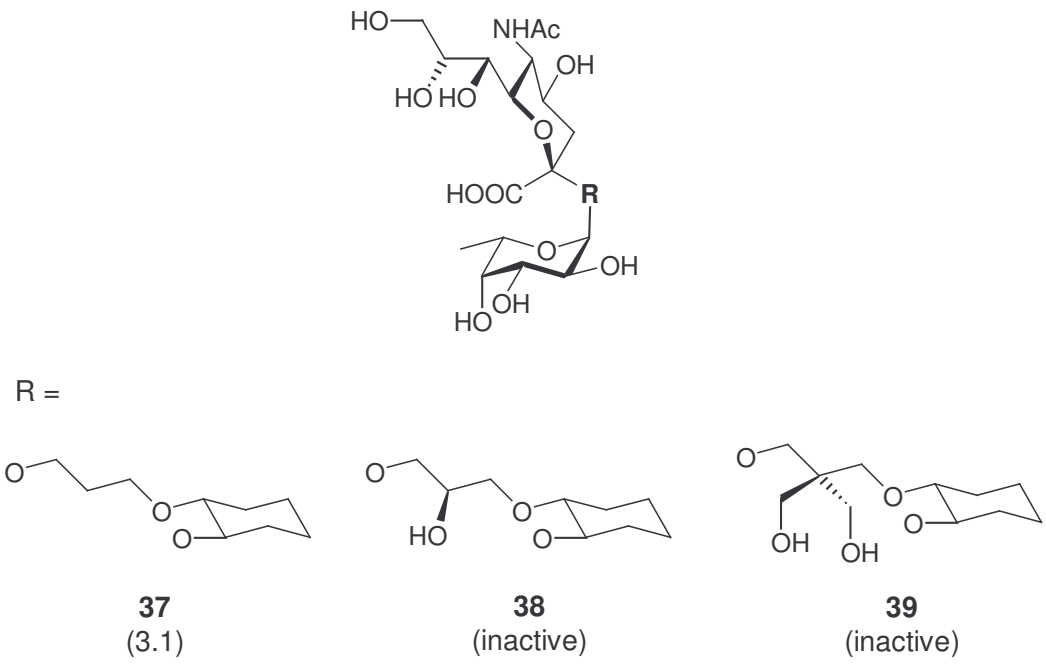


Figure 20 : Mimics containing different spacers in replacement to Gal-Glc*N*Ac [96]. rIC_{50} values are shown in brackets.

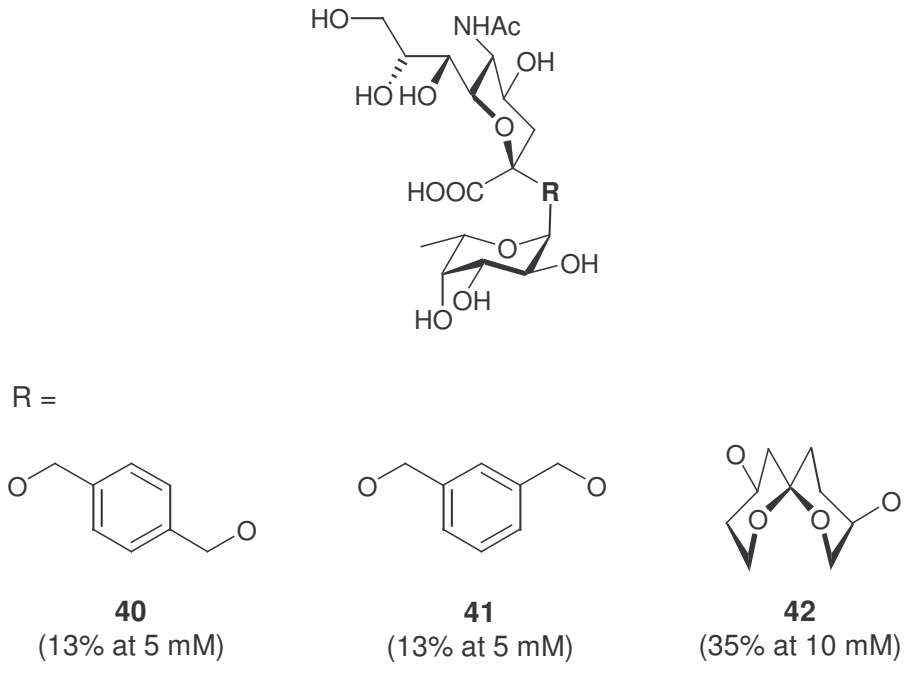
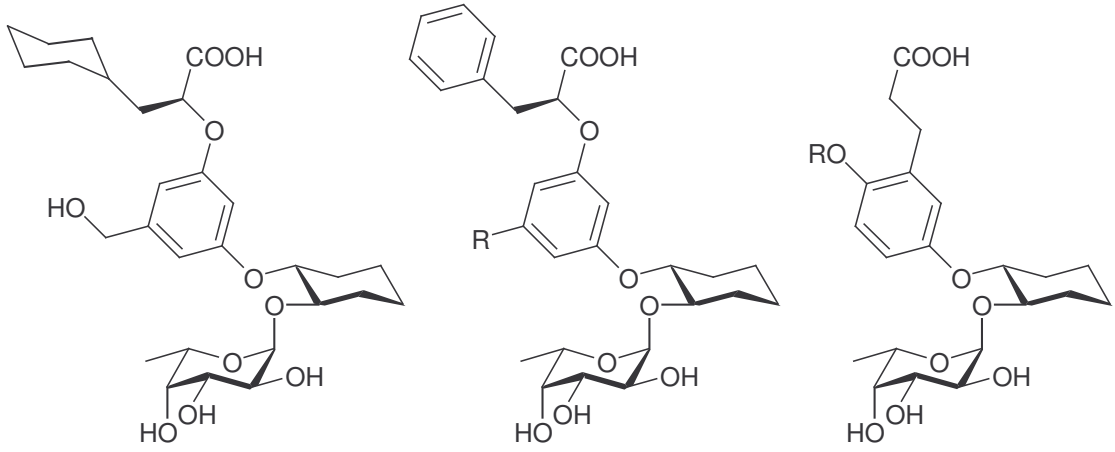


Figure 21 : Mimics containing different spacers in replacement to Gal-Glc*N*Ac [109-111]. Inhibition ratios are shown in brackets.

Additional, more rigid linkers linkers, were therefore tried, that unfortunately did not lead to better results (**40**-**42**, figure 21).

A.III.3. Replacement of three sugar units

The L-fucose unit of sLe^x (**2**) binds to the calcium ion embedded in the CRD of the selectins and contain three of the six pharmacophores of the molecule. It is probably the most difficult residue to substitute. Therefore, the largest and most drug-like group of selectin antagonists is based on structures containing only this sugar unit, with the exception of some cases, where it is replaced by D-mannose or L-galactose. The sugar unit is then substituted by additional groups that aim to mimick the other pharmacophores of the reference ligand (Gal-4 and Gal-6 hydroxyls, Neu*N*Ac carboxylic acid).



43
(inactive) [56]

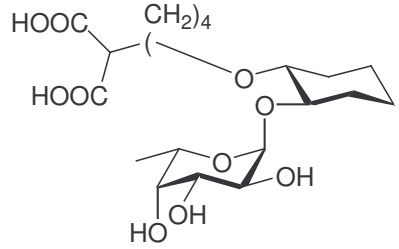
44 : R = H
45 : R = Ph
46 : R = *i*-Pr
47 : R = COOH
48 : R = CH_2OH
all inactive [56]

49 : R = H (inactive)
50 : R = Bn (0.87)
[121]

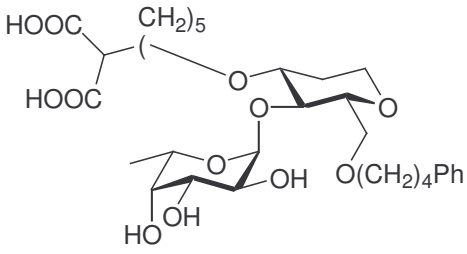
Figure 22 : Monosaccharide mimics of sLe^x : Use of an aryl spacer. rIC_{50} values are shown in brackets.

Some approaches used the substituents that already proved efficient in former mimics, like the cyclohexanediol replacing Glc*N*Ac, and the cyclohexyllactic acid replacing Neu*N*Ac. Most common linkers (replacing galactose) were polyaryl (e.g. biphenyl) [112-114], or polyamides (e.g. glycopeptides) [115-120] and met various success.
Single aryl groups (differently substituted) were studied independently by Ernst *et al.* [56] (compounds **43**-**48**) and Liu *et al.* [121] (compounds **49**-**50**). Among the molecules that were synthesized (see figure 22), few showed a faint activity (at best equivalent to the one of sLe^x).

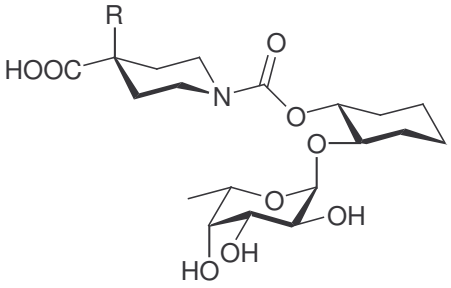
Mimics using malonic acid [122] or piperidine carboxylic acid [123] derivatives in replacement to Neu*N*Ac were not more potent (examples are disclosed in figure 23, compounds **51**-**55**).



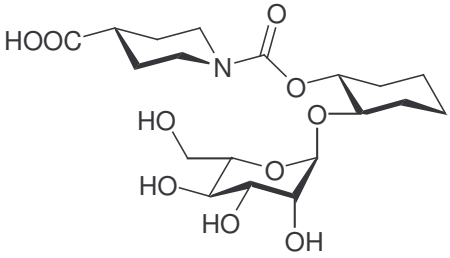
51
(1.0) [122]



52
(2.6) [122]



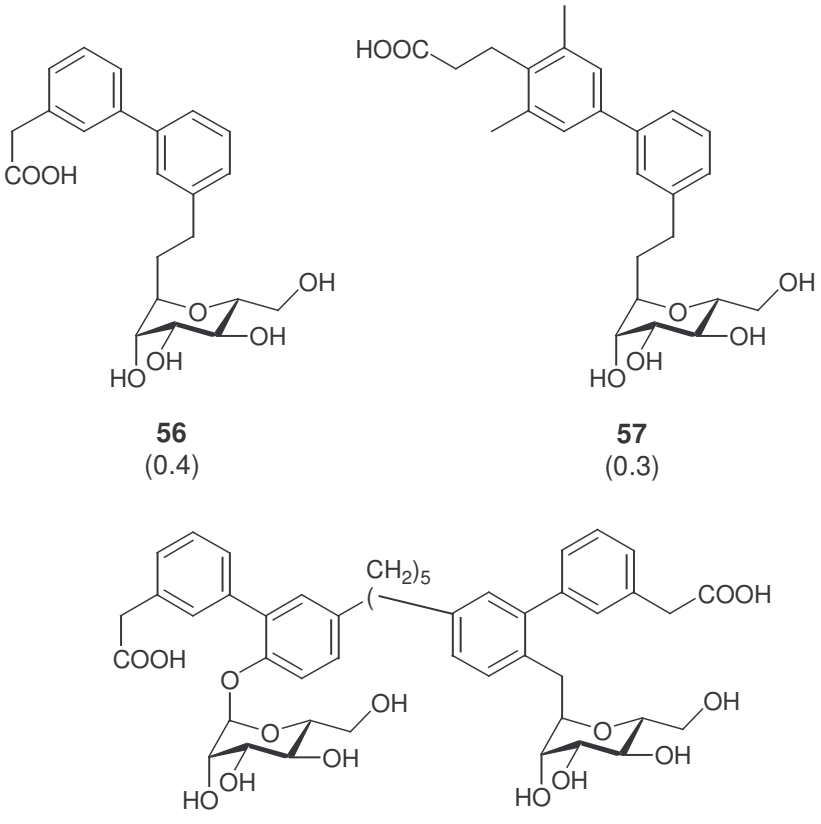
53 : R = H (> 5)
54 : R = COOH (1.6)
[123]



55
(5) [123]

Figure 23 : Monosaccharide mimics of sLe^x : Use of malonic or piperidine carboxylic acids as Neu*N*Ac-Gal replacement. rIC_{50} values are shown in brackets.

The first breakthrough among the class of monosaccharidic selectin antagonists was published by Kogan *et al.* [112-114], who developed a library containing as much as 45 different structures based on derivatized biphenyl residues linked to the anomeric position of mannose (replacing fucose). With the exception of three of them (compounds **56**-**58**, figure 24), all were less active against E-selectin than sLe^x. However, many showed increased activity against P-selectin, namely two to twenty times better than sLe^x. Noteworthy is also the dimeric structure of compound **58**, mimicking the extended sialyl di-Le^x structure of the natural ligands isolated from human neutrophils [56] (see also part **A.I**). The synthetic dimer **58** was found to be six times more active than sLe^x. Consequently, the molecule is now in phase II clinical trials, carried out by Texas Biotechnology, Houston, TX, USA, for the treatment of asthma, reperfusion injury and psoriasis [115].



56
(0.4)

57
(0.3)

58
(0.13)

Figure 24 : Monosaccharide mimics of sLe^x [112-114]: Use of biphenyl spacers. rIC_{50} values are shown in brackets.

Large efforts have been invested by Wong *et al.* [97,115-120] in the creation of libraries of fucose- mannose- or galactose-based glycopeptides. For that purpose, he pointed at two key elements that influence the inhibitor's potency, namely *turns*, mimicking the Glc*N*Ac unit, and *hydroxyls*, that should replace the galactose pharmacophores. Figure 25 gives examples of both, and a selection of the most potent inhibitors discovered by this approach (**59**-**62**) is shown in figure 26. In general, activity improvement was much better against P-selectin than E-selectin.

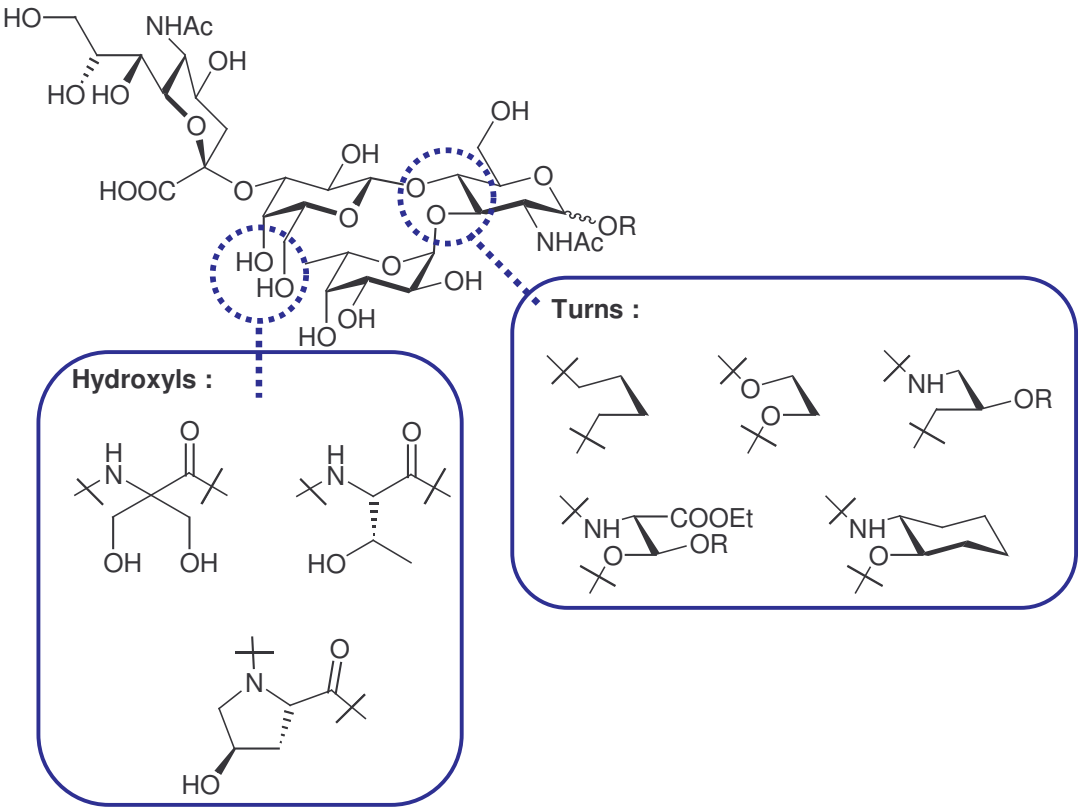


Figure 25 : sLe^x mimics library design, from Wong *et al.* [97].

Another approach, based on combinatorial synthesis, has been studied by Armstrong *et al.* [122] and Wong *et al.* [123]. They used two- and four-component Ugi reactions using an aldehyde, a primary amine, an isonitrile and a carboxylic acid, thereby producing a variety of small glycopeptide structures. Here again, affinity was mainly gained against P-selectin, and little against E-selectin.

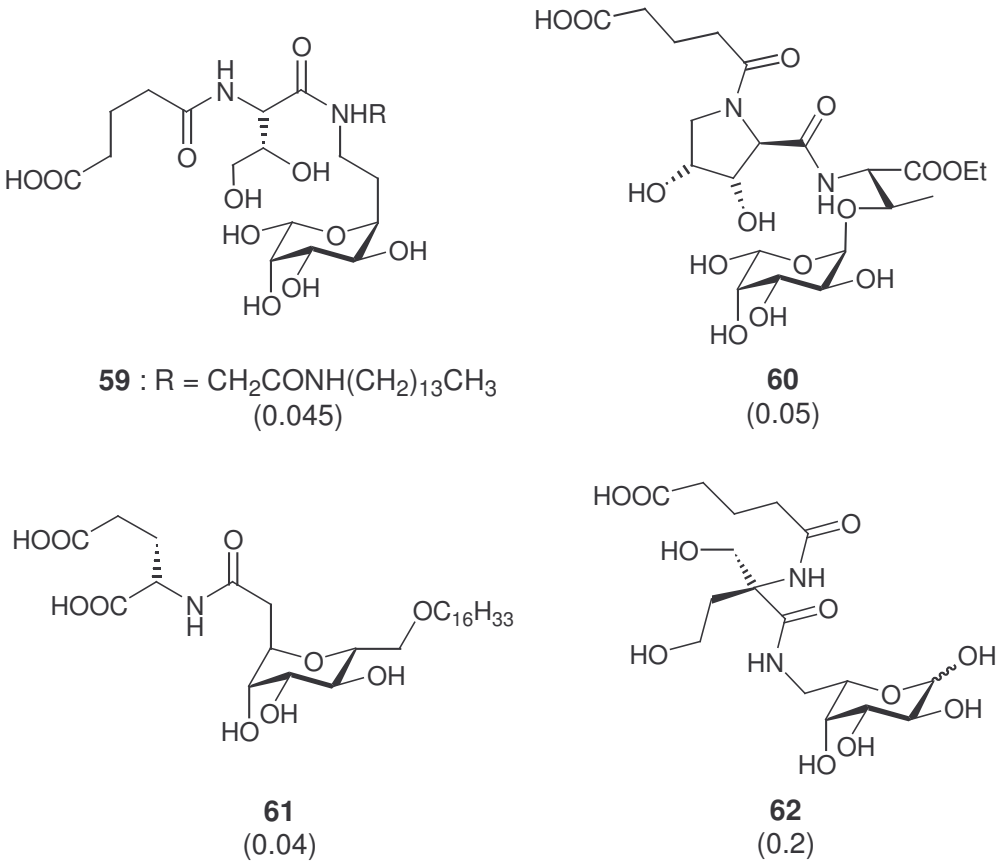
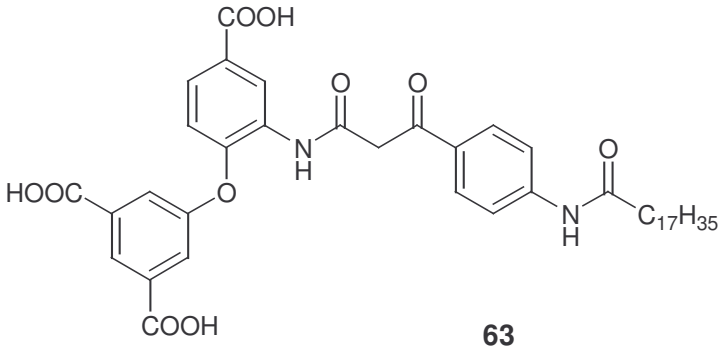


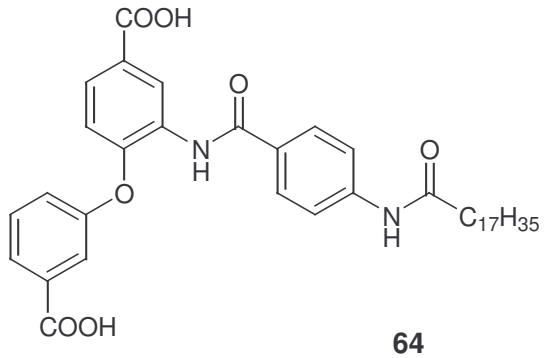
Figure 26 : sLe^x mimics library of Wong *et al.* [97], examples. rIC$_{50}$ values are shown in brackets.

A.III.4. Non-carbohydrate sLe^x mimics

The group of Kondo *et al.* [124] was the first to introduce a non-carbohydrate sLe^x mimic (**64**, figure 27). Using their own models, they carried out a pharmacophore search, that identified the possible inhibitory molecule **63**. The compound was then optimized using conformational analysis and medicinal chemistry methods, resulting in compound **64**. However, the high molecular weight of this compound is a drawback to its possible development as a drug. In addition, **64** probably forms micelles because of its long lipophilic tail, leading to a polyvalent interaction towards target proteins.



63
(2.0)



64
(0.15)

Figure 27 : Non-glycosidic high molecular weight sLe^x mimics [124]. rIC_{50} is shown in brackets.

More recently, two groups, using crystallography experiments and molecular modeling, independently came across the possibility to replace the fucose moiety with quinic acid derivatives [125-126]. They both developed small-molecular weight E- or P-selectin inhibitors, some examples of which are disclosed in figure 28. Kaila *et al.* [125] focused on molecule libraries where the 2-hydroxy group of quinic acid is substituted (compounds **65**-**66**), whereas Girard *et al.* [126] preferred amide formation at the carboxylic acid site (compounds **67**-**68**). However, although the activities are acceptable for P-selectin inhibition, good E-selectin non-glycosidic inhibitors remain elusive.

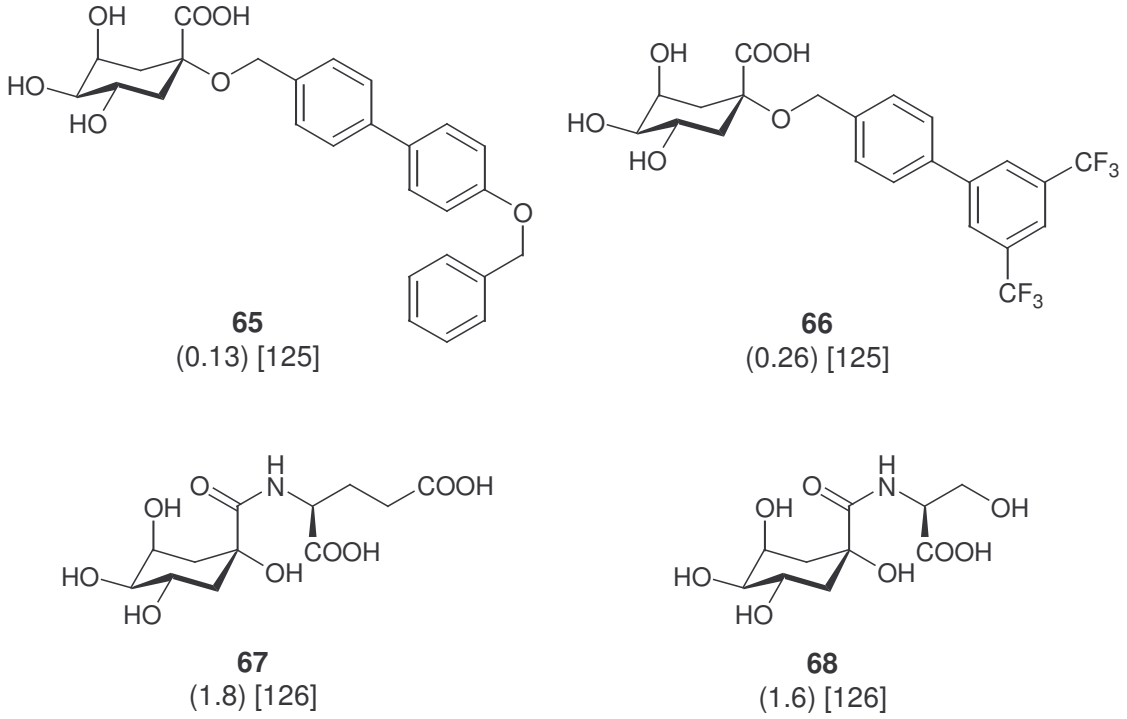
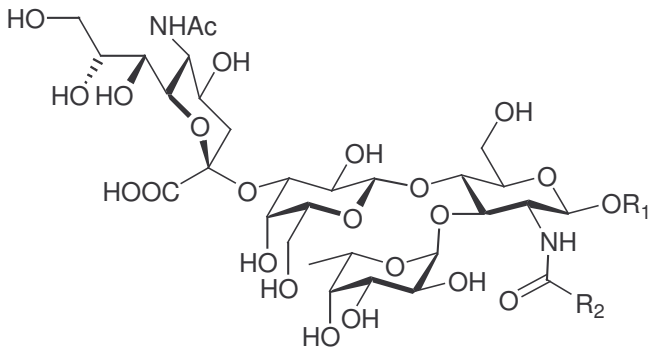


Figure 28 : Non-glycosidic sLe^x mimics based on quinic acid. rIC$_{50}$ (against P-selectin) is shown in brackets.

A.III.5. Addressing secondary binding sites

Many of the rational design approaches used in the examples described so far aimed at the improvement of the binding affinity by minimizing entropic costs. This resulted in the development of inhibitors in which the pharmacophores were pre-organized as close as possible to the bioactive conformation of sLe^x, thus avoiding energetically costly conformational changes prior to binding. Another possibility to improve affinity is the enhancement of the enthalpic contribution by additional protein-ligand interactions. It was therefore suggested that the introduction of lipophilic groups could lead to new interactions with hydrophilic patches on the surface of the protein, close to the binding site.

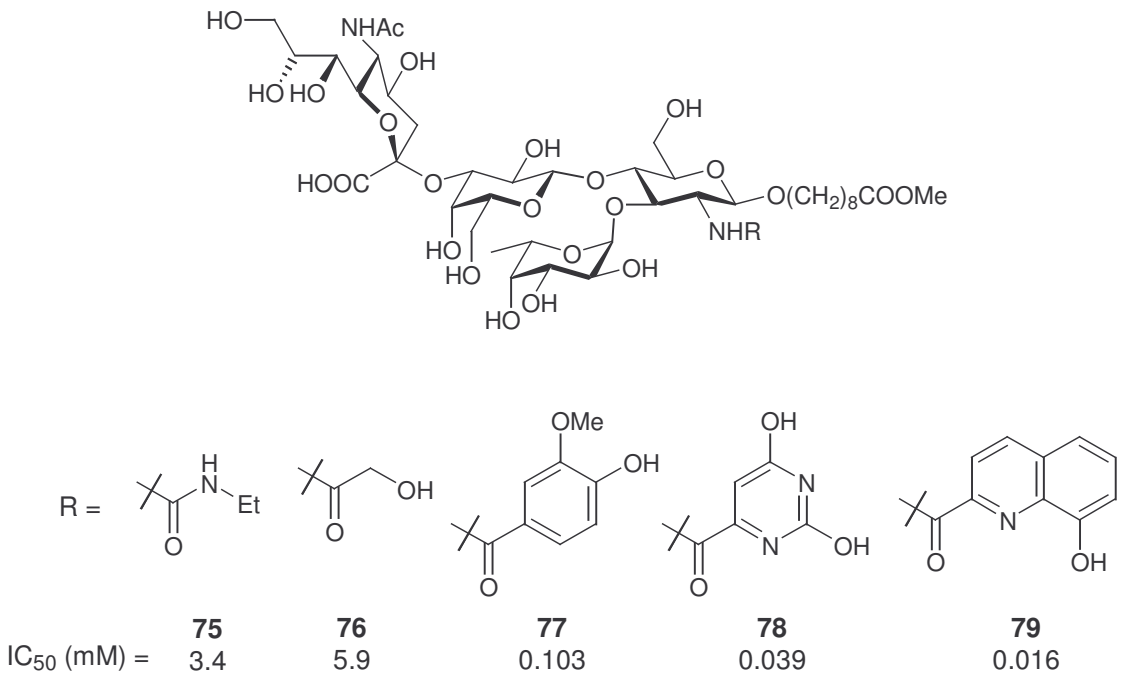
Hayashi *et al.* [127,128] published the structure of sLe^x derivatives functionalized with hydrophobic groups, either at the reducing end, or at the *N*-acetyl of Glc*N*Ac position on sLe^x. Some of the products showed increased binding affinity (figure 29).



	R_1	R_2	IC_{50} (mM)
69	$C_{12}H_{25}$	C_4H_9	2.0
70	Et	C_4H_9	1.0
71	$C_{12}H_{25}$	Me	1.6
72	Et	Me	1.5
73	$C_{12}H_{25}$	Napht	0.1
74	Et	Napht	0.1

Figure 29 : Mimics of sLe^x adressing secondary binding pockets [127,128].

Ernst *et al.* [78] further investigated the effect of aliphatic, aromatic and heteroaromatic acyl substituents at the Glc*N*Ac nitrogen atom, resulting in a substantial increase of the binding affinities (figure 30).



	75	**76**	**77**	**78**	**79**
IC_{50} (mM) =	3.4	5.9	0.103	0.039	0.016

Figure 30 : Mimics of sLe^x adressing secondary binding pockets [78].

All the examples presented in this chapter allow us to draw some important features about the conception of potent selectins (E-selectin in particular) inhibitors.

A.III.6. Conclusions

- It has been proven really difficult to devise a good inhibitor without keeping the fucose and galactose units untouched.
- Studies where Glc*N*Ac was replaced by a spacer have shown that pre-organization of the ligand in solution is required for good binding.
- It has also been found that further steric constraints (added on Glc*N*Ac or Neu*N*Ac mimics) play an important role for pre-organization.
- Finally, recent results have shown that additional interactions with lipophilic pockets close to the binding site of the protein may significantly improve ligands' affinities.

A.IV. Bioassays evaluating ligands affinity to E-selectin

Different bioassays have been devised by the various teams working in the selectin field. Some are based on ELISA, Biacore or NMR. In the case of the present work, bioassays were carried out at GlycoMimetics Inc., Gaithersburg, MD, USA. The company developed two assays as standards for the evaluation of ligands' activity towards E-selectin [129,130]. One of them is a static polymer-based cell-free assay based on ELISA techniques, whereas the second one is a cell-based flow assay. Both are described hereafter.

A.IV.1. Static cell-free bioassay

Thoma *et al.* [129,130] prepared a glycopolymer based on a polylysine backbone, which side-chains are substituted with sLe^a (**4**, 20%), glycerol (75%) and biotin (5%) (figure 31). The glycopolymer is used as a competitive ligand in a binding assay.

The assay uses an E-selectin/hIg construct [129], which is coated on a microtiter plate. The bound protein is then incubated first with the glycopolymer, different concentrations of the ligand to test. and labeled streptavidin. After washing, fluorescence intensity is measured allowing to calculate the affinity of the tested ligand.

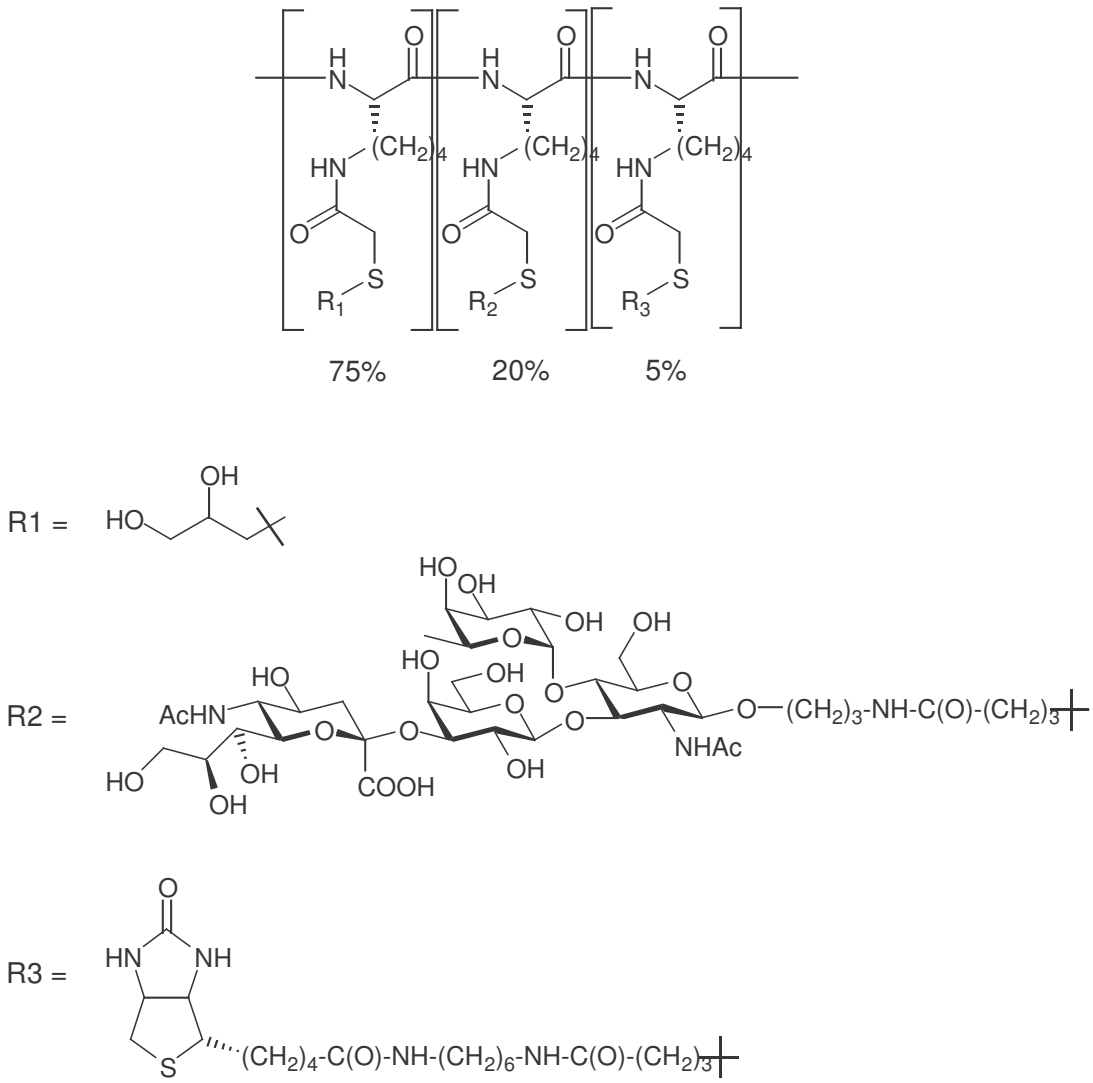


Figure 31 : Structure of the sLe[a] polymer used in the static cell-free bioassay [129].

A.IV.2. Cell-based flow assay

Considering that the IC_{50} values determined in a static assay may not reflect correctly the conditions occurring *in vivo*, the same team devised a second bioassay [130], based on flowing cells. For that purpose, a parallel plate flow chamber coated with human umbilical vein endothelial cells (HUVECs) was used to study the rolling of polymorphous neutrophils (PMNs) in contact with selectins in a hydrodynamic flow, therefore mimicking

the non-equilibrium conditions found *in vivo*. Digital image acquisition was used to determine the number of interacting cells in the presence or absence of any potential E-selectin inhibitor [131] (figure 32).

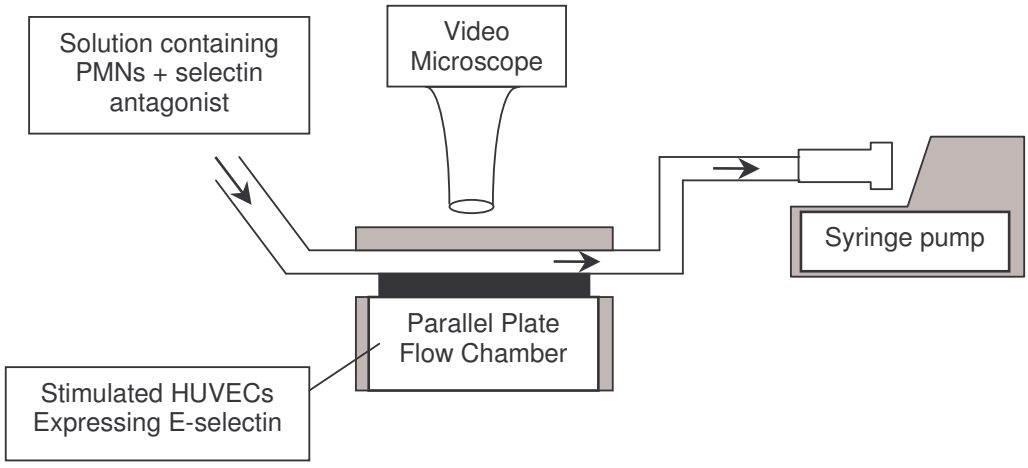


Figure 32 : Set-up of the cell-based flow assay [130].

B. Results and discussion

B.I. Thesis

In the present work, we focused on two axes of research :

- The first one is the targeting of lipophilic patches of the protein localized near the sLe^x binding site, that could be reached by substituting the galactose unit at position 2, thereby gaining binding affinity from enthalpic contributions. A library of mimics is thus planned, taking advantage of the properties of Huisgen 1,3-dipolar cycloaddition of alkynes to an azide [132].

- Our second research axis is to force our ligands into a solution conformation expected to be as close as possible to the bioactive conformation, thereby minimizing entropic costs upon binding.

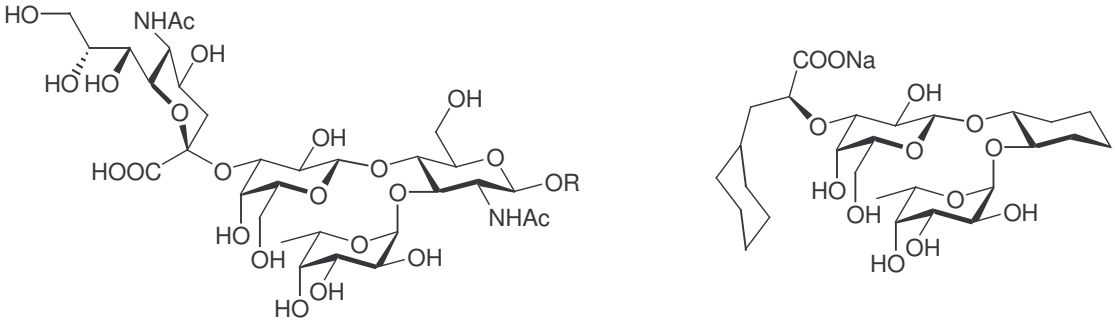
B.II. First hypothesis : New interactions in non-specific binding pockets

B.II.1. Introduction

B.II.1.1 Crystal structure and modeling studies

Our first hypothesis is based on the published crystal structure of the Lec-EGF construct co-crystallized with sLe^x [82] and molecular modeling studies derived thereof.
Based on the crystal structure, a computer model of the Lec-EGF domain of E-selectin has been developed at our Institute by Dr. Michele Porro [133]. The model was then used for the virtual docking of several structures to the binding site of the protein, beginning with sialyl Lewis^x (sLe^x, **2**), which is assumed to be one of the natural ligands

(cf. part **A.I.3**). A second compound of great importance is our reference compound, CGP69669 (**22**), one of the best ligands known at the time and therefore a suitable lead compound. Our main expectation from these docking studies was to find areas close to the binding site that could be reached easily by new substituents, hopefully leading to supplementary interactions, thereby resulting in an increase of the affinity.



sLe^x (**2**) CGP69669 (**22**) [103]

Figure 33 : Formulas of sLe^x (**2**) and CGP69669 (**22**) [134].

The docking results are illustrated in the following figures (figure 34 and figure 35), where the pharmacophore is highlighted. In a summarized way (for more details, see part **A.II.1**), the 3-OH and 4-OH of the fucose moiety (green circles) are expected to bind the calcium ion embedded in the protein, whereas the carboxylic acid, the 4-OH and 6-OH of the galactose moiety, and even the 2-OH of the fucose (yellow circles) interact with several amino acids of the protein. Our interest was focused on the region 1 of the protein, which does not belong to the generally accepted binding site. It is, however, close to the core of the ligand, and possibly a good opportunity to gain further interaction with the protein and hence better affinity.

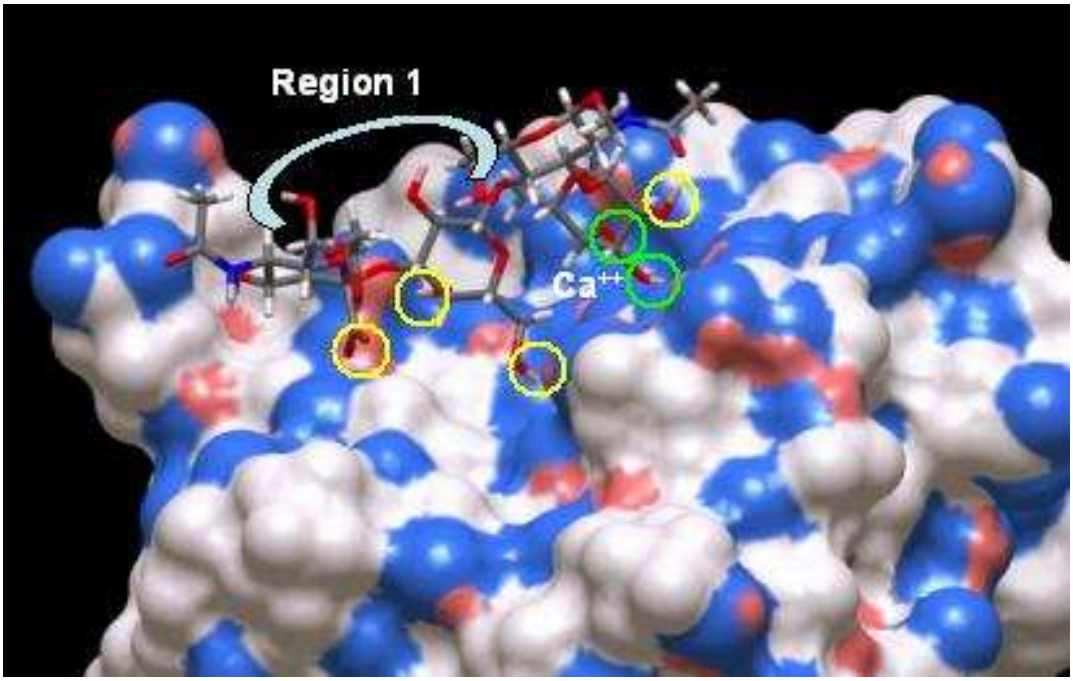


Figure 34 : sLe^x (**2**) docked in Lec-EGF.

It is interesting to note that the complexity of the sLe^x molecule is not required for good binding. Indeed, the simpler molecule **22** has an experimental relative IC_{50} (rIC_{50}) of 0.08, whereas rIC_{50} of sLe^x has been normalized to 1 (12 times less).

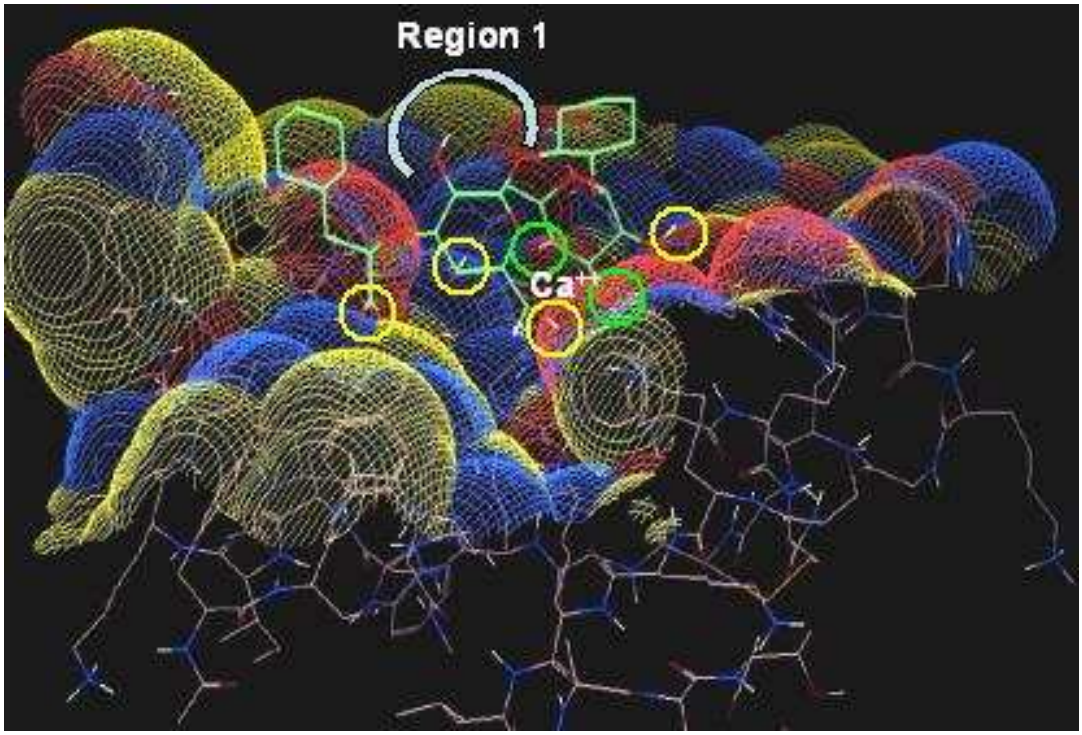


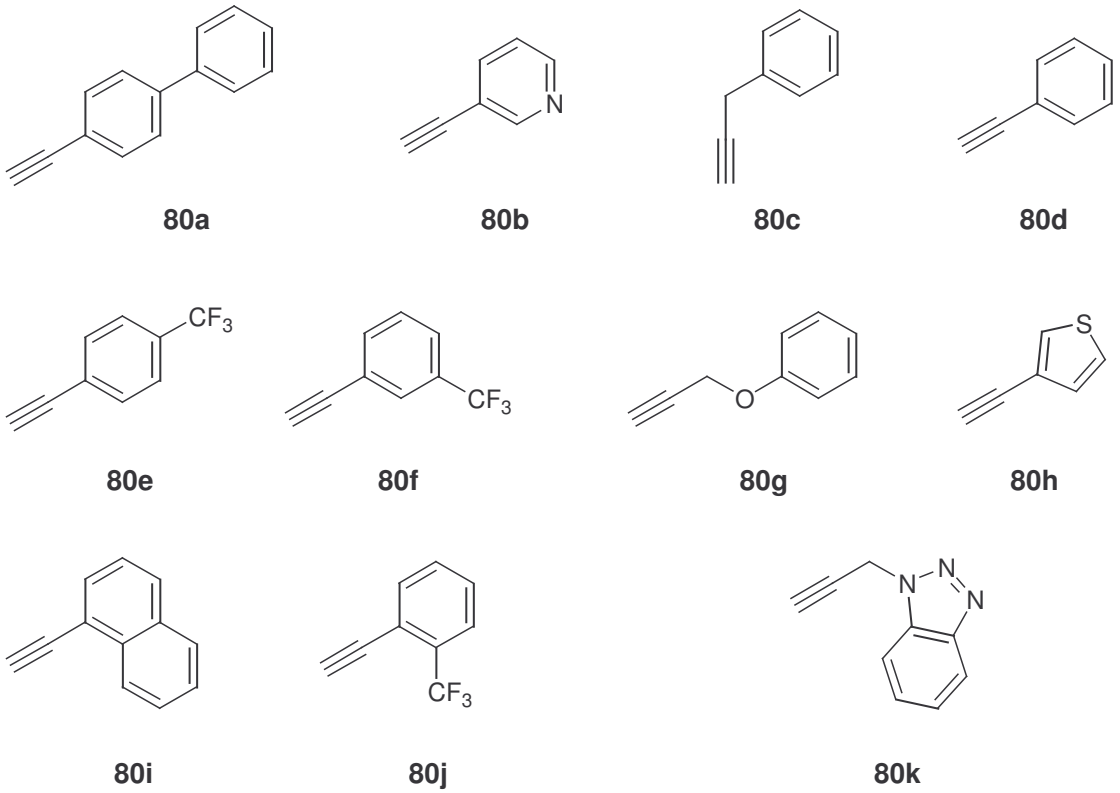
Figure 35 : Compound **22** docked in Lec-EGF.

B.II.1.2 Adressing the lipophilic pocket facing Gal-2

Modification in region 1, which can be reached by modifying the position 2 of the galactose unit is the subject that will be addressed in the following. The idea arose from molecular modeling studies, themselves based on the published crystal structure [82]. They showed that the position 2 of the galactose was facing a lipophilic patch on the surface of E-selectin. It was therefore assumed that aromatic groups at this position would trigger an increase in the affinity.

As it is discussed later (see **B.II.2.2**), 1,3-dipolar cycloaddition of various alkynes to an azide was considered as a valuable reaction for modifying the position 2 of the galactose with a broad range of chemical entities. Further considering that aromatic (substituted or unsubstituted) groups seemed promising in modeling studies, we used the following commercially available alkynes (**80**a-k, figure 36).

In the following, the synthesis of the protected 2-azido-2-deoxy-galactoside precursor will first be discussed in details, focusing on the key coupling steps. Considerations about click chemistry and the very last steps (final deprotection) will follow.



80a **80b** **80c** **80d**

80e **80f** **80g** **80h**

80i **80j** **80k**

Figure 36 : Alkynes for the planned triazole library.

B.II.2. Synthesis

The target molecule is the protected precursor **96** having an azido group at the position 2 of the galactose moiety (figure 37).

The retrosynthetic study shows the linear coupling sequence of specifically made building blocks. The sequence starts with the suitably protected thioethyl fucoside **81** [135], which was first coupled with the optically pure (*1R*,*2R*)-cyclohexane-1,2-diol **83**. The molecule obtained (**84**) was further coupled with the 2-azido-2-deoxy-galactoside unit **90**, and after deprotection the cyclohexyllatic acid moiety **99** was added.

Once the target precursor has been obtained, two routes are possible : Either the 1,3-dipolar cycloaddition could be carried out directly on the protected molecule **96** (before final catalytic hydrogenation), or the molecule could first be deprotected, 1,3-dipolar cycloaddition remaining the last step.

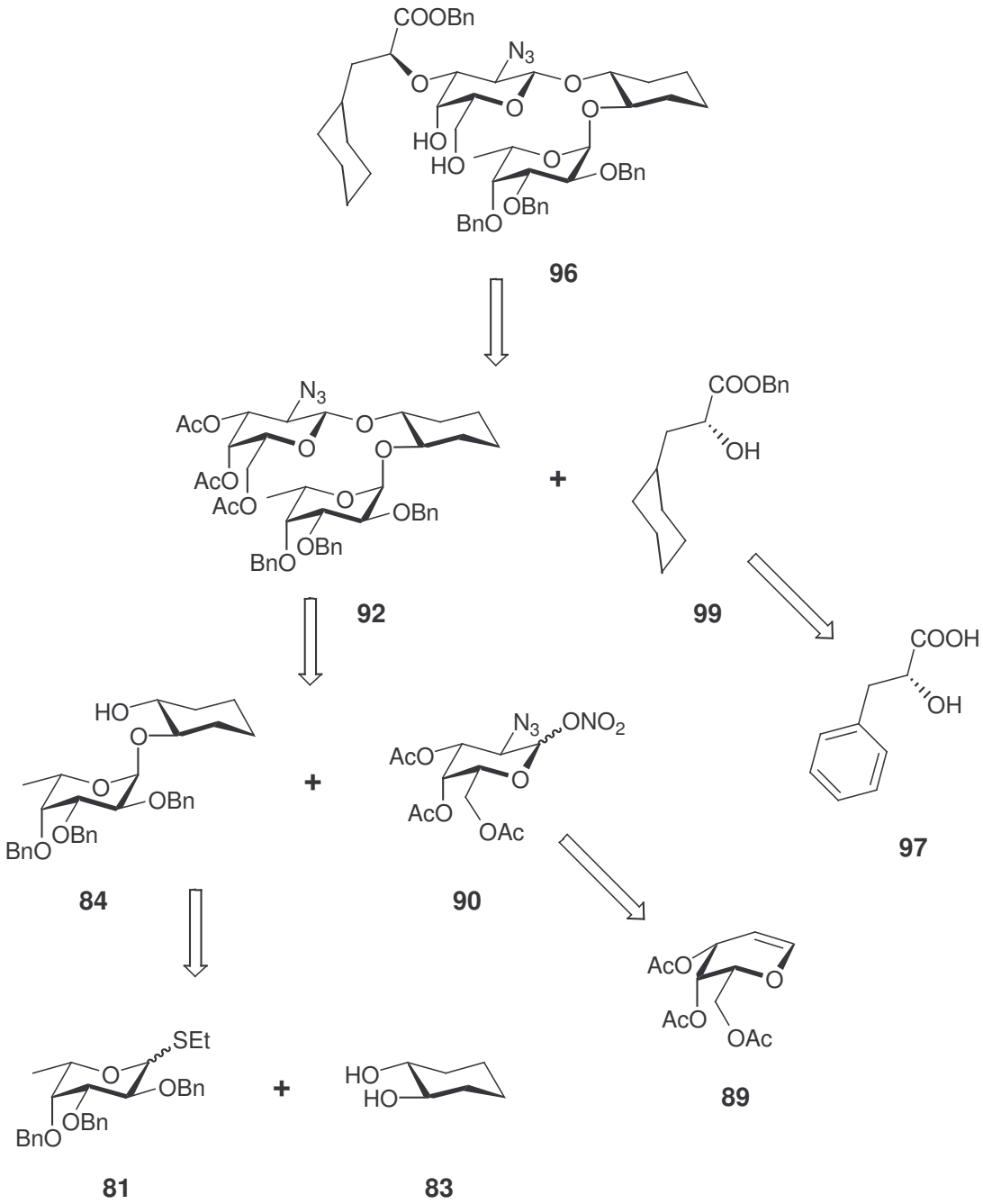


Figure 37 : Target molecule **96**, and retrosynthetic pathway to it.

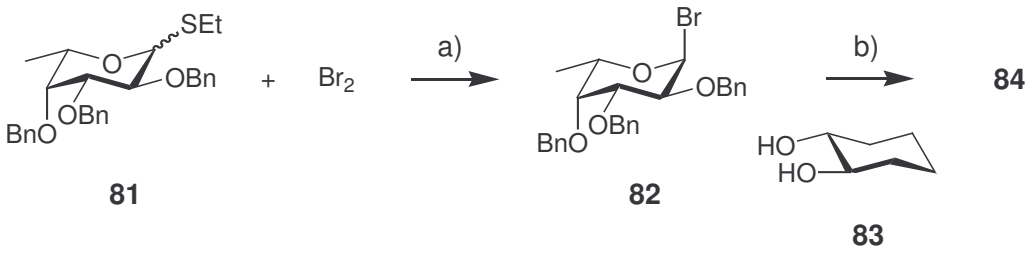
In the next pages, the synthesis will be developed step by step, focusing on the different coupling reactions.

B.II.2.1 Coupling steps

Coupling (glycosidation) steps are key steps in carbohydrate chemistry, and therefore, the different protecting groups have to be conscientiously chosen for their stability towards coupling conditions and their “orthogonal” character. This means that a part of them will have to be cleaved selectively in the presence of the other protecting groups.

One of the main properties of a coupling reaction in carbohydrate chemistry is its reliability in respect to regio- and/or stereoselectivity [136-145]. In our synthesis, three key coupling steps are used. The first one should lead to the α-fucoside anomer, the second one has to build the β-galactoside, and the last one should lead specifically to the attachment of cyclohexyllactic acid at the position 3 of the galactose moiety. Other coupling orders were envisioned too, for example with an enzymatic fucosylation as the last step.

Fucose protection and coupling to (1*R*,2*R*)-cyclohexane-1,2-diol :



Scheme 1 : Synthesis of the fucose-diol building block **84** [134,96,146]: a) Br_2, DCM, 0°C to r.t., 1h (quant.) ; b) TEAB, DMF. r.t., 3h (70%).

Before carrying out the coupling, the fucose had to be suitably protected. Our starting material was D-fucose.

The protection pattern is partly dictated by the coupling reaction which should yield the α-anomer. Therefore, in the 2-position, protective groups with so-called “neighboring group participation” [147-150] properties should be avoided.

Participating groups, for example, are esters and amides, and trigger the formation of *trans*-glycosides (β-anomer in the case of fucose) via the mechanism presented in figure 38. The bromide is removed with a promoter (e.g. silver salt) leading to the corresponding oxonium ion which reacts intramolecularly to form an intermediate dioxolenium ion. The access of the alcohol to the α position is now blocked. Therefore, the alcohol attacks from the β direction, selectively forming the β-anomer of the product.

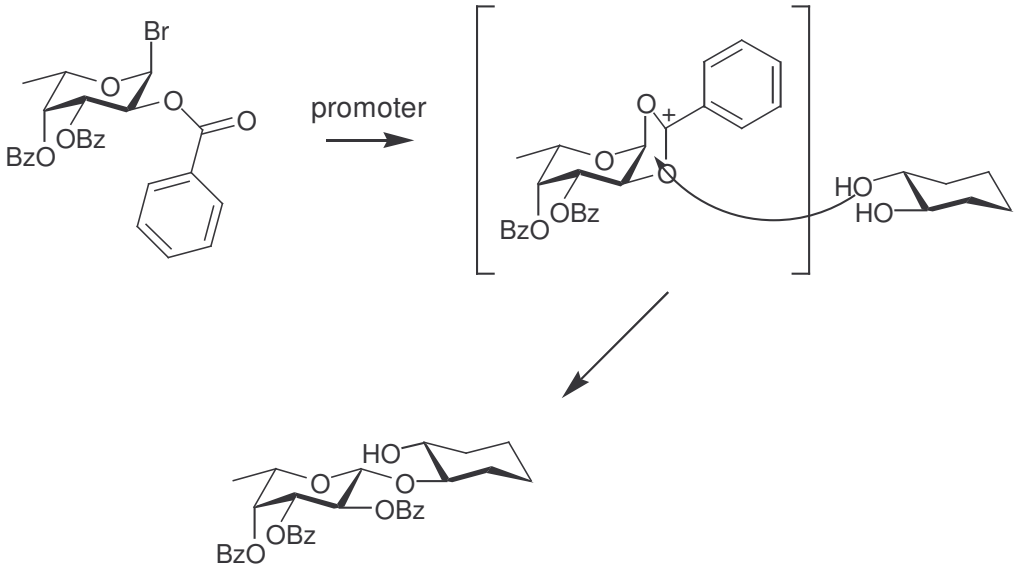
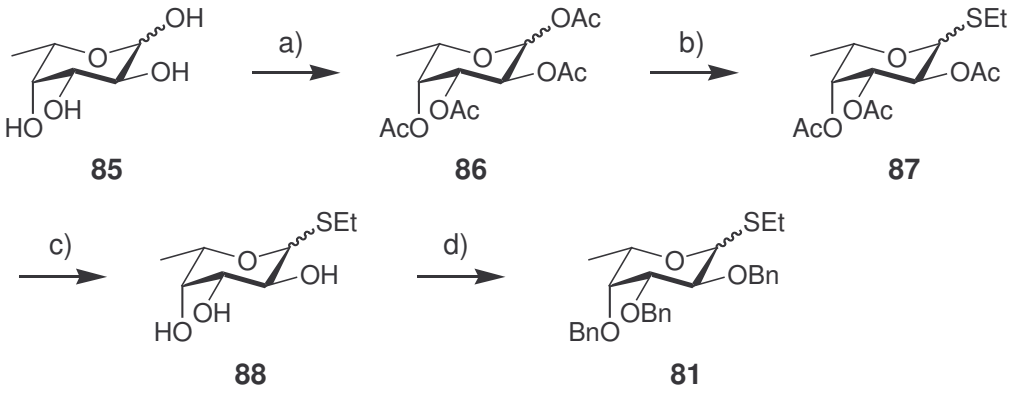


Figure 38 : Glycosidation mechanism with a participating group [149].

Since we planned to synthesize an α-fucoside (*cis* product), which is thermodynamically favorable, a non-participating group at position 2 of the fucose is needed. Ethers are perfectly suited for that role. Among them, benzyl and silyl ethers are the most preferable, as they are relatively easy to cleave (moreover selectively) afterwards.

In the course of the synthesis, the last coupling step is mediated by dibutyltin oxide (see below) and cesium fluoride. As silyl ethers can be cleaved by fluoride ions, their use is thus not recommended in this synthesis. Benzyl ethers can selectively be removed by hydrogenation. They are stable under basic and acidic conditions, and therefore suited for our purpose. There are different types of benzyl ethers. The most used among them are simple benzyl and *para*-methoxybenzyl ethers [151-155]. In our case, simple benzyl ethers were chosen. The issue is further discussed in part **C.III.3**.



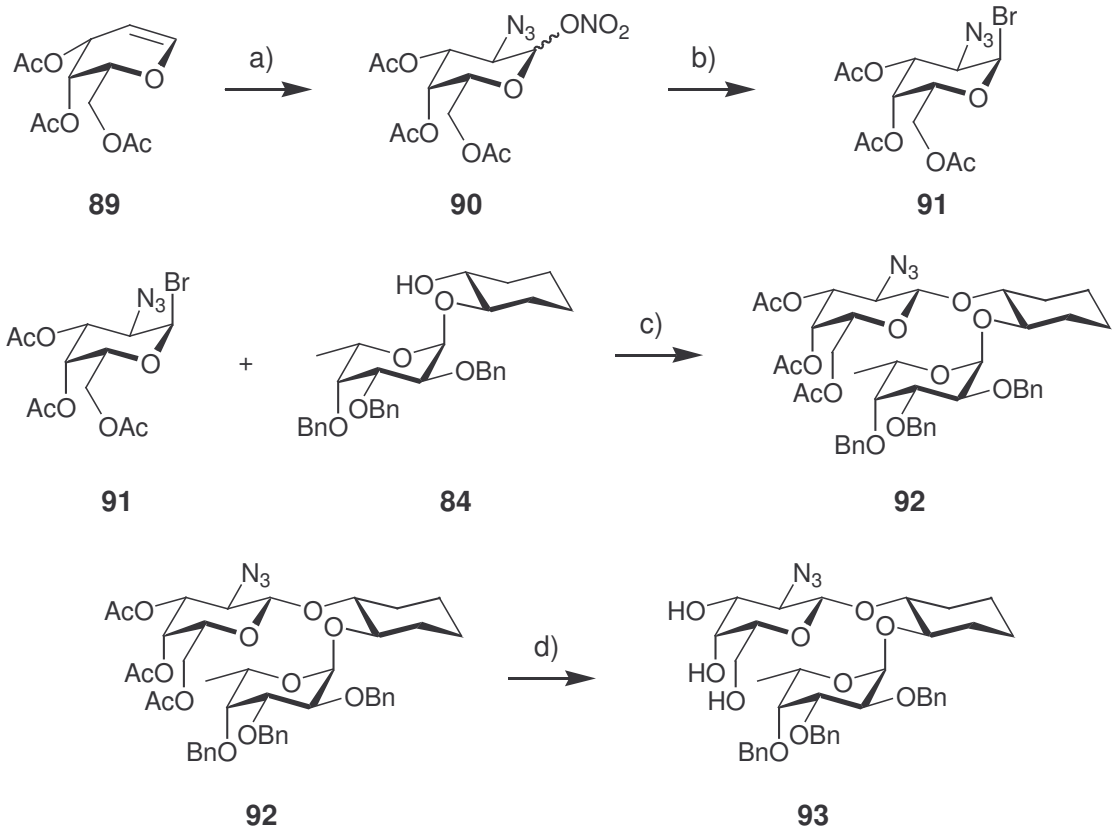
85 **86** **87** **88** **81**

Scheme 2 : Protection of the fucose [135] : a) Ac_2O, pyridine, 0°C to r.t., 17h (quant.) ; b) EtSH, $SnCl_4$, DCE, 0°C, 4h (78%) ; c) K_2CO_3, aq. MeOH, 19h (86%) ; d) BnBr, NaH, DMF, 0°C to r.t., 16h (80%).

Several methods are described in the literature for the synthesis of anomeric thioethers [156-159], using various Lewis acid promoters. After some trials using boron trifluoride diethyl etherate [156,158] that were not completely satisfying, tin tetrachloride [157] was chosen for allowing a cleaner reaction, also with a higher yield.

Once the suitably protected fucose (**81**, scheme 2) was obtained, coupling using the in situ anomerization procedure [160] was executed. In a first step, **81** is reacted with bromine to form an anomeric mixture of the corresponding fucosyl bromides. With TEAB the α-anomer was then anomerized into the more reactive β-anomer which was then coupled with the (*1R*,*2R*)-cyclohexane-1,2-diol (**83**) to give (*1R*,*2R*)-2-(2,3,4-tri-*O*-benzyl-L-fucosyl)cyclohexane-1,2-diol (**84**). As expected, the α-anomer was the main product. But the minor β-anomer was still present in a 10%-20% amount, and proved difficult to separate on classical column chromatography.

Preparation and coupling of the galactose mimic :



Scheme 3 : Synthesis of **93** : a) NaN_3, CAN, MeCN, -20°C, 18h, 53% ; b) LiBr, MeCN, r.t., 15h (94%) ; c) silica gel supported Ag_2SiO_3, DCM, r.t., 185h (54%) ; d) K_2CO_3, MeOH / H_2O 1:1, 41h (76%).

The main feature of this galactose mimic is the presence of an azide at position 2, instead of a hydroxy group. The azide could be introduced easily, starting from peracetylated D-galactal (**89**). Azidonitration [161-163] was achieved with sodium azide and cerium ammonium nitrate (CAN) in acetonitrile (the mechanism [162,164] of the reaction being presented in figure 39).

Cerium(IV) from CAN first oxidizes an azide ion into an azide radical. In the absence of an acceptor, these radicals would decompose into three molecules of nitrogen. However, in a suitable solvent like acetonitrile [164], an olefin would be competitively azidated, resulting in a carbenium ion after the radical intermediate is deactivated by a second cerium(IV) ion. The high stereoselectivity of the reaction (90% of the azide ends

up in equatorial – galactose – configuration and 10% in the axial – talose – configuration) is specific to the galactose series, as interactions with the axially positioned 4-O-acetyl group prevent the axial positioning of the azide. The obtained oxycarbenium ion can react in two different ways. Either (i, figure 39) it is attacked directly by the nitrate ion present predominantly forming the more stable α-anomer of the product, or (ii, figure 39) it forms an intermediate imine-carbenium ion with acetonitrile. S_N2-substitution with a nitrate then yields the β-anomer.

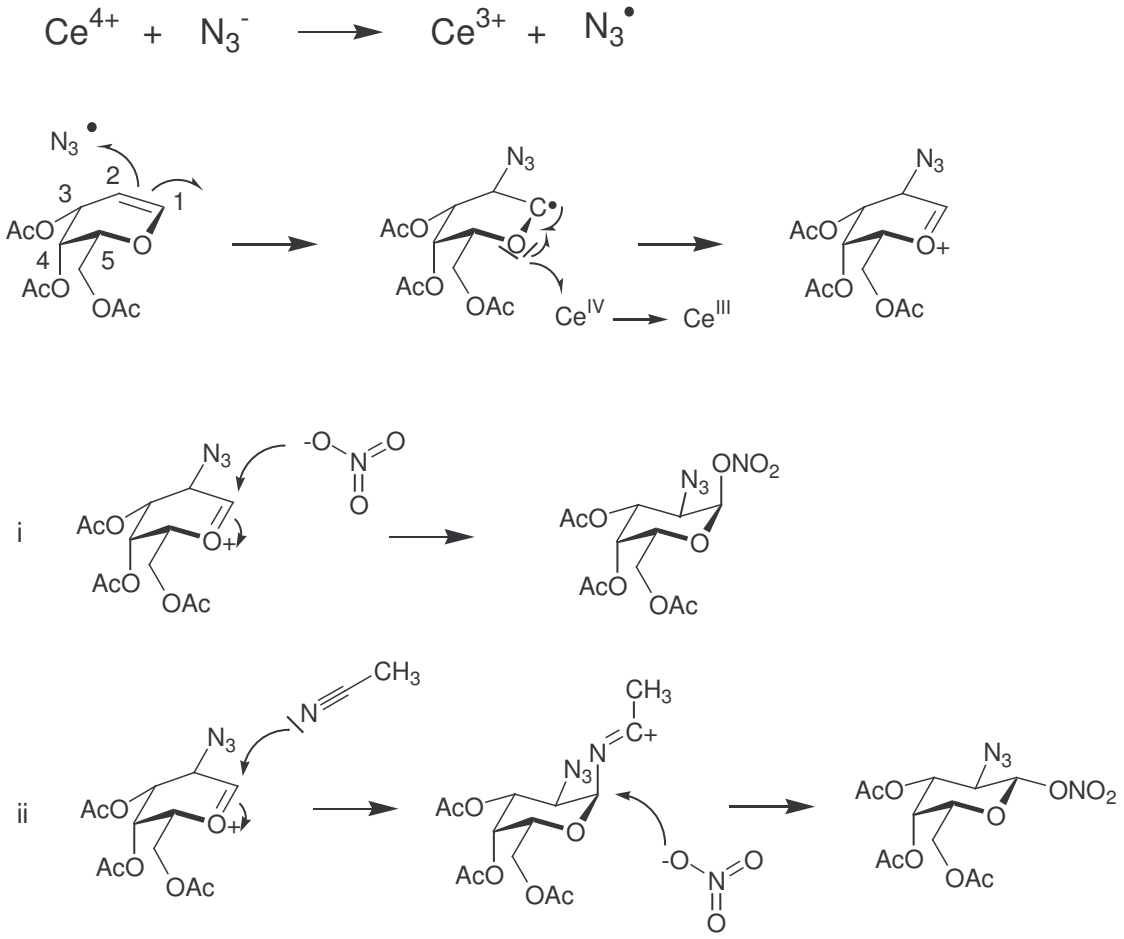


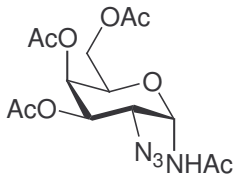
Figure 39 : Azidonitration mechanism.

From the mechanism, it looks that the α/β ratio of the product could be influenced by modifying the dilution. In our case a typical α/β ratio was from 1:1 to 2:1. However, as both anomers of **90** could be used in the next step, increased stereoselectivity was not required. As already mentioned above, the reaction also produced some 10% of the by-

product having an axial azide (resulting in an α-talose derivative), which could not be properly separated. It was, however, easily separable after the coupling step.

The nitro group is a very good leaving group and could readily be displaced with lithium bromide, to form quantitatively the α-galactosyl bromide (**91**, see Scheme 3) needed for the coupling with **84**.

It is to note that both the galactosyl nitrate and the galactosyl bromide are reactive species, and hence expected to undergo decomposition upon normal phase column chromatography. We therefore envisaged the use of both **90** and **91** without purification. In a recent paper, however, Liu *et al.* [163] showed that the azidonitration step might also produce *N*-acetyl-3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-α-D-galactopyranosylamine (**94**, figure 40), mainly during work-up procedure. **94** is prone to binding alkali metal ions, and thus makes the subsequent formation of **91** using lithium bromide more difficult to run to completion. Consequently, chromatographic purification of **90** is mandatory, even if some of it is lost through decomposition, as it eliminates by-product **94**.



94

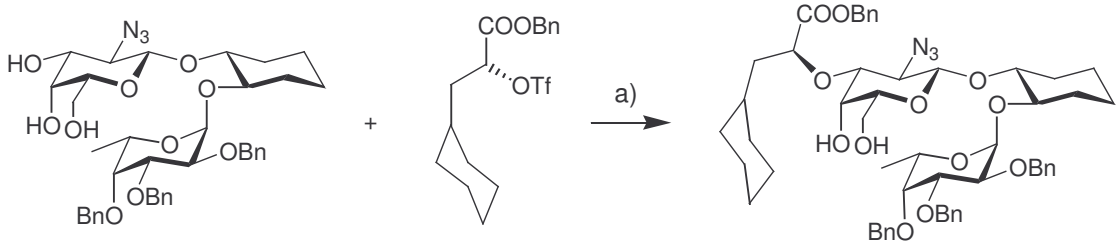
Figure 40 : By-product **94**.

In the subsequent galactosylation of **84**, no participating neighboring group is present. Therefore, glycosidation will lead to the more stable *cis*-anomer, which is α. From the work of Paulsen *et al.* [165-171], it is known that insoluble silver salts are very efficient in promoting the formation of β-galactosides.

Coupling was thus performed with silica gel supported silver silicate, resulting in product **92**, which was then treated without purification with potassium carbonate to give the partially deprotected product **93**. Unfortunately, yields over both steps never exceeded 45%, whereas the literature [168,170,172] sometimes describes yields of 70% or over.

The issue is discussed longer in part **C-II**. However, it was always possible to recover most of the unreacted starting material **84**.

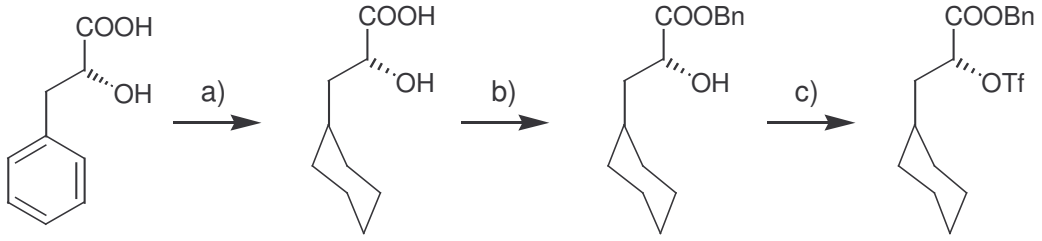
Preparation and coupling of the lactic acid derivative :



93 **95** **96**

Scheme 4 : Synthesis of the target protected tetrasaccharide mimic **96** [78,104]: a) **93**, Bu_2SnO, MeOH, reflux, 18h ; **95**, CsF, DME, r.t., 20h (71% over 2 steps).

Our starting material was D(+)-phenylactic acid (**97**, (*2R*)-2-hydroxy-3-phenyl-propionic acid). The aromatic ring was first hydrogenated using 5% Rhodium on alumina. The benzyl ester was then formed, and the hydroxyl group was activated through triflation prior to coupling (see scheme 5 for details).



97 **98** **99** **95**

Scheme 5 : Synthesis of the lactic acid building block **95** [173,104] : a) Rh/Al_2O_3, H_2 (4 bar), water/dioxane/AcOH 4:2:1, r.t., 72h (93%) ; b) Cs_2CO_3, MeOH/water 1:1, r.t., 1h ; BnBr, DMF, r.t., 20h (58% over 2 steps) ; c) Tf_2O, 2,6-di-*tert*-butylpyridine, DCM, -20°C to 0°C, 3h (70%).

The alkylation step was mediated by the formation of a stannylene acetal [174-183] on the deprotected galactosyl moiety of the molecule **93**. For thermodynamic reasons, even with a free hydroxyl group in position 6, the stannylene acetal mainly forms between position 3 and 4 favoring the building of a 5-member-ring over a 6-member ring.

In solution, the stannylene acetal can be found in two different forms [178], either dimers with 5-coordinate tin atoms, or even oligomers with 6-coordinate tin atoms. In figure 41, the mechanism focuses on the dimers. Due to the presence of the n-butyl groups, the covalently linked oxygens of the galactose are either in an equatorial (4-OH here) or an axial (3-OH) position, based on the tin atom. In that configuration, only axial oxygen atoms are activated, resulting in the regioselectivity of the reaction.

Fluoride ions have a good affinity for tin, comparable to their affinity for silicon. When cesium fluoride is added, fluoride ions will strongly coordinate with the tin atom, thus weakening its bond with oxygen. The more nucleophilic oxygen then readily attacks the triflate activated carbon on the cyclohexyllactic acid resulting in a S_N2 reaction.

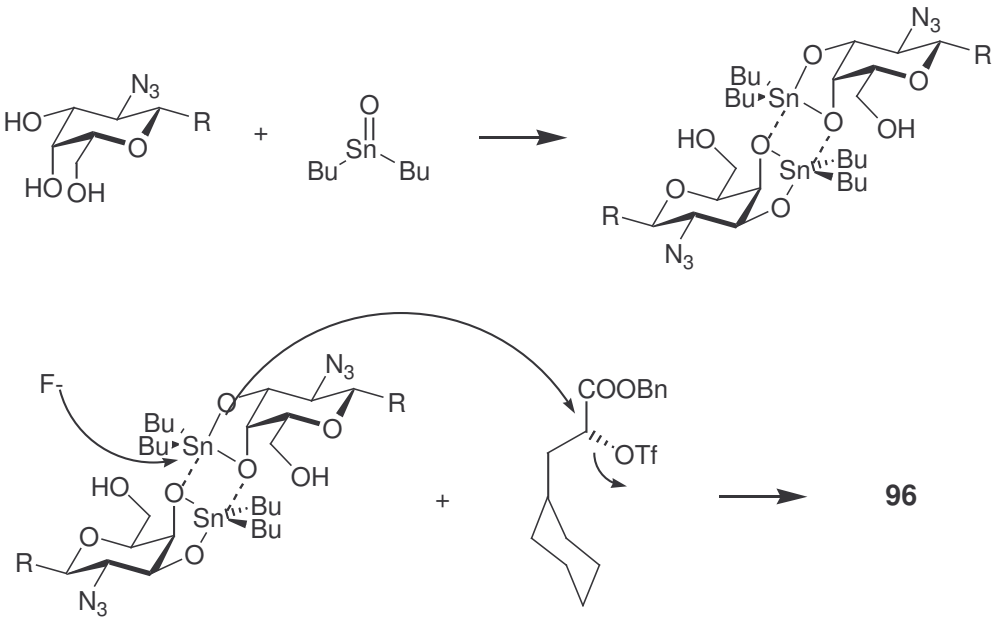


Figure 41 : Mechanism of the stannylene acetal mediated coupling.

96 is a key building block for the synthesis of a library of triazoles, by two different pathways. Either triazoles were built on protected molecule **96**, as presented below (**B.II.2.2**) or deprotection could have been carried out first, library synthesis remaining the very last step of the synthesis (**B.II.2.3**).

B.II.2.2 Construction of a triazole library via click chemistry

Introduction to Click Chemistry :

The concept of “Click Chemistry” was first introduced by Sharpless *et al.* in 1999 [184]. Referring to chemical reactions taking place in Nature, it aims to find a set of powerful, reliable and selective reactions that could easily and rapidly couple small building blocks via heteroatom links. In addition, such reactions should meet a range of stringent criteria :

> “The reaction must be modular, wide in scope, give very high yields, generate only inoffensive byproducts that can be removed by nonchromatographic methods, and be stereospecific (but not necessarily enantioselective). The required process characteristics include simple reaction conditions (ideally, the process should be insensitive to oxygen and water), readily available starting materials and reagents, the use of no solvent or a solvent that is benign (such as water) or easily removed, and simple product isolation.”[185, p. 2008]

In our case, the reaction chosen is the Huisgen 1,3-dipolar cycloaddition of alkynes to an azide, resulting in triazoles [132,186-188] (see figure 42). Until the last couple of years, this reaction did not obtain the deserved attention, probably because of concerns about the safety of working with azides. However, azides belong to the most convenient groups in synthetic chemistry. They are insensitive to acids, bases, or oxidants, which makes them very well suited for carbohydrate chemistry, and are consequently more and more often used as protecting groups for primary amines (see below **B.II.2.3** for amine-azide interchange). On the other hand, a broad range of alkynes are commercially available.

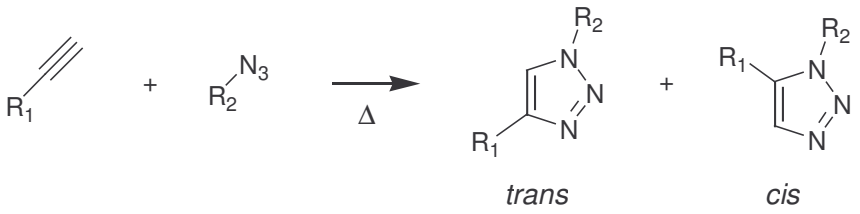


Figure 42 : 1,3-dipolar cycloaddition of alkynes to azides.

As is shown in figure 42, a given alkyne might couple to the azide giving two different regioisomers, so-called 1,4- and 1,5-regioisomers (*trans* and *cis* respectively) [186]. Therefore, when a mixture of sterically unhindered azides and alkynes is heated, it is likely that a 1:1 mixture of both isomers is obtained. Efforts to control this selectivity have met varying success, until Rostovstev *et al.* [186] and Tornøe *et al.* [189] reported the use of copper(I) as a catalyst, yielding selectively the 1,4-*trans*-regioisomer at ambient temperature. Furthermore, Cu(I) can be prepared *in situ*, using copper(II) sulfate which is reduced with sodium ascorbate. Even metallic copper has been shown to catalyse the regioselective reaction [190].

Synthesis of the triazole library :

The triazoles were synthesized mostly from the protected **96**, containing an azido group at position 2 of the galactose unit. The standard method described by Rostovstev *et al.* [186] used catalytic amounts (~10% eq.) of copper(II) sulfate and excess sodium ascorbate, in a 1:1 mixture of water and *tert*-butanol. In our case, the acetylenes that were among the most promising substituents according to molecular modeling, contain a triple bond conjugated with an aromatic system, and are thus deactivated. In addition, the benzyl protected **96** is relatively lipophilic and did not dissolve well in the reported solvent mixture. Therefore, the reaction times were much longer than reported. They ranged from 3 days in the best case (benzylacetylene, or 3-phenyl-1-propyne, **80c**) to no reaction at all (biphenylacetylene, or 4-ethynylbiphenyl, **80a**). In order to increase the reaction rate, methanol was used as the only solvent, and copper(II) sulfate and sodium ascorbate added in stoichiometric amounts. With these conditions 4-ethynylbiphenyl **80a** reacted to completion in two days.

B.II.2.3. Final deprotection

The idea behind :

Libraries of compounds are often built by parallel synthesis. Basically, one common scaffold is synthesized in large amounts, and then separated into small batches that are elaborated into the different members of the library.

In our case we planned the 1,3-cycloaddition to be carried out at the end of the synthesis, ideally as the last step. Moreover, we aimed to have as little as possible work-up and purification steps of the end-products. In practice, it means that :

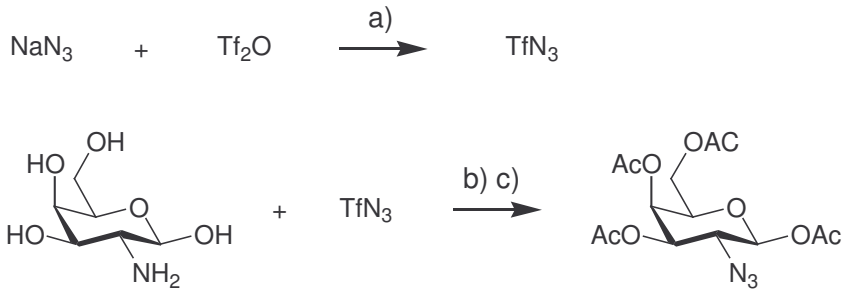
- The library precursor should be fully deprotected ;
- The last reaction, building the library, should be clean and quantitative ;
- Few, ideally no purification steps should be necessary.

We thus planned to perform the last step of the synthesis (copper(II) catalysed 1,3-cycloaddition of alkynes to the azide) in a 96-well microtiter plate, where ideally the static bioassays could be performed without any more purification step.

The chosen reaction looks perfectly suited for this approach, as it could be run in water with no organic co-solvent (or few enough to be acceptable for the assay). Moreover, the reactants (copper(II) sulfate, sodium ascorbate) should be used in catalytic quantities, and are not expected to poison the protein. And finally, a high yield of the reaction should lower the need for further purification. The prerequisite, therefore, is to deprotect the target compound **96** prior to triazole formation.

Amine-azide interchange :

The remaining protecting groups of **96** are three benzyl ethers on the fucose moiety and the benzyl ester protecting the lactic acid moiety. The most common method for the cleaving of benzyl groups is catalytic hydrogenation, using palladium on activated charcoal. Under these conditions, the azido group will be reduced to an amine. Fortunately, procedures have been described for the synthesis of an azide from a primary amine [191-196]. The reactant used is trifluoromethanesulfonyl azide (triflyl azide, TfN_3), which is freshly prepared before use (scheme 6). The reaction was used as described in the cited literature [191,193], using both protected and unprotected galactosamine as test compound. The major drawback of the method is the use of dichloromethane as solvent for the TfN_3 preparation, as it is known that azides (sodium azide in particular) may turn into explosives (CH_2ClN_3, $CH_2(N_3)_2$) in chlorinated solvents. A. Titz from our Institute developed a new method, using toluene as solvent, which proved as efficient as dichloromethane [197].



100 **101**

Scheme 6 : Amine to azide exchange applied to galactosamine [191,193] : a) DCM/water 2:1, 0°C, 2h ; b) TfN_3, K_2CO_3, cat. $CuSO_4$, MeOH/water/DCM 6:3:1, 16h ; c) Ac2O, pyridine, r.t., 16h (58% over two steps.

Final deprotection and library synthesis :

With all the tools in hand, deprotection of **96** under reducing conditions was tried. The azido group was first reduced into a primary amine, which turned to be nucleophilic enough to react with the benzyl ester, thus forming a 6-membered ring lactam (as illustrated in figure 43). It turned out impossible to cleave with methods soft enough to keep the integrity of the entire molecule.

Different methods (other hydrogenation conditions, DDQ oxidation, Lewis acid mediation) were tried for the cleavage of the benzyl groups, aiming to prevent the formation of the lactam (further discussed in part **C.III**). Up to now, none of them give the expected results.

Therefore, the triazole library was formed from the protected **96**, before the final deprotection by hydrogenolysis was carried out. This was carried out successfully with a small set of five triazoles, that were then tested in bioassays to test their E-selectin antagonist activity.

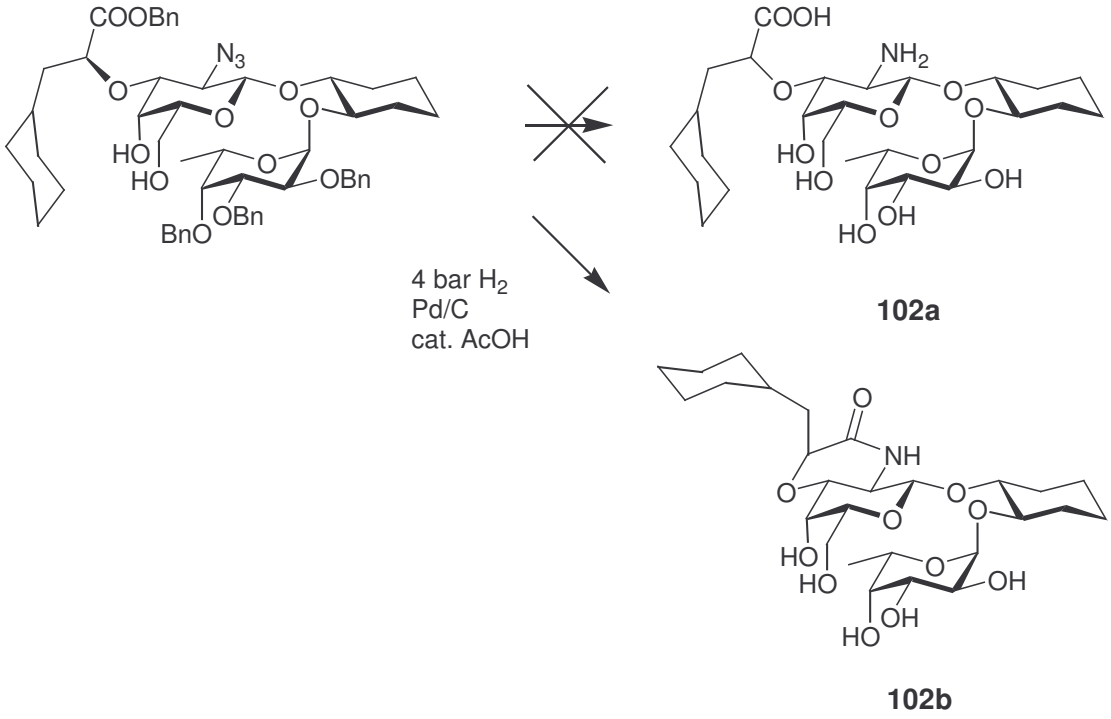
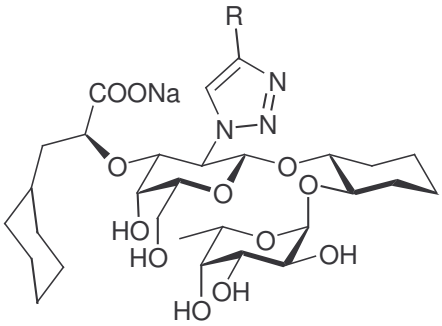


Figure 43 : Lactam formation during the hydrogenation of **96**.

B.II.3. Results of the bioassays

Activity tests of all antagonists were performed at Glycomimetics Ltd., Gaithersburg, MD, USA. The assay chosen is static, and cell-free [129], as described in part **A.IV.1**.

Five triazoles (figure 44 and table 2) were synthesized and sent for testing.



103a-e

Figure 44 : General formula of the triazoles **103**.

As stated in part **A.III**, all IC_{50} values are relative to the activity of sLe^x, (rIC_{50} = 1). The rIC_{50} of our reference compound, namely CGP69669, is 0.08.

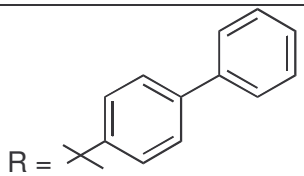
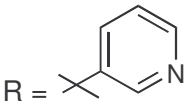
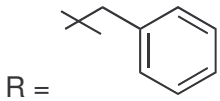
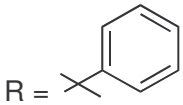
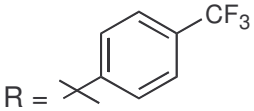
ID	Formula	IC_{50} (µM)
22	CGP69669	80
103a		> 1000
103b		275
103c		90
103d		125
103e		350

Table 2 : Inhibitory activity of the individual triazoles.

None of the triazoles was more active than the reference compound. Therefore, the main conclusion of this work is that the substituents do not establish an additional lipophilic contact with the protein and therefore do not show improved binding. Some substituents (e.g. in **103a**) are even deleterious for the activity.

However, these results offer some insight into the binding site of E-selectin, in particular the part of it facing the Gal-2 position. First of all, the size of the Gal-2 substituent seems important. Indeed, the smaller phenyl substituent (**103d**) binds better than pyridinyl (**103b**), which is again better than trifluorophenyl (**103e**), while biphenyl (**103a**)

completely prevents binding. Secondly, flexibility seems important too, as the benzyl group (**103c**) has the best binding affinity among all, although it is sterically more demanding than phenyl or pyridinyl.

The affinity of the benzyltriazole may also fit well with one hypothesis exposed in next part, namely that lipophilic interactions between the substituents in the Gal-2 and Gal-3 positions may stabilize the acid orientation in the bioactive conformation, and thus enhance activity.

B.III. Second hypothesis : Stabilization of the bioactive conformation

B.III.1. Introduction.

The bioactive conformation of sLe^x (**2**) is known with certainty from trNOE NMR [80,81] and the crystal structure of Lec-EGF co-crystallized with sLe^x [82]. To facilitate the evaluation of new ligands, Kolb and Ernst [104,105] developed and validated a 2D internal coordinate system, allowing to visualize the spatial orientation of the functionnalities relevant for the pharmacophore. Two particular *torsion angles* were defined to describe the 3D-structure of sLe^x (see figure 45).
The first one, called *acid orientation*, is defined by (Fuc-C1;Fuc-O1) ; (Acid-Cα;Acid-C=O), while the second one, called *core orientation*, is defined by (Fuc-C4;Fuc-C1) ; (Fuc-O1;Acid-Cα). For a given ligand, molecular dynamics simulation give the probability of the possible conformations of the molecule (see figure 46), which are then plotted relatively to those angles. Based on the NMR results of Peters *et al.* [75,76] for the bioactive conformation, Kolb and Ernst [105] defined a *bioactive window*, visualizing an ensemble of conformations surrounding the bioactive one.

Our hypothesis is that the conformation of a potentially active molecule in solution should be as close as possible to the bioactive conformation, in order to minimize entropic penalties upon binding.
Different approaches to constrain the conformation of sLe^x mimics have been studied so far : (i) The introduction of bulky substituents at different positions of the molecule [78,127,128] ; (ii) The use of C-glycosides [198] ; (iii) The formation of macrocycles [107,199] (see also the existing E-selectin inhibitors, part **A.III**).

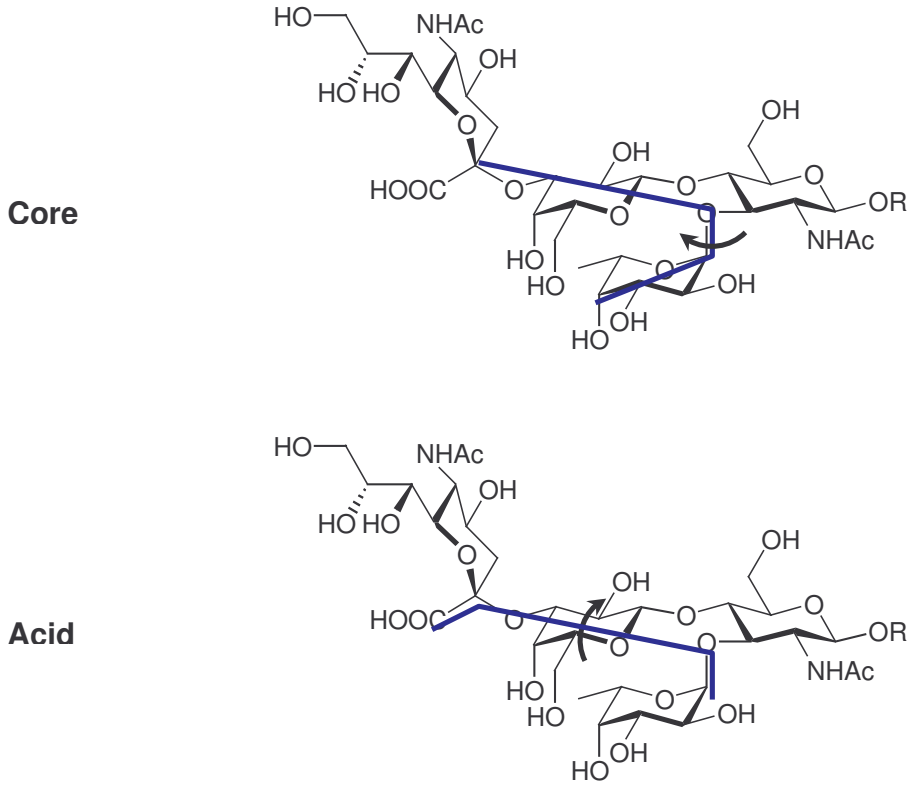


Figure 45 : Torsion angles, as defined based on sLe^x.

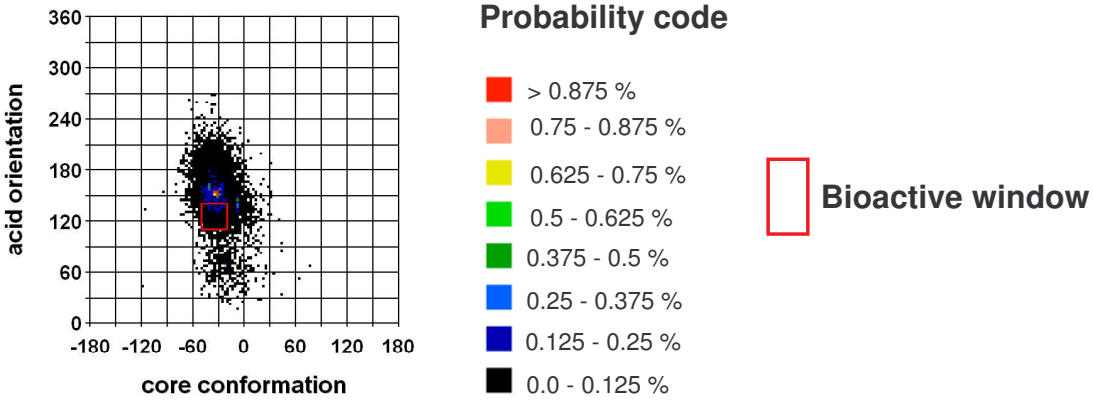


Figure 46 : Probabilistic distribution of the conformation of a compound [105].

In this work, we planned to study the influence of Gal-2 substituents on the pre-organization of the bioactive conformation. We will focus on two research axes, namely the bulkiness of the substituent, and its possible intramolecular interactions with the lactic acid derivative mimicking the Neu*N*Ac group of sLe^x.

B.III.1.1. Relation between the α-protons at the 2-position of Gal and the 5-position of Fuc

For antagonists with core conformation and acid orientation in the bioactive window, the proton in the 2-position of Gal and the 5-position of Fuc are in close spatial relationship. Therefore, they are also expected to influence each other, and the influence should be noticeable with ^{1}H NMR experiments, particularly by using the nuclear Overhauser effect (nOe). In such an experiment, a ^{1}H NMR spectrum of the molecule is first recorded under normal conditions, showing the chemical shifts of the protons of interest. In a second analysis, the spectrum is recorded while the molecule is irradiated specifically at the resonance frequency of one of these two protons, canceling all influences the chosen proton has on the rest of the molecule. If the second proton is close enough to the irradiated one, a substantial nOe can be observed. As close the spatial relationship, as large is the expected low field shift for H-5 of Fuc.
In conclusion, if we assume that in the bioactive conformation Gal-H2 and Fuc-H5 protons are close to each other, ^{1}H NMR spectra with nOe should be an easy way to reveal how close the conformation of a given sLe^x mimic is relative to the bioactive one.

B.III.1.2. Examples

The corresponding investigation already has been published [103] for a number of selectin antagonists (figure 47).

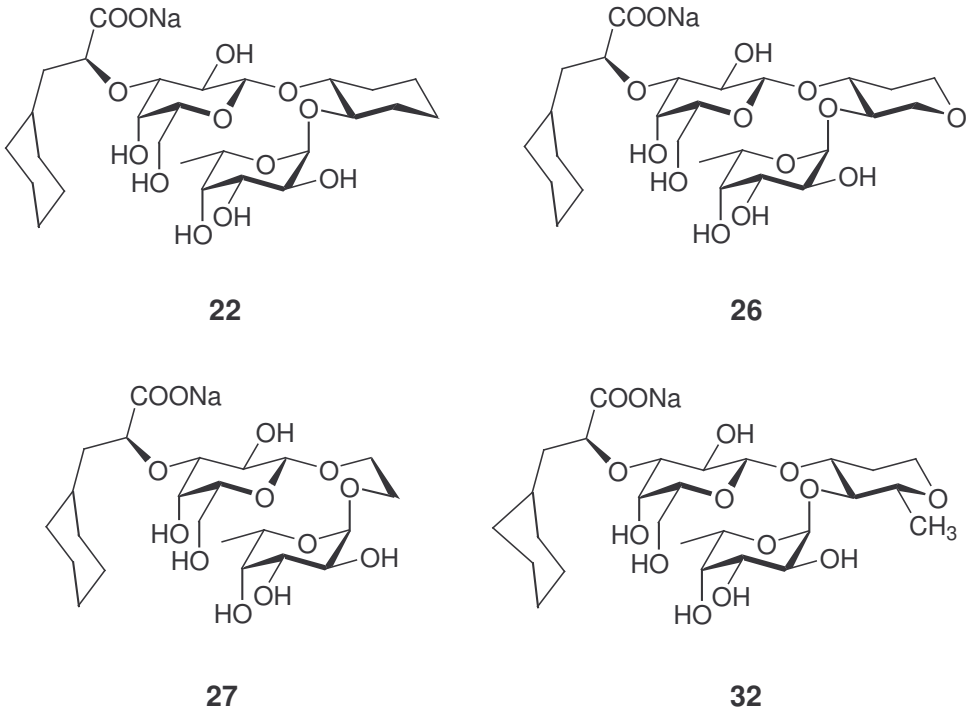


Figure 47 : Structures of some of the molecules described in [103].

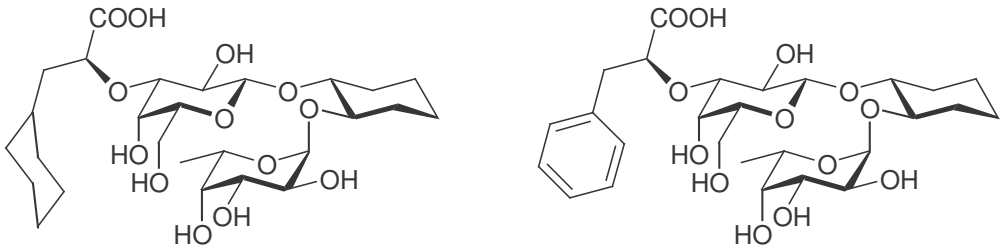
Molecule	**22** (CGP69669)	**26**	**27**	**32**
Chem. shift Fuc-H5 (in D_2O)	4.60 ppm	4.42 ppm	4.12 ppm	4.77 ppm
nOe between Gal-H2 and Fuc-H5	0.094	0.043	0.003	0.108
rIC_{50} (rel. to sLe^x)	0.08	0.08	> 10	0.04

Table 3 : Chemical shifts of the Fuc-H5 proton and normalized nOe values in the molecules of figure 47 [103], in regard to their rIC_{50}.

The results clearly show that **32** is the most constrained molecule exhibiting the strongest nOe. The methyl group of the tetrahydropyran ring obviously forces the fucose in a position close to galactose, thereby stabilizing the core in the bioactive conformation. Consequently, the entropy cost upon binding is reduced and the binding affinity hence increased.

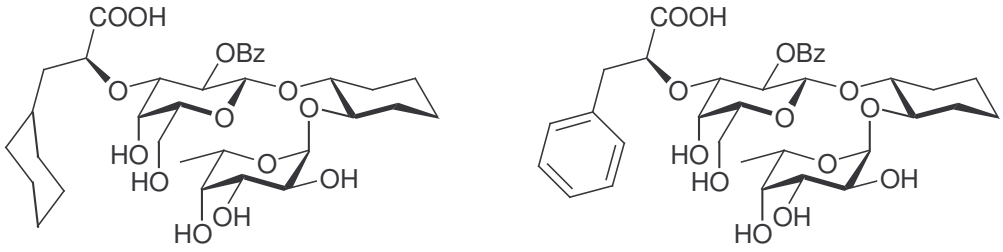
Moreover, looking to the normal ^{1}H NMR spectra of these molecules without irradiation, it appears that, even there, the chemical shift of Fuc-H5 is also correlated to the constraint. Indeed it changes from δ = 4.12 ppm for compound **27** to δ = 4.77 ppm for compound **32**. We therefore have a reliable tool to evaluate new molecules in regard to their conformational stability.

B.III.1.3. <u>The molecules studied</u>



22
(0.08)

23
(0.35)



104
(?)

105
(?)

Figure 48 : Molecules compared using the modeling tool developed by Ernst *et al.* [104,105]. Known rIC_{50} values are disclosed in brackets.

Using the modeling tool described above, four different molecules were studied (see figure 48), in order to compare the effect of the presence of a benzoyl group at galactose

2-position, in combination with either a cyclohexyl- or phenyl-lactic acid moiety. It was expected that π–π interactions between both aromatic groups would enhance the pre-organization of the molecules into the bioactive conformation.

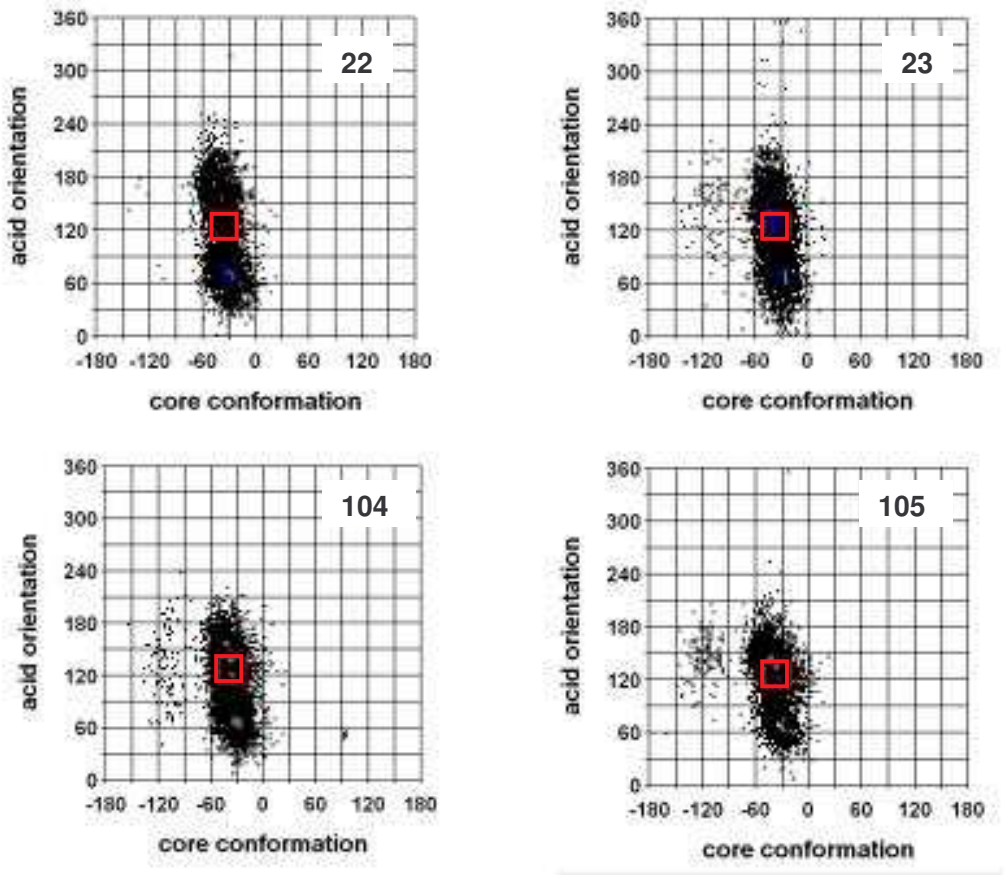
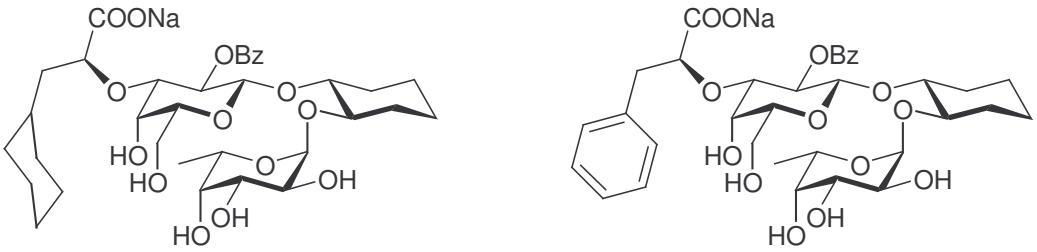


Figure 49 : 2D-plots of the molecules of figure 48.

As can be seen from the plot of figure 49, the possible conformations of molecule **104** (resp. **105**) concentrate more and closer to the bioactive window (red square) than in the case of the reference molecule **22** (resp. **23**). Compound **22** has a measured rIC_{50} of 0.08 and we thus expect the one of compound **104** to be higher. The same effect is expected in the case of compound **105**. Increased interactions due to π-stacking may even improve more the affinity.

Modeling results were encouraging, and we thus tried to validate them experimentally. The following molecules (**104** and **105**, figure 50) were therefore synthesized, in order to confirm or at least complete *in silico* results.

This synthesis is relatively close to the one used for compound CGP69669 [105,200]. The said synthesis will be extensively described in the following pages, focusing again on the different coupling steps.



104 **105**

Figure 50 : Target molecules **104** and **105**.

B.II.2. Synthesis

Figure 50 shows the target molecules. We will concentrate on the synthesis of **105**, knowing that the synthesis is the same for compound **104**, provided that cyclohexyllactic acid (**98**, cf. **B.II.2.1**) is used instead of phenyllactic acid (**97**). With the exception of Gal-2 position, the target molecules are not protected.

As shown in the retrosynthesis (figure 51), **105** will be synthesized in a parallel way. Conditions of the final deprotection steps should be tuned in order to retain the last benzoyl ester.

In the next pages, the synthesis will be developed step by step, focusing on the different coupling reactions.

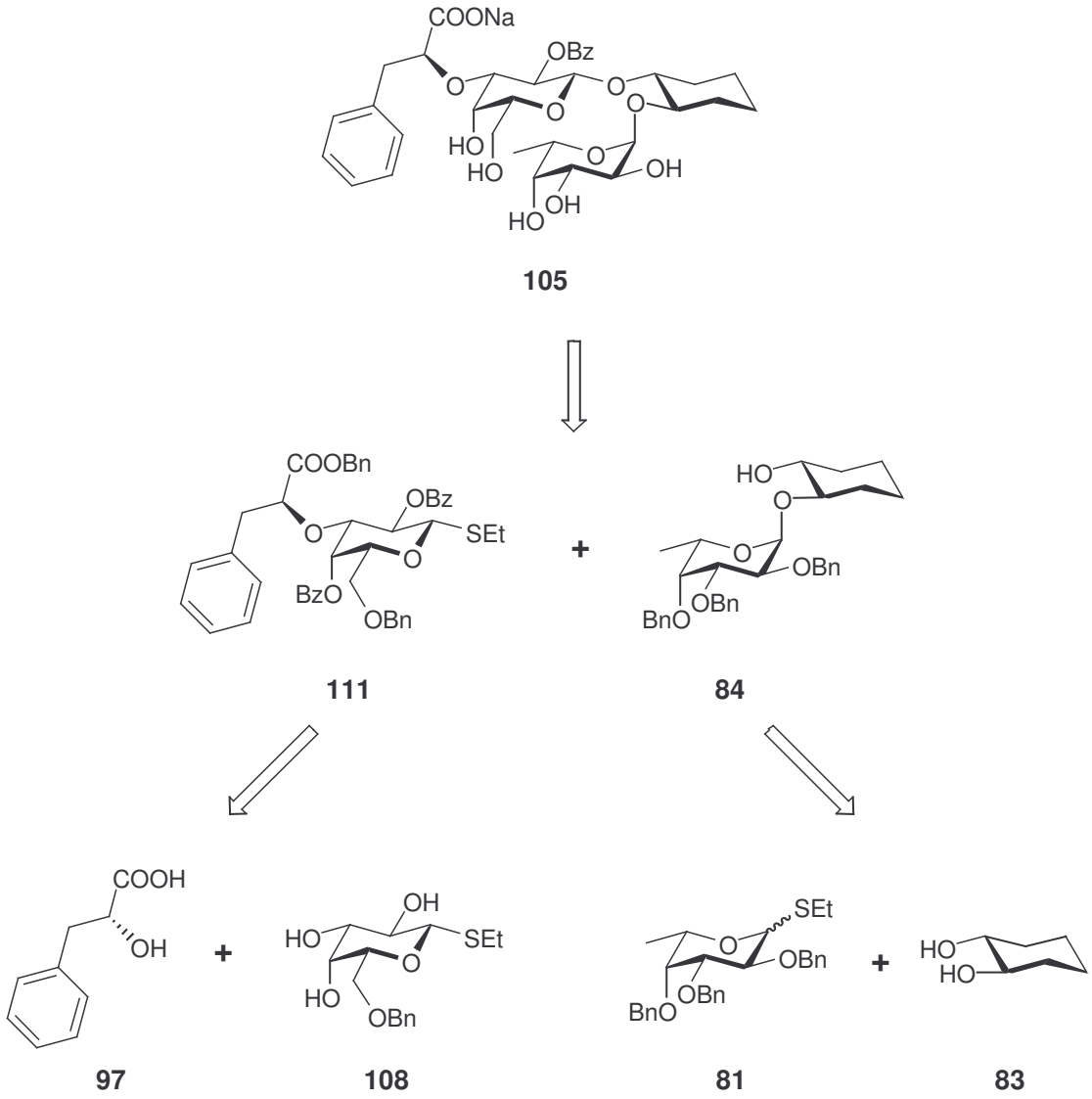
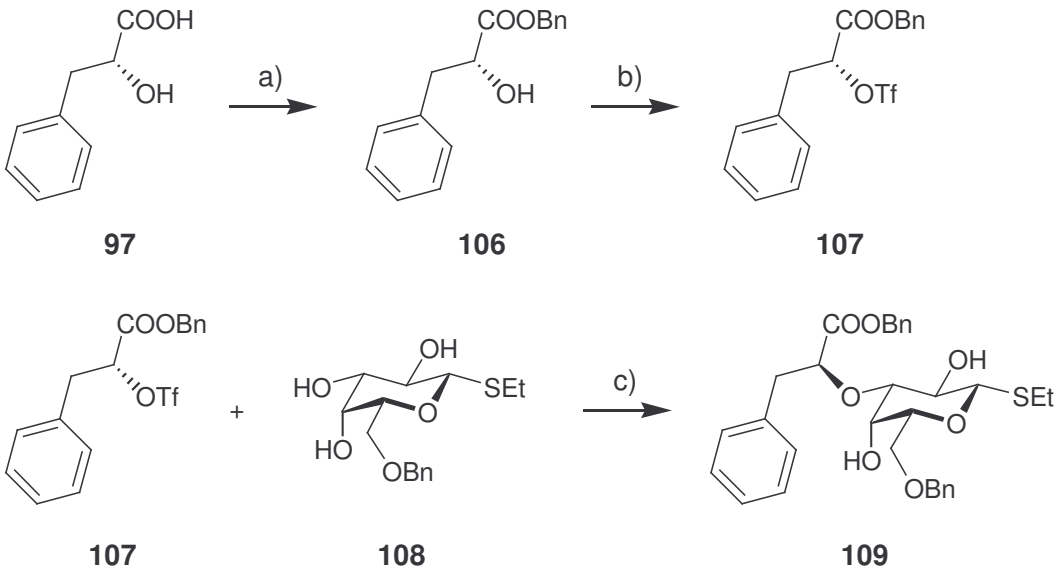


Figure 51 : Target molecule **105** and retrosynthetic pathway to it.

B.III.2.1. Coupling steps

The synthesis of **84** has already been described before (part **B.II.2.1**). Therefore, we will concentrate on the other steps.

Preparation of the galactose building block :

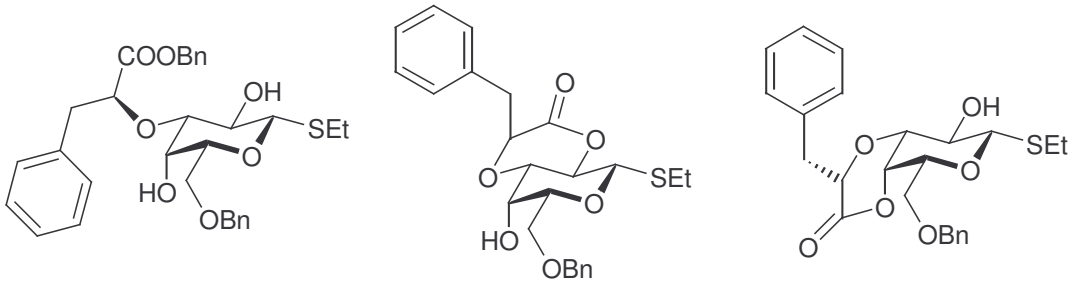


Scheme 7 : Synthesis of the galactose building block **109** [78,104] : a) Cs_2CO_3, DMF, r.t., 1h ; BnBr, DMF, r.t., 5h (one-pot, 88% over 2 steps) ; b) Tf_2O, pyridine, DCE, -20°C to -5°C, 2h (84%) ; c) **108**, Bu_2SnO, MeOH, reflux, 18h ; **107**, CsF, DME, r.t., 24h (36% over 2 steps).

Our starting materials were 6-*O*-benzyl-1-ethylthio-β-D-galactose (**108**) and D(+)-phenyllactic acid (**97**). **97** was first protected with a benzyl ester and activated by forming a triflate, leading to benzyl (*2R*)-3-phenyl-2-*O*-triflyl-propionate (**107**). It was then coupled with the galactose derivative via the formation of a stannylene acetal which reaction is still described in part **B.II.2.1**.

In that case however, yields were dramatically lower (36% for the best one, compared to 71% for the compound of part **B.II.2.1**). No real explanation has been found for the versatility of this stannylene acetal mediated reaction. A competitive reaction may occur, the fluoride ions present in solution being able to react with the lactic acid triflate. Indeed, benzyl 3-phenyl-2-fluoropropionate was found in a significant amount among the reaction's products.

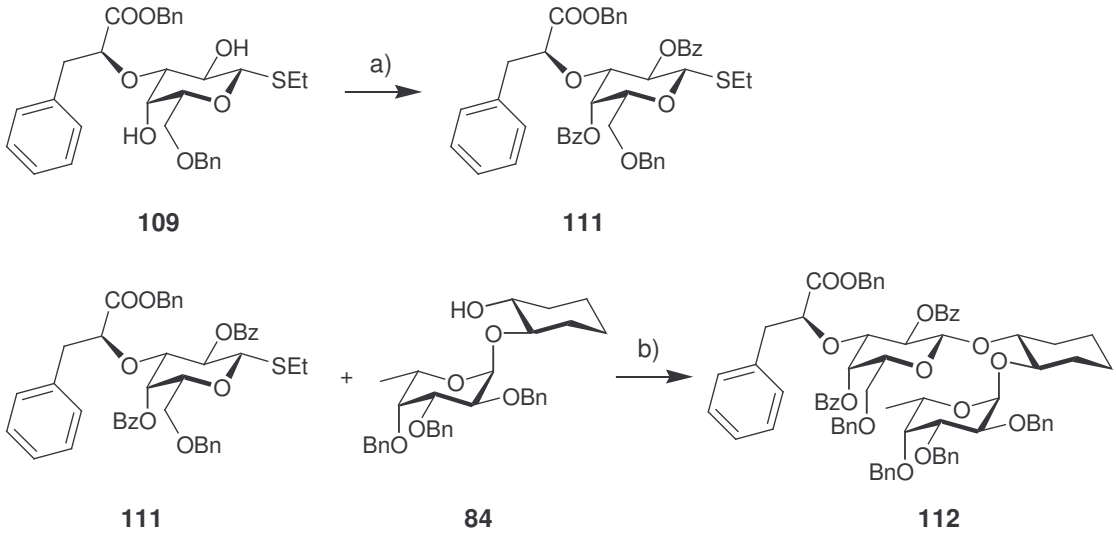
Moreover, the product is partly (~30%) found in the form of a lactone, essentially built between the carboxylic acid and the galactose 2-hydroxyl, but also in some extent with the galactose 4-hydroxyl (figure 52). Reaction product was therefore a mixture of three substances. The expected one was then separated from the lactones.



109 **110a** **110b**

Figure 52 : Molecule **109** and the two possible lactones (**110a** and **110b**).

Protection and coupling of the galactose building block :



111 **84** **112**

Scheme 8 : Synthesis of the target protected tetrasaccharide mimic **112** [78,104] : a) BzCl, pyridine, r.t., 16h (46%) ; b) DMTST, DCM, r.t., 19h (52%).

Protection is carried out with benzoyl chloride, using pyridine as a solvent. As in the previous step, part of the starting material **109** (about 50%) was converted into the derived lactones. It is obvious that lactone formation dramatically lowered the yields over the last two steps (coupling with lactic acid and protection with benzoyl groups). Indeed, up to 65% of the starting galactose derivative (**108**) was converted. Therefore, efforts have been made in order to transform the lactones into protected molecule **111**. Their description and outcome is exposed in next part (**C.IV**).

This coupling step was carried out with aid of dimethyl(methylthio)sulfonium triflate (DMTST). The mechanism of the reaction [201] is shown in figure 53.
The group present at galactose 2-position is a benzoyl ester, thus participating to the coupling (cf. **B.II.2.1**). The glycosidation is therefore expected to be β-selective. Indeed, no α-anomer was produced, although yields were moderate (about 60%).

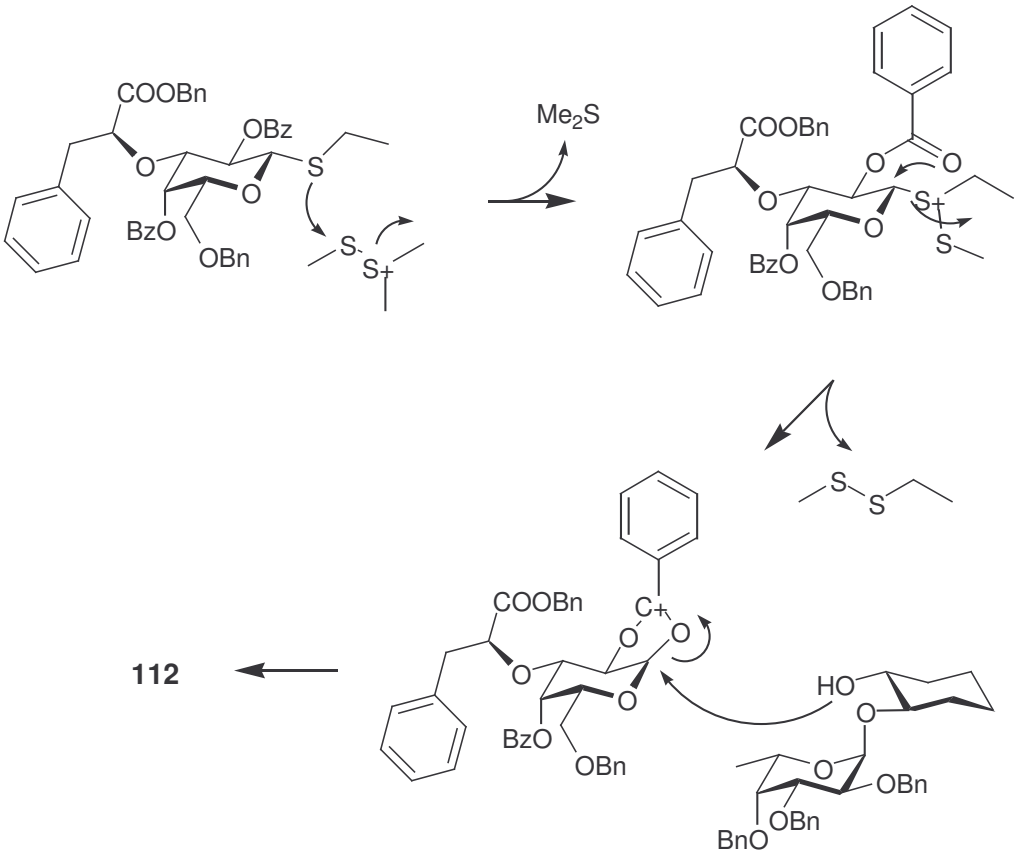
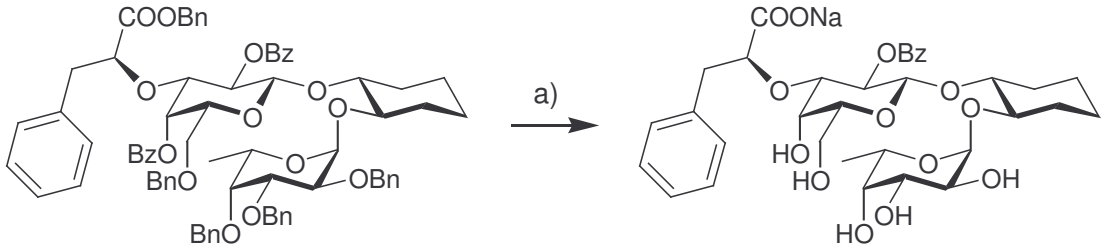


Figure 53 : Mechanism of the DMTST promoted coupling [201].

B.III.2.2 Deprotection, selective benzoyl cleavage



112 **105**

Scheme 9 : Preparation of the target molecule **105** : a) 10% Pd/C, H_2 (4 bar), dioxane, r.t., 48h ; MeONa, 1M in MeOH, r.t., 3h (36% over 2 steps).

The first step of the deprotection procedure is catalytic hydrogenation, cleaving the benzyl ester, and the four benzyl ethers protecting the molecule (Gal-6, Fuc-2,3,4). The reaction was carried out with 10% palladium on charcoal and lasted 24 h to 48 h under 4 bar hydrogen overpressure.

The second deprotection step is the cleavage of only one of the two remaining benzoyl groups. The standard reaction uses a solution of sodium methanolate in methanol and is expected to cleave all the benzoyl esters. However, it was already performed by C. Müller in our Institute on very similar molecules [198], where it was found by serendipity that the benzoyl ester at position-2 of the galactose was not cleaved, or at least, was retained much longer than any other benzoyl ester. Tests using model compounds then showed [198] that this behavior was due to the lactic acid bulky substituent (cyclohexyl in the cited study).
This effect may be due to the steric hindrance of the lactic acid lipophilic substituent. It may even be enhanced in our case, if we consider the possible π–π interactions between the phenyllactic acid moiety and the benzoate.

When applied to our molecule, sodium methanolate selectively cleaved Gal-4 benzoyl ester, leaving as expected the Gal-2 benzoate untouched. The obtained **105** as well as its cyclohexyllactic acid containing counterpart (**104**) were then tested in bioassays for their E-selectin antagonist activity, and ^{1}H NMR spectrum compared to the ones of the other molecules of interest.

B.III.3. Results of bioassays and NMR experiments

B.III.3.1 Bioassays

Activity assays of the new molecule were again carried out at Glycomimetics Ltd, Gaithersburg, MD, USA, using the static cell-free assay. Next table (see table 4) compares the result to the activity of molecules **22** and **23** (**B.III.1.3**, figure 48). As stated in part **A-III**, all IC_{50} values are relative to the activity of sLe^x, which IC_{50} is by definition fixed at 1 mM.

Molecule ID	rIC_{50}
22 (CGP69669)	0.08
104	0.04
23	0.35
105	0.17

Table 4 : Compared affinity of the four products (**22**, **23**, **104**, **105**).

As expected from *in silico* evaluation, affinity of the new compounds **104** and **105** was higher than the ones of their unprotected counterparts. In both cases the benzoyl ester at Gal-2 induced a 2-fold decrease of the IC_{50}.

We will now look at the ^1H NMR spectra, looking for confirmation of our assumptions about the correlation between conformational constraints and binding affinity.

B.III.3.2 Chemical shifts of the Gal-2 and Fuc-5 protons in ^{1}H NMR.

ID	Chemical shift Fuc-H5 (ppm, in D_2O)	Binding affinity (µM)
triazole **103a**	4.09	> 1000
27 [103]	4.12	> 10 000
23 [103]	n.p.	350
26 [103]	4.42	230
triazole **103c**	4.45	90
triazole **103b**	4.53	275
105	4.54	175
104	4.58	47
CGP69669 **22** [104]	4.60	80
32 [103]	4.77	39

Table 5 : Chemical shift of the Fuc-5 proton in several molecules, and their binding affinities, sorted through chemial shift values (n.p. : not published).

A good correlation between the chemical shifts of the Fuc-H5 proton of the various molecules and their binding affinity is noticeable. The results confirm the predictions of Ernst *et al.* [103], namely that the binding affinity of the studied ligand is strongly dependent on their pre-organization into the bioactive conformation.

It allows us to discriminate between the low binders (the too flexible molecule **27** or the bulky biphenyl triazole **103c** in this case) and the good binders (lead compound **22** or molecule **32**) with a single ^{1}H NMR experiment. It is therefore a very valuable tool to a preliminary evaluation of the activity of similar molecules.

C. Synthetic difficulties and other considerations

This part aims at describing some of the synthetic hurdles and secondary routes that paved the way to the results presented in the previous parts **B.II** and **B.III**. The first issue will be the order of the coupling steps used in both synthesis. It is followed by discussions about the coupling steps themselves. At the end the problem of the eventual formation of a lactam (first synthesis, part **B.II.1**) or a lactone (second synthesis, part **B.III.1**) between the carboxylic acid of the lactic acid unit and the position 2 (or 4) of the galactose unit is tackled.

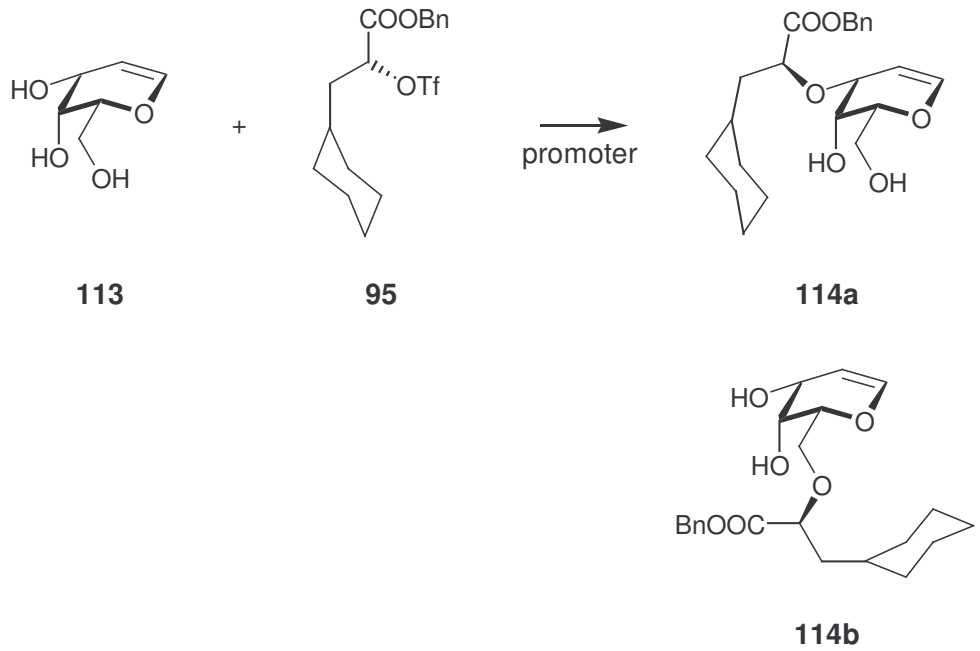
C.I. Order of the coupling steps

Any chemical synthesis (of more than 3 steps) might be carried out in two different ways, so-called sequential or parallel. However, it is well-known that the sequential process is less efficient in terms of overall yield than the parallel one. If we now look at both syntheses described in the previous parts, in respect to the ready-to-use building blocks, we note that they are quite different. The second one is parallel [(Fuc<->diol)<->(Gal<->Lac)->**103a-e**], and thus already optimal, whereas the first one is fully sequential starting from the correctly protected fucose [(Fuc<->diol)<->Gal)<->Lac)->**105**]. Let us have a look at the possibilities for a parallel synthesis instead of the sequential one for **103a-e**.

C.I.1. First synthesis, parallel

As already seen, there is no difficulty to fucosylate diol (**83**) with glycosyl donor **81** giving **84**. The problem is different with galactose and lactic acid. Because of the introduction of the azide, the synthesis of the galactose building block begins with peracetylated D-galactal (**89**) that is transformed in a galactosyl nitrate (**90**) followed by the corresponding bromide, which is then coupled to the pseudodisaccharide **84**. Galactosyl nitrate and bromide are however too reactive to envisage their stannylene acetal mediated alkylation with the lactic acid moiety. Therefore, this coupling must be done on the starting galactal.

As stated in part **B.II.2.1**, the reaction is expected to be regioselective, even with a fully deprotected sugar. However, when tried with D-galactal, it resulted in a mixture of 3-substituted and 6-subsituted product (see figure 54) in a 1:3 ratio.



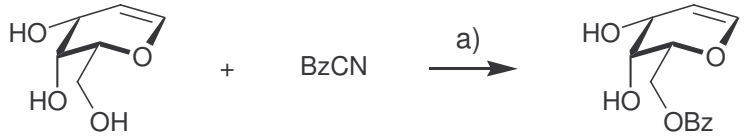
113 **95** **114a** **114b**

Figure 54 : Products formed in the stannylene acetal mediated alkylation of **113** with **95**.

This result can be explained by the big difference existing between the galactal and galactose spatial configurations. From the mechanism (figure 41), due to the different

ring structures and constraints of both species, the formation of a 5-member-ring acetal is kinetically favored in the case of galactose, whereas the thermodynamically favored 6-member ring formation is formed on the galactal.

To avoid the formation of the 4,6-acetal, the 6-position of D-galactal is protected. From the many selective methods to protect a primary alcohol benzoyl protection with benzoyl cyanate was chosen [202] (scheme 10).



113 **115**

Scheme 10 : Monobenzoylation of D-galactal at position 6 [202] : a) BzCl, MeCN/Et_3N 3:1, -45°C, 4h (39%).

The reaction worked, although not as selectively as expected : It yielded only 40% of the 6-monobenzoylated product **115**, whereas after 4h reaction, a relatively large amount of several polybenzoylated products could be isolated. Unfortunately, when **115** was used in a stannylene acetal mediated coupling, 6-*O*-benzoyl-D-galactal yielded no isolable product.

C.I.2. Enzymatic fucosylation as the last synthetic step.

Another coupling sequence was envisaged, with the fucose moiety added at the end of the synthesis. In this case, the first coupling involves 3,4,6-*O*-acetyl-2-azido-2-deoxy-D-galactosyl bromide and cyclohexanediol, followed by the addition of the lactic acid moiety. The subsequent addition of L-fucose is carried out enzymatically using human fucosyl transferase III (FucT III) [203-205]. The major advantage of the method is the possibility of working with unprotected starting material. The fucosylated product would thus be ready for triazole library synthesis, and for subsequent bioassays, without any deprotection steps as originally planned (see **B.II.2.3**).

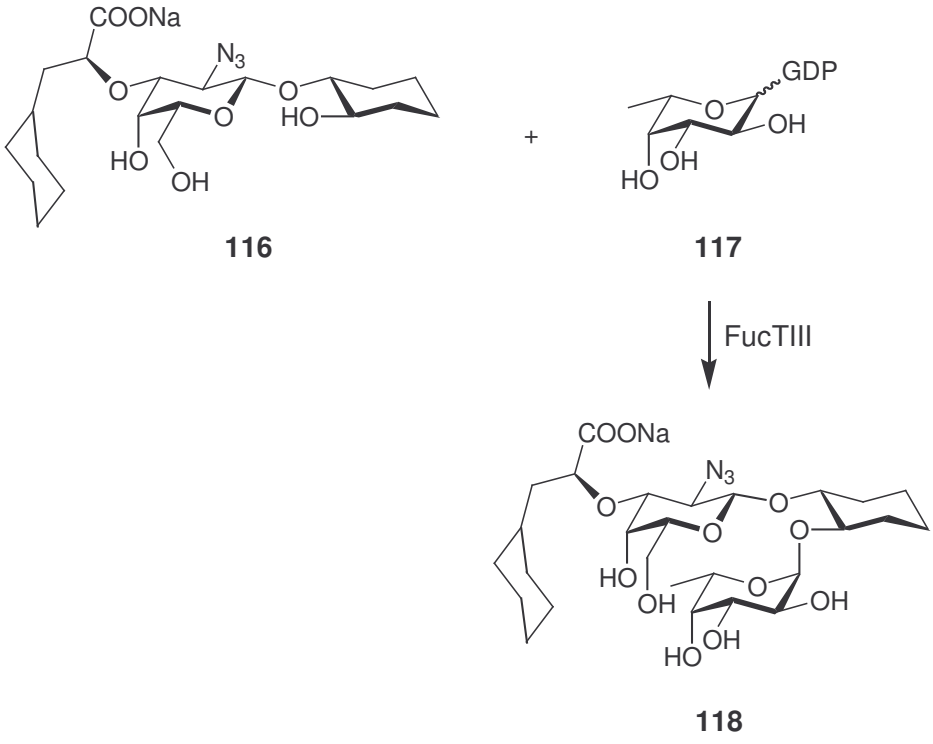


Figure 55 : Planned enzymatic fucosylation.

The method also has drawbacks, the first of them being the cost of the enzyme and the fucosyl donor, GDP-fucose (guanosyl-diphosphate-fucose). Because of these costs, the reaction is limited to very small batches (< 100 mg). This, however, should be sufficient for a screening library (<10 mg of each product needed), but will be limiting in case a bigger batch has to be synthesized for extended studies.

The major problem, however, was the unsolvability of the starting material under the conditions of the enzymatic reaction. The reaction was first tried on an analytical scale, using radio-labeled ^{14}C-GDP-fucose. Various batches showed upon scintillation a 64% conversion. However, when run at preparative scale, the starting material (**116**) precipitated as soon as the buffer was added (sodium cacodylate and magnesium(II) chloride) and neither heating nor dilution led to a complete dissolution. As a consequence, no significant amount of the product could be isolated, even after some days of incubation.

C.II. Supported silver silicate as a glycosylation promoter

It was already discussed in part **B.II** that a major issue of glycosylation reactions is the stereoselectivity at the anomeric center. Most of the time, it is directed via neighboring group participation of the protecting groups. Whereas non-participating groups at position 2 are leading to *cis*-anomers (thermodynamically stable), participating groups are yielding *trans*-anomers.

In the case of 2-deoxy-2-azido saccharides, glycosylation will mainly give the α-anomer, and specific techniques have to be chosen in order to invert the stereochemical outcome if the *trans*-isomer is desired. For that purpose, reactions in a heterogeneous medium using an insoluble solid glycosylation promoter are often efficient. Two different types of promoters are mainly used, the most recent ones being solid acids of the zeolith class [206]. The classical ones are insoluble silver salts [171,207].

The galactose coupling of our first synthesis (**B.II.2.1**) is such a case. Our building block was a 2-azido-2-deoxy-galactose derivative (**91**), the azido group being non-participating, and we needed exclusively the β-anomer (*trans*) of the product. The aglycon coupling partner was molecule **84**. Fearing that acidic promoters could drive the hydrolysis of the fucoside, the insoluble silver salts method was applied.

C.II.1. The published results

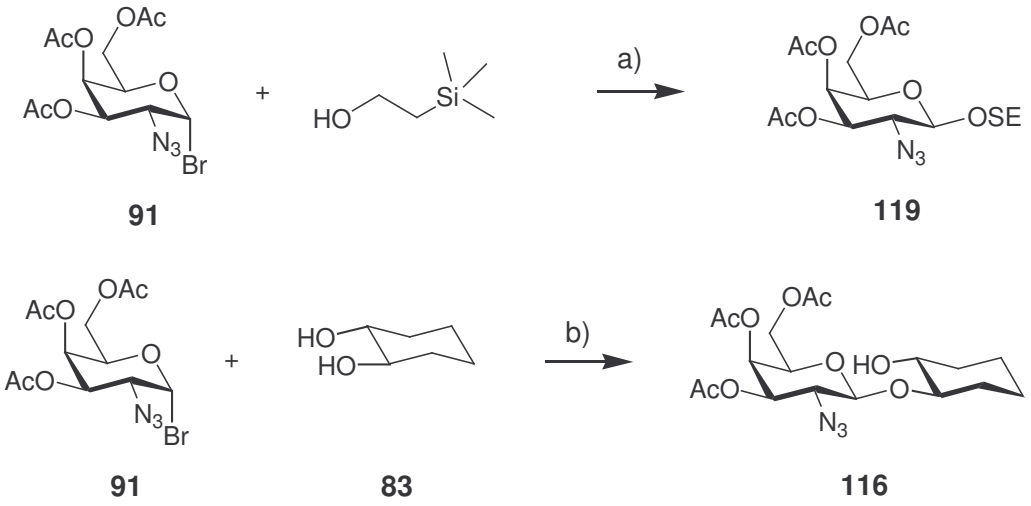
Among insoluble silver salts, silver carbonate is used from time to time [208,170], but with variable results regarding selectivity. On the other hand, supported silver silicate raised a lot of interest. Most of the publications refer to the seminal work of Paulsen *et al.* [165-171] and Van Boeckel *et al.* [207,209,210]. Both used silver silicate (Ag_2SiO_3) supported either on silica gel, or on alumina. Interestingly, no study has been carried out to compare the efficacy of the respective approaches.

Among all the published results, very different reaction conditions were used (reaction temperature, reaction time, dilution, presence of molecular sieve, etc.) and resulted in variable yields (from 35% to 85%) and stereoselectivities. We therefore compared different reaction conditions in order to find the optimal conditions.

C.II.2. Finding the most relevant reaction conditions

Our first trial with reactions promoted by supported silver silicate was the anomeric protection of our galactosyl derivative with a trimethylsilylethyl (OSE) group [211]. We used a silica gel supported promoter with additional molecular sieve, at room temperature (scheme 11).

Wilstermann *et al.* [211] reported up to 72% yield for this reaction, whereas we only could reach 33%. No information, however, was given regarding the method to produce the silver silicate promoter, which to our knowledge is not commercially available. We used the method reported by Paulsen *et al.* [167].



Scheme 11 : Protection of galactose anomeric position with an OSE group [211] : a. silica gel supported Ag_2SiO_3, DCM, r.t., 16h (33%); b. silica gel supported Ag_2SiO_3, DCM, r.t., 16h (33%).

In subsequent trials cyclohexane-1,2-diol was used as an aglycon, towards the synthesis of compound **116** (**C.I.2**) using the same batch of promoter and leading to up to 60% yield. These new results suggest that the quality of the promoter was not the main cause of the limited success of the first reaction (that yielded **119**). We therefore wanted to find more reliable reaction conditions.

Two interesting and convergent facts were first noted :

- No by-products are formed during the reaction ; Almost the entire uncoupled aglycon could be recovered, leading to yields relatively to the consumed aglycon of close to 100%.
- TLC and MS in the course of the reaction never showed any trace of the galactosyl bromide.

Both facts suggest that the glycon moiety reacts easily with the promoter, this reaction being hence competitive with the coupling (see figure 56).

The mechanism shows, in the case of silica gel, how the competitive reaction might proceed. Indeed, strong interaction between silver and glycosyl bromide leads to a further polarization of the C-Br bond. The activated intermediate is then attacked by the alcohol functions present in the medium, here the aglycon (trimethylsilylethanol or

cyclohexane-1,2-diol) or the silanol groups that could be present at the surface of the promoter.

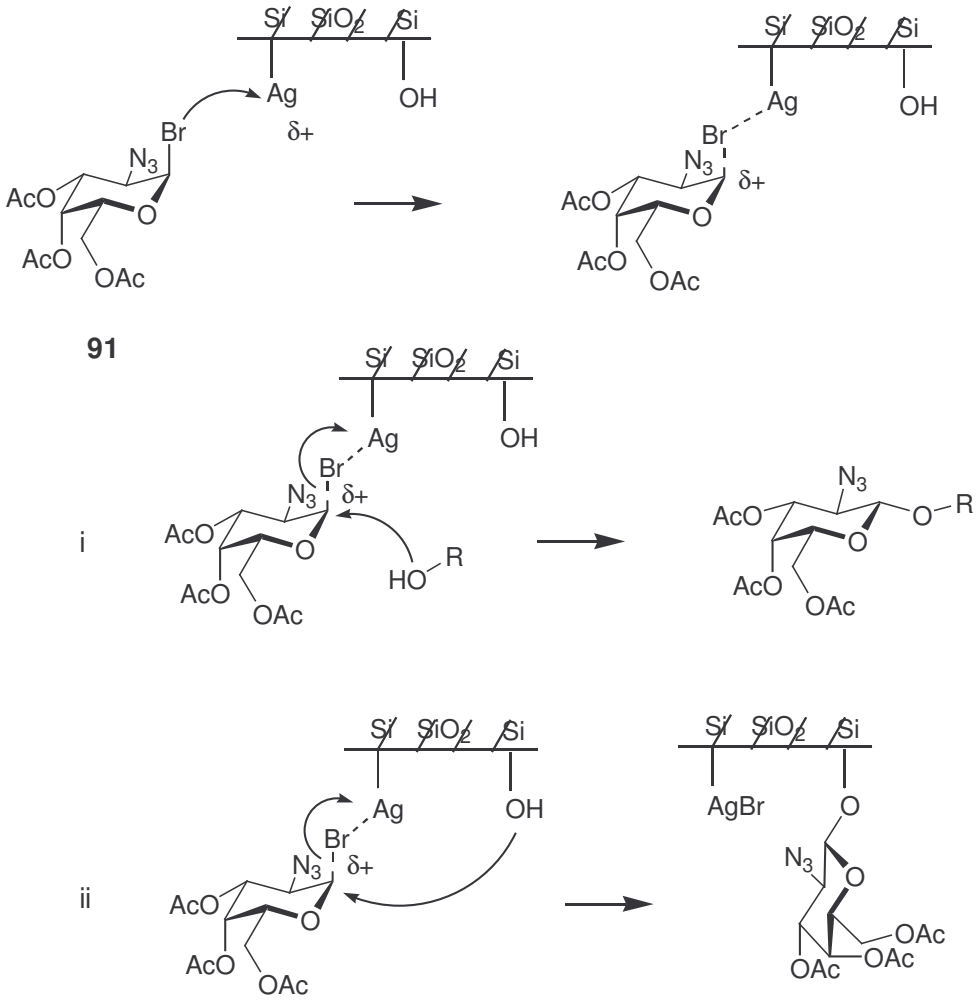


Figure 56 : Proposed mechanism for the supported silver silicate mediated coupling.

This mechanism, along with various entries of the literature [165,168,172,212-216] allow us to devise relevant reaction conditions. In order to avoid the competitive reaction with the promoter support, a high concentration of the aglycon coupling partner is needed close to the reaction site. This can be reached by either using a large excess of the aglycon **84** or by adding the donor 91 very slowly to the reaction mixture. It might also be useful to reduce the reaction temperature, working at 0°C, or even less.

C.II.3. Evaluation of both reported silver silicate supports

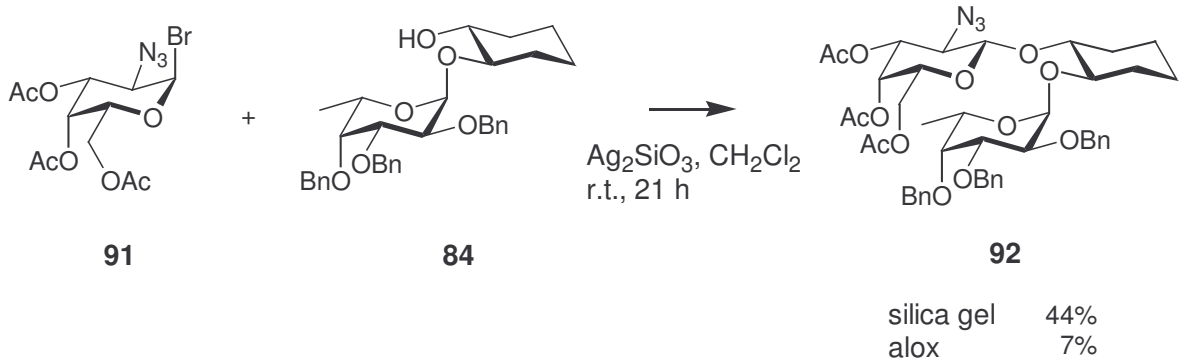
Silver silicate was synthesized on both reported supports using the protocol published by Paulsen *et al.* [167]. Details are summarized in table 6 below.

		Silica gel supported silver silicate	Alumina supported Silver silicate
Starting materials	$AgNO_3$	excess	excess
	$Na_2Si_3O_7$	4.06 mmol	4.06 mmol
	Support	0.38 g silica gel	0.38 g alox
	Product amount	0.64 g	1.20 g
	Silver load (est.)	2.21 mmol/g	3.72 mmol/g
	Yield (test reaction)	44%	7%

Table 6 : Amounts of materials used for the synthesis of both supported silver silicate promoters.

The silver load is estimated based on the recovered silver nitrate, which is used in excess. We further assumed that the sodium trisilicate should be converted completely into silver silicate. More precise estimations should be possible using X-ray powder diffraction. Based on the estimated silver load, there is a significant difference between the two promoters (3.72 vs 2.21 mmol/g).

For the test reaction, galactose derivative **91** is coupled to the pseudo-disaccharide **84** (see scheme 13 below). Again, the yields are relatively low, but the difference between both promoters is significant. When alumina supported silver silicate was used, only 7% of the starting aglycon was converted (all the glycon unit was consumed however), whereas silica gel supported promoter gave a 44% yield.



Scheme 12 : Silver silicate mediated coupling of galactosyl bromide **91** to **84**.

Because silver cations are probably more concentrated on alumina than on silica, the reverse result was expected. It is likely that the competitive reaction with the support was more prominent in the case of alumina, consuming the galactosyl bromide before it was able to react with the alcohol. However, whatever the support, the competitive reaction could never be prevented, even by varying the reaction conditions. Therefore, it may be useful to explore the use of different supports. In that sense, recent publications report the synthesis of silica supported silver silicate in the form of nanotubes [217,218]. the synthesis is relatively easy, and thus accessible without specific material. However, they have not been tested yet as glycosidation promoters. Another possible support is activated charcoal, with the great advantage that it lacks reactivity. As a consequence the competitive reaction with the support would not take place.

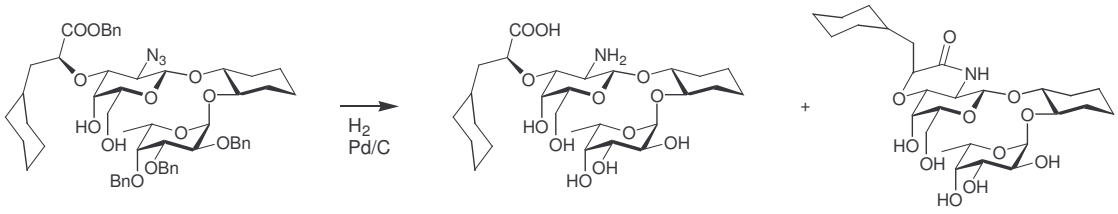
C.III. Lactam formation

As it is already described in part **B.II.2.3**, hydrogenation of **96** unexpectedly lead to the formation of a 6-membered ring lactam by nucleophilic attack of the intermediate primary amine at position 2 of the galactose at the benzyl ester of the lactic acid moiety.

The first hydrogenation was carried out using 10% palladium on activated charcoal at a hydrogen pressure of 4 bar in ethanol with a catalytic amount of acetic acid. The reaction typically took 3-5 days, until the last benzyl ethers were completely cleaved. Under these

conditions, the major amount of the product was in the lactam form (**102b**, figure 57), whereas the minor fraction consisted of the free acid (**102a**).

As the lactam is difficult to cleave while keeping the integrity of the rest of the molecule, this result is not acceptable. Therefore, in order to prevent this side-reaction to occur, other methods were tried for the cleavage of the benzyl groups.



96 **102a** 57% **102b** 35%

Figure 57 : Formation of a lactam during Pd-catalysed hydrogenation of **96**.

C.III.1. Optimizing the hydrogenation conditions

In the course of the hydrogenation, the azide is first reduced to the amine, followed by the cleavage of the benzyl ester. From molecular modeling, the most probable position of the lactic acid residue for the molecule in solution is a gauche(-) conformation, where amine and ester/acid spatially separated. Hence, the reaction can only take place after a major conformational shift. However, both catalysts (palladium or palladium hydroxide on activated charcoal), with or without acetic acid, gave the same result.

Due to the benzyl ester group, the electrophilicity of the carbonyl carbon is enhance, hence favoring the lactam formation. Since the corresponding acid has a reduced electrophilicity compared to the benzyl ester, we first cleaved first ester, simply using 0.1 eq. sodium carbonate in aqueous dioxane. The free carboxylic acid was then subjected to hydrogenation, using dioxane as solvent. However, the lactam was still formed, in the same proportion as before.

Independently of the catalytic system and the ester activation, other factors facilitate the formation of the lactam, among them the irreversibility of the reaction (especially in

contrary to lactone formation, see below **C.IV**) and the thermodynamically favored formation of a 6-membered ring.

As a result of this investigation, it became clear that the formation of the amino group should be avoided by applying non-reducing conditions for the debenzylation.

C.III.2. Non-reductive methods

We first turned our attention toward oxidative cleavages. One of particular interest was performed with 2,3-dichloro-5,6-dicyano-*p*-benzoquinone (DDQ) under photoirradiation [219]. Two batches were set up. In the first one, the reaction was carried out under direct UV irradiation (100W, 365 nm). However, after 4h reaction, MS analysis showed complete decomposition of the starting material. In the second attempt, the reaction was carried out for several days under normal light. In this one the reaction worked very well at the beginning, and three out of four benzyl groups were readily cleaved in less than 48h. However, the last one proved much more stable and in the mean time, oxidation of the primary 6-hydroxyl of the galactose moiety was detected.

Another well-studied method for cleavage of the benzyl groups is the use of Lewis acids, e.g. Because of the acid-sensitivity of the fucoside, side reactions may occur. Three out of four benzyl groups were cleaved within 24h and only traces of decomposition did occur. Unfortunately, the fourth benzyl group again turned out to be much more stable, and with prolonged reaction times the competitive fucose hydrolysis became predominant.

Because both approaches for the removal of the benzyl protection failed, we tried to solve the problem by modifying the protecting groups.

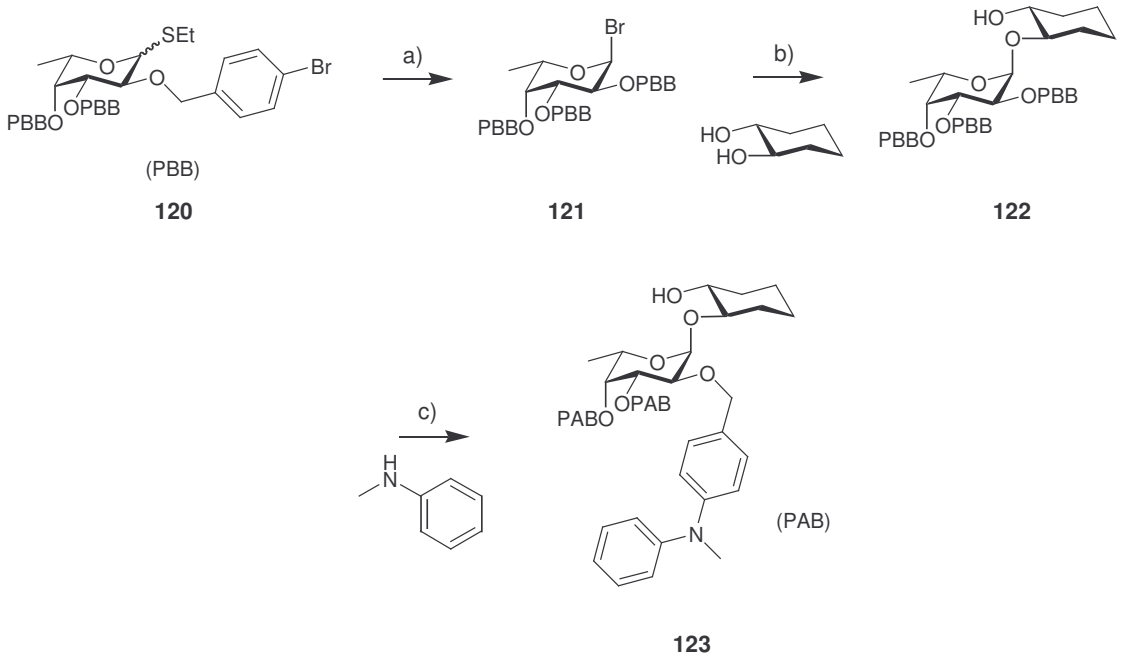
C.III.3. Other protecting groups for fucose

As already stated in part **B.II.2.1**, ether protecting groups are the best suited because they are non-participating (mandatory to get α-directed coupling), Silyl ethers are expected to be unstable under the conditions used later in the synthesis.
Another possible ether protecting group are *p*-methoxybenzyl (PMB) ethers [221-223]. Due to the electronic effect of the *para*-positionned methoxy group, those ethers should be more sensitive to oxidative (iode, DDQ or CAN [151,152,154]) or mild Lewis acid mediated cleavages [224-226]. Unfortunately, the fucose protecting PMB ethers were not stable enough in the glycosidation reaction of cyclohexane-1,2-diol (Br_2, followed by the alcohol and TEAB), and was partially cleaved.

A second approach is derived from the work of Buchwald *et al*. [227,228] on Palladium catalysed amination reactions. Fucose should first be protected with *p*-bromobenzyl groups (PBB), which properties (introduction, stability and cleavage) basically the same than for unsubstituted groups. The coupling with cyclohexane-1,2-diol would then be performed in standard conditions (**B.II.2.1**). PBB groups could now be coupled with *N*-methylalanine by Pd-catalysis. The presence of an anilin nitrogen changes the properties of the benzyl ethers, which now have a reactivity comparable to the one of PMB ethers, and should thus be cleavable using mild Lewis acids.

In preliminary tests the fucose building block, protected with PBB groups was coupled to 1R,2R-cyclohexane-1,2-diol, followed by amination using $Pd_2(dba)_3$ as catalyst. Unfortunately, the reaction was uncomplete, leading to an unseparable mixture of mono-, di- and tri-aminated compounds (5:30:65 resp.). Modified reaction conditions (e.g. different catalyst and/or ligand) could lead to better results.

It would be even more comfortable if this reaction could be carried out in the last steps of the synthesis. Unfortunately, the phosphine ligands used in amination are incompatible with an azide. The reaction thus has to be carried out before the introduction of the azide, *id est* before the coupling with the galactose building block.



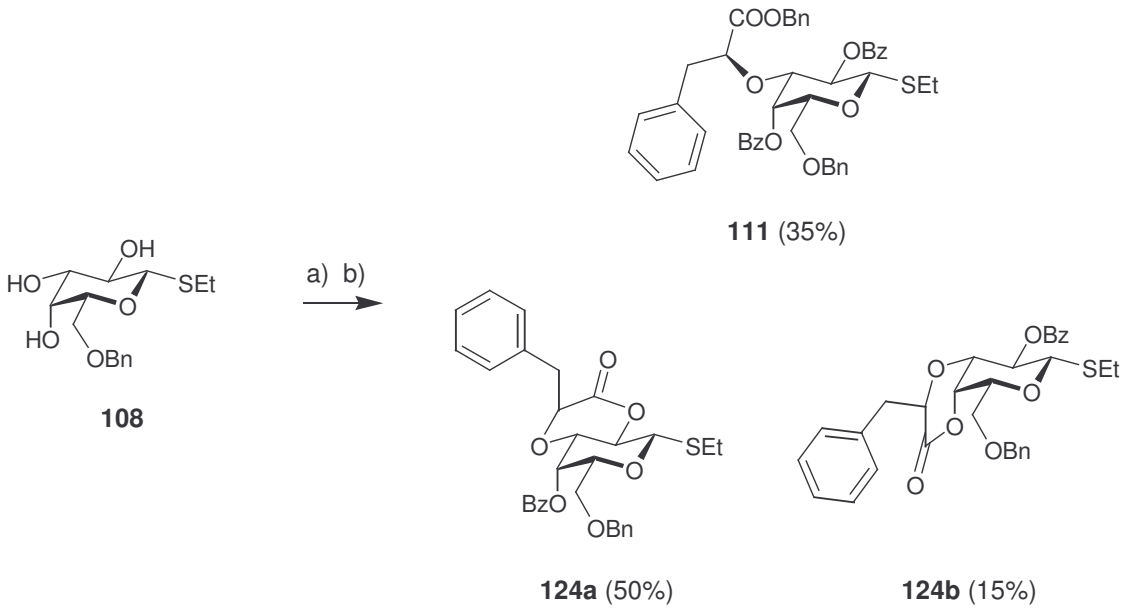
120 **121** **122**

123

Scheme 13 : Pd-catalysed amination used as protective strategy : a) Br_2, DCM, 0°C to r.t., 1h ; b) TEAB, DMF, r.t., 2h (59% over 2 steps) ; c) $Pd_2(dba)_3$, di-(*t*-butyl)-*o*-biphenylphosphine, *t*-BuONa, toluene, r.t., 42h (59%).

C.IV. Lactone formation

As it is already described in part **B.III.2.1**, two intramolecular lactones were unexpectedely formed on the galactose moiety of molecules **109** and **111**. Lactones can be seen as suitable protecting groups. In our case, however, it is not a suitable solution. Indeed, when a lactone is formed between the Gal-2 hydroxyl and the lactic acid, the position 2 of the galactose becomes non-participating, the carbonyloxy group being constrained in the direction opposite to the anomeric position. Therefore, the main coupling product would be the α-anomer. Among the three compounds possibly formed after two steps (scheme 14), only **111** and **124b** (50% of the total) will be able to form β-anomers in the subsequent glycosidation.



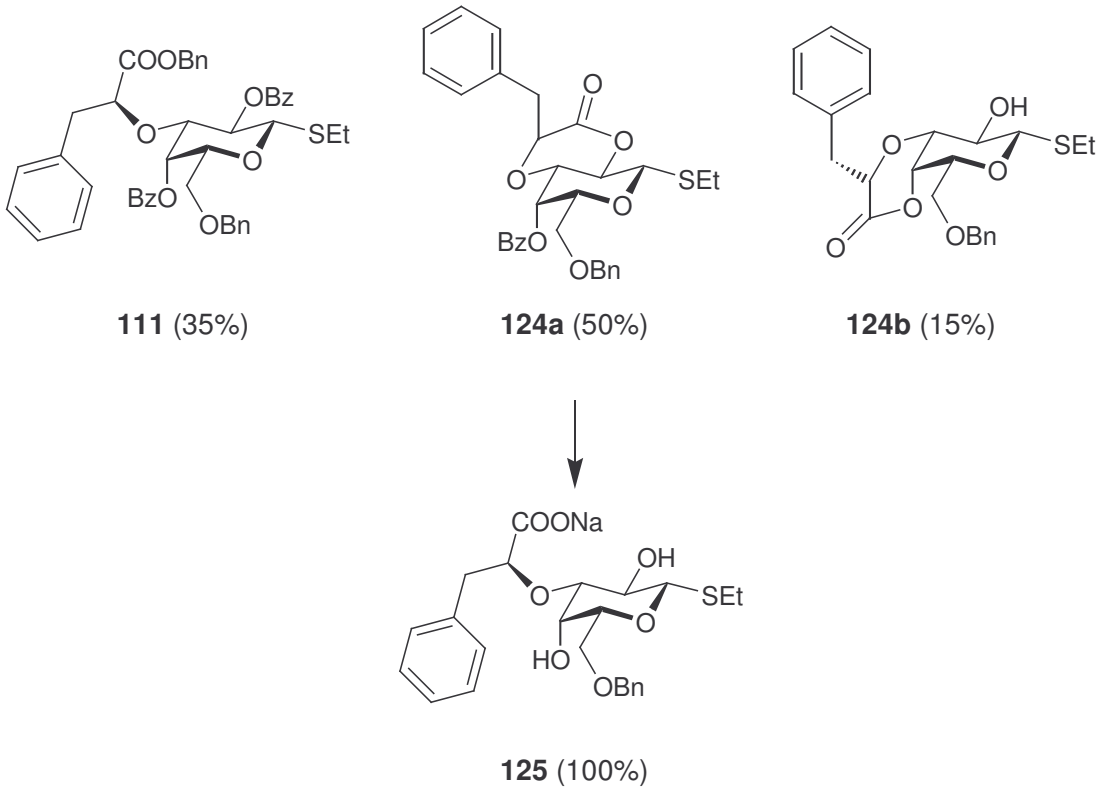
Scheme 14 : Formation of the lactones over two reaction steps : a) **108**, Bu_2SnO, MeOH, reflux, 18h ; **107**, CsF, DME, r.t., 24h (36% over 2 steps) ; b) BzCl, pyridine, r.t., 16h (88%).

C.IV.1. Chromatographic separation

A first solution would be to separate the three products after benzoyl protection, via chromatography. Unfortunately only one lactone could be readily isolated, namely the one formed with the Gal-4 hydroxyl (**124b**), which is the minor product. The second one (**124a**) did not separate from the fully protected product **111**.

C.IV.2. Lactone opening and re-protection

Treatment with diluted sodium hydroxide readily cleaved the lactone, as well as the other esters in the molecules, resulting in only one product (**125**, figure 58). This product then has to be protected again.



111 (35%) **124a** (50%) **124b** (15%)

125 (100%)

Figure 58 : Cleavage of the lactones with diluted aqueous sodium hydroxide.

We expected that the lactone formation was favored by the presence of the carboxylic acid protecting benzyl ester. As for the lactam formation discussed in the previous chapter (**C.III**), the ester is expected to activate the carbonyl group, making it more prone to reacting with the free hydroxyl groups on the molecule. However, benzoyl protection of **125**, using benzoyl chloride under esterification conditions, still yielded the lactones. The reasons for this reactivity were shown by MS analyses that showed the presence of the mixed anhydride formed between the carboxylic acid and the benzoyl chloride. The acid function was consequently activated again, and hence reacted towards lactone formation.

C.IV.3. Selective protection of the starting material

The last solution envisaged would then be to protect the starting galactosyl derivative **108** selectively at position 2. Indeed, position 4 should not be protected, otherwise the stannylene acetal mediated coupling would not be possible.

The selective protection could certainly be carried out by first simultaneously protecting the Gal-3 and Gal-4 hydroxyl groups with a benzylidene acetal, followed by a suitable protection of the Gal-2 hydroxyl and subsequent cleavage of the acetal.

This protection/deprotection sequence was however not tried in the course of this work, and we preferred the straightforward coupling of the three-product mixture with compound **84**, despite the resulting material loss. Noteworthy is that the needed compound **112** was easily isolable from the mixture.

D. Experimental part

D.I. General methods

D.I.1. Optical rotation

Optical rotations were measured with a Perkin-Elmer *341* polarimeter at a temperature of 20°C. Na-D optical rotations were extrapolated from the measured Hg values (546 and 578 nm) with the help of Drude's equation :

$$[\alpha]_D^{20} = \frac{[\alpha]_{579}^{20} \bullet 3.199}{4.199 - \frac{[\alpha]_{579}^{20}}{[\alpha]_{546}^{20}}} \qquad \text{with} \qquad [\alpha]_\lambda^{20} = \frac{\alpha \bullet 100}{c \bullet d}$$

α = Measured optical rotation

c = Concentration in g/L

d = Recipient length in m

λ = Wavelength in nm

The solvents used for the measurements were CH_3Cl p.a. (Fluka) and MeOH p.a. (Fluka).

D.I.2. Infrared spectroscopy (IR)

IR spectra were recorded on a *Perkin Elmer Spectrum One FT-IR* spectrometer as KBr pellets or films. Most characteristic absorption bands of the spectrum were given in cm^{-1} and specified as vs (very strong), s (strong), m (medium), w (weak), b (broad).

D.I.3. Nuclear Magnetic Resonance Spectroscopy (NMR)

Proton (^{1}H NMR) and carbon-13 (^{13}C NMR) nuclear magnetic resonance spectra were recorded on a Bruker *UltraShield™ Supraconducting NMR 500/70B* spectrometer.

^{1}H NMR spectra were recorded at 500.1 MHz and ^{13}C NMR spectra were recorded at 125.8 MHz in $CDCl_3$ or MeOD. Chemical shifts are given in δ units, parts per million (ppm) downfield from tetramethylsilane (TMS).

Values [229] for **$CDCl_3$** : **^{1}H NMR: 7.26 ^{13}C NMR: 77.03**

Values [229] for **CD_3OD** : **^{1}H NMR: 3.31 ^{13}C NMR: 49.00**

Value [229] for **D_2O** : **^{1}H NMR: 4.79**

Multiplicities were specified as follows: s (singlet), d (doublet), dd (double doublet), t (triplet), q (quartet), dq (double quartet), m (multiplet). Interpretation of the spectra was done according to 1st order.

For assignment of resonance signals to the appropriate nuclei the following abbreviations have been used: Fuc (fucose), Gal (galactose), Lac (lactic acid), Tzl (triazole), Diol (cyclohexane-1,2-diol). In cases where the numbering of nuclei does not accord to the numbering in IUPAC nomenclature, the differences are illustrated in a formula scheme of the corresponding substance.

D.I.4. Elementary analyses

Elementary analyses were performed at the Institute of Organic Chemistry (University of Basel, Switzerland).

D.I.5. Mass spectrometry coupled with high-pressure liquid chromatography (MS and LC/MS)

Mass spectra were recorded on a Waters *Micromass ZQ*^{TM} ESI spectrometer. Although otherwise stated, ionization parameters were the following : 60 V cone voltage and 3.50 kV capillary voltage, and the solvent being acetonitrile / water 1:1 with 0.2% formic acid (10 µL/min).

The spectrometer is coupled to an HPLC system from Waters (coupled injector/collector *2767 Sample Manager* with *AutoPurification*^{TM} system, *2996* PDA detector, *Sunfire*^{TM} columns). Analytical column is a C_{18}, 3.5 µm, 2.1x50 mm. Preparative column is a C_{18} *OBD*^{TM}, 5 µm, 19x150 mm.

Analytical (15 min, 0.5 mL/min) as well as preparative (30 min, 15 mL/min) runs were carried out with a gradient from acetonitrile / water 5:95 to 95:5, with 0.2% formic acid.

D.I.6. Thin Layer Chromatography (TLC)

Reactions were monitored by TLC using glass plates coated with silica gel 60 F254 (Merck). The spots were detected under short wavelength UV light (254 nm) or charring with Mostain, a molybdate solution (a 0.02 M solution of ceric ammonium sulfate and ammonium molybdate tetrahydrate in aqueous 10% H_2SO_4. The plates were then heated for 2 min at 150°C.

D.I.7. Filtration and chromatography

Flash chromatography was performed with 200-400 mesh silica gel 60 under air pressure. Thin layer chromatography was used to monitor column fractions.

D.I.8. Hydrogenations

Hydrogenations under pressure were performed in a protected mixing device from Parr Instrument Company, (Moline, IL, USA) in 250 mL bottles.

D.I.9. Drying of the solvents

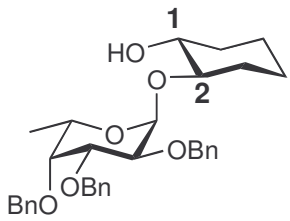
Absolute solvents like dichloromethane (DCM), acetonitrile (MeCN), 1,2-dichloroethane (DCE), 1,2-dimethoxyethane (DME) were prepared by filtration over basic aluminium oxide (Fluka, type 5016 A basic).

Absolute solvents like methanol, ethanol or dioxane were dried by refluxing with sodium and distilled immediately before use.

Pyridine was freshly distilled under argon in the presence of CaH_2, and stored on KOH platelets.

N,N-Dimethylformamide (DMF) was stirred during 4 h before use on activated molecular sieve 4 Å beads.

D.II. Description of the reactions



(*1R,2R*)-2-*O*-(2,3,4-Tri-*O*-benzyl-α-L-fucosyl)-cyclo-hexane-1,2-diol (84)

(1_148)

Thioethyl 2,3,4-tri-*O*-benzyl-L-fucoside (**81**) (8.00 g, 16.7 mmol) was dissolved in dry DCM (80 mL), and cooled to 0°C under argon. Bromine (1.00 mL, 19.6 mmol) was added slowly at 0°C, then the mixture was heated slowly to r.t.. After 1 h, the remaining bromine was quenched using cyclohexene (2.0 mL, 30 min stirring at r.t.).

(*1R,2R*)-Cyclohexane-1,2-diol (**83**, 2.40 g, 20.7 mmol) and tetraethylammonium bromide (5.30 g, 25.2 mmol) were dissolved in DMF (25 mL). Activated powdered molecular sieves 3 Å (1.0 g) was suspended in the solution which then was stirred for 30 min under argon at r.t.. Then, the fucosyl bromide solution was added dropwise to the suspension. After stirring for 3h, the reaction mixture was neutralized with pyridine (2 mL), diluted with DCM (50 mL), and washed with saturated aqueous $NaHCO_3$ solution (30 mL) and water (2x30 mL). The organic layer was dried with Na_2SO_4 and concentrated. Chromatography on silica gel (petrol ether / ethyl acetate 2:1, with 0.1% Et_3N) yielded **84** (6.19 g, 70%) as a yellowish oil.

(1_148)

$[\alpha]_D$ = -121.4 (c = 2.06, MeOH).

(1_148, 21/01/05)

^{1}H NMR (500 MHz, $CDCl_3$) : δ = 1.14 (d, 3H, $^3J_{5,6}$ = 6.5 Hz, Fuc-H6), 1.18-1.33 (m, 4H, diol), 1.70 (m, 2H, diol), 2.00 (s, 2H, diol), 3.24 (m, 1H, diol-H1), 3.44 (m, 1H, diol-H2), 3.69 (s, 1H, Fuc-H4), 3.93 (d, 1H, $^3J_{2,3}$ = 10.1 Hz, Fuc-H3), 4.07 (m, 2H, fuc-H2, Fuc-H5), 4.64-4.97 (m, 6H, C$\underline{H}_2$-Ph), 4.98 (d, 1H, Fuc-H1), 7.26-7.39 (m, 15H, 15 Ar-H).

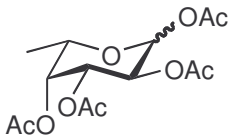
(1_148 2101/05)

^{13}C NMR (125 MHz, $CDCl_3$) : δ = 16.7 (Fuc-C6), 24.0, 24.4, 30.1, 32.5 (4C, diol), 67.0 (Fuc-C5), 73.2 (diol-C2), 73.3, 73.4, 74.9 (3C, 3 $\underline{C}H_2$-Ph), 76.2 (Fuc-C2), 77.5 (Fuc-C4), 79.3 (Fuc-C3), 83.9 (diol-C1), 96.2 (Fuc-C1), 127.4, 127.5, 127.7, 128.0, 128.2, 128.4 (15C, 15 Ar-$\underline{C}$H), 138.5, 138.6, 138.9 (3C, 3 quat. Ar-C).

(1_182)

Elemental analysis : Calculated for $C_{33}H_{40}O_6$: C, 74.41% ; H, 7.57%. Found : C, 74.31% ; H, 7.54%.

1,2,3,4-Tetra-*O*-acetyl-L-fucose (86)



(2_004)

L-fucose (0.60 g, 3.65 mmol, **85**) was dissolved in pyridine (15 mL), and cooled to 0°C under argon. Acetic anhydride (3.0 mL, 31.7 mmol) was added slowly to the cold solution, which was then heated slowly to r.t. and stirred for 17 h. The reaction mixture was diluted with ethyl acetate (30 mL), washed with a saturated aqueous $NaHCO_3$ solution (3x20 mL), and with water (2x 20 mL). The organic layer was dried with Na_2SO_4 and concentrated to yield **86** (1.20 g, quant.) as a yellowish solid. The compound was used in next step without further purification.

(1_201, 01/09/05)

α-anomer : ^{1}H NMR (500 MHz, $CDCl_3$) : δ = 1.15 (d, 3H, $^3J_{5,6}$ = 6.5 Hz, H-6), 2.00, 2.01, 2.14, 2.17 (4s, 12H, C$\underline{H}_3$C=O), 4.27 (q, 1H, $^3J_{5,6}$ = 6.5 Hz, H-5), 5.29-5.35 (m, 3H, H-2, H-3, H-4), 6.33 (d, 1H, $^3J_{1,2}$ = 2.9 Hz, H-1).

(1_201, 01/09/05)

^{13}C NMR (125 MHz, $CDCl_3$) : δ = 16.0 (C-6), 20.1-21.1 (4C, $\underline{C}H_3$C=O), 67.7 (C-5) 66.4, 69.9 (C-3, C-4), 70.6 (C-2), 90.4 (C-1), 169.2, 169.5, 170.0, 170.2 (4C, $CH_3\underline{C}$=O).

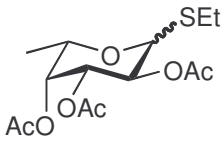
(1_201, 01/09/05)

β-anomer : ^{1}H NMR (500 MHz, $CDCl_3$) : δ = 1.22 (d, 3H, $^3J_{5,6}$ = 6.4 Hz, H-6), 1.99, 2.03, 2.11, 2.18 (4s, 12H, C$\underline{H}_3$C=O), 3.96 (qd, 1H, $^3J_{4,5}$ = 0.9 Hz, $^3J_{5,6}$ = 6.4 Hz, H-5), 5.07 (dd, 1H, $^3J_{3\text{-}4}$ = 3.3 Hz, $^3J_{2,3}$ = 10.4 Hz, H-3), 5.26 (dd, 1H, $^3J_{4,5}$ = 0.9 Hz, $^3J_{3,4}$ 3.3 Hz H-4), 5.32 (dd, 1H, $^3J_{1,2}$ = 8.3 Hz, $^3J_{2,3}$ = 10.4 Hz, H-2), 5.68 (d, 1H, $^3J_{1,2}$ = 8.3 Hz, H-1).

(1_201, 01/09/05)

^{13}C NMR (125 MHz, $CDCl_3$) : δ = 15.8 (C-6), 20.1-21.1 (4C, $\underline{C}H_3$C=O), 70.0 (C-4), 70.5 (C-2), 70.7 (C-5), 71.3 (C-3), 92.6 (C-1), 169.3, 170.0, 170.2, 170.6 (4C, $CH_3\underline{C}$=O).

2,3,4-Tri-*O*-acetyl-1-ethylthio-L-fucoside (87)



2_005

1,2,3,4-tri-*O*-acetyl-L-fucose (1.20 g, 3.61 mmol) (**86**) was dissolved in dry DCE (15 mL), and cooled to 0°C under argon. Ethanethiol (0.35 mL, 4.73 mmol) was added to the cold solution, followed by tin tetrachloride (0.65 mL, 5.53 mmol). After stirring at 0°C for 4 h, the reaction mixture was diluted with DCM (20 mL), washed with an aqueous KF/KH_2PO_4 solution (10%, 2x 20 mL), and water (2x 20 mL). The organic layer was dried on Na_2SO_4 and concentrated. Chromatography on silica gel with petrol ether and ethyl acetate 3:1 as eluent afforded **17** (0.94 g, 78%) as a yellowish oil

(1_206, 09/09/05)

α-anomer : ^{1}H NMR (500 MHz, $CDCl_3$) : δ = 1.15 (d, 3H, $^3J_{5,6}$ = 6.5 Hz, H-6), 1.25 (t, 3H, ^3J = 7.4 Hz, S-CH_2-C$\underline{H}_3$), 1.98, 2.06, 2.16 (3s, 9H, C$\underline{H}_3$C=O), 2.51 (dq, 1H, ^3J = 7.4 Hz, ^2J = 12.9 Hz, S-CH$\underline{H}$-CH_3), 2.57 (dq, 1H, ^3J = 7.4 Hz, ^2J = 12.9 Hz, S-C$\underline{H}$H-CH_3), 4.48 (q, 1H, $^3J_{5,6}$ = 6.6 Hz, H-5), 5.21 (dd, 1H, $^3J_{3,4}$ = 3.2 Hz, $^3J_{2,3}$ = 10.8 Hz, H-3), 5.26 (dd, 1H, $^3J_{1,2}$ = 5.4 Hz, $^3J_{2,3}$ = 10.8 Hz, H-2), 5.28 (dd, 1H, $^3J_{4,5}$ = 0.8 Hz, $^3J_{3,4}$ = 3.2 Hz, H-4), 5.69 (d, 1H, $^3J_{1,2}$ = 5.4 Hz, H-1).

(1_206, 09/09/05)

^{13}C NMR (125 MHz, $CDCl_3$) : δ = 14.8 (-S-CH_2-$\underline{C}H_3$), 15.9 (C-6), 20.7, 20.9, (3C, $\underline{C}H_3$C=O), 24.2 (-S-$\underline{C}H_2$-CH_3), 64.6 (C-5), 67.3 (C-2), 68.7 (C-3), 70.5 (C-4), 81.9 (C-1), 169.7, 170.2, 170.7 (3C, $CH_3\underline{C}$=O).

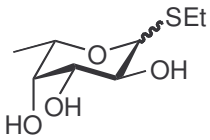
(1_206, 13/09/05)

β-anomer : ^{1}H NMR (500 MHz, $CDCl_3$) : δ = 1.21 (d, 3H, $^3J_{5,6}$ = 6.3 Hz, H-6), 1.27 (t, 3H, ^3J = 7.5 Hz, S-CH_2-C$\underline{H}_3$), 1.98, 2.06, 2.17 (3s, 9H, C$\underline{H}_3$C=O), 2.70 (dq, 1H, ^3J = 7.6 Hz, ^2J = 12.6 Hz, S-CH$\underline{H}$-CH_3), 2.75 (dq, 1H, ^3J = 7.5 Hz, ^2J = 12.6 Hz, S-C$\underline{H}$H-CH_3), 3.81 (qd, 1H, $^3J_{4,5}$ = 0.8 Hz, $^3J_{5,6}$ = 6.3 Hz, H-5), 4.43 (d, 1H, $^3J_{1,2}$ = 9.9 Hz, H-1), 5.04 (dd, 1H, $^3J_{3,4}$ = 3.4 Hz, $^3J_{2,3}$ = 10.3 Hz, H-3), 5.21 (dd, 1H, $^3J_{1,2}$ = 9.9 Hz, $^3J_{2,3}$ = 10.3 Hz, H-2), 5.27 (d, 1H, $^3J_{3,4}$ = 3.4 Hz, H-4),

(1_206, 13/09/05)

^{13}C NMR (125 MHz, $CDCl_3$) : δ = 14.8 (-S-CH_2-$\underline{C}H_3$), 16.4 (C-6), 20.7, (3C, $\underline{C}H_3$C=O), 24.2 (-S-$\underline{C}H_2$-CH_3), 73.2 (C-5), 68.0 (C-2), 72.4 (C-3), 71.0 (C-4), 83.5 (C-1), 169.9, 170.2, 170.6 (3C, $CH_3\underline{C}$=O).

1-Ethylthio-L-fucoside (88)



2_009

2,3,4-Tri-*O*-acetyl-1-ethylthio-L-fucoside (1.56 g, 4.67 mmol) (**87**) was dissolved in MeOH (15 mL) and water (1 mL). Potassium carbonate (65 mg, 0.47 mmol) was then added. After stirring for 19 h, the reaction mixture was concentrated. Chromatography on silica gel with DCM and MeOH 9:1 as eluent afforded **88** (0.84 g, 86%) as an off-white solid.

(2_009, 01/09/05)

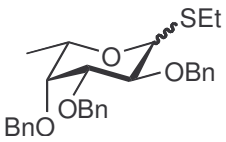
α-anomer : ^{1}H NMR (500 MHz, MeOD) : δ = 1.22 (d, 3H, $^{3}J_{5,6}$ = 6.6 Hz, H-6), 1.28 (t, 3H, ^{3}J = 7.5 Hz, S-CH_2-C$\underline{H}_3$), 2.49-2.63 (m, 2H, S-C$\underline{H}_2$-CH_3), 3.59 (dd, 1H, $^{3}J_{3,4}$ = 3.1 Hz, $^{3}J_{2,3}$ = 10.1 Hz, H-3), 3.66 (broad s, 1H, H-4), 4.28 (q, 1H, $^{3}J_{5,6}$ = 6.6 Hz, H-5), 5.26 (dd, 1H, $^{3}J_{1,2}$ = 5.6 Hz, $^{3}J_{2,3}$ = 10.1 Hz, H-2), 5.34 (d, 1H, $^{3}J_{1,2}$ = 5.6 Hz, H-1).

^{13}C NMR (125 MHz, MeOD) : δ = 15.5 (C-6), 16.6 (-S-CH_2-$\underline{C}H_3$), 25.2 (-S-$\underline{C}H_2$-CH_3), 67.9 (C-5), 69.5 (C-2), 71.2 (C-3), 76.0 (C-4), 87.4 (C-1).

β-anomer : ^{1}H NMR (500 MHz, MeOD) : δ = 1.25 (d, 3H, $^{3}J_{5,6}$ = 6.5 Hz, H-6), 1.28 (t, 3H, ^{3}J = 7.4 Hz, S-CH_2-C$\underline{H}_3$), 2.64-2.78 (m, 2H, S-C$\underline{H}_2$-CH_3), 3.46 (dd, 1H, $^{3}J_{3,4}$ = 3.0 Hz, $^{3}J_{2,3}$ = 9.2 Hz, H-3), 3.50 (t, 1H, $^{3}J_{1,2}$ = $^{3}J_{2,3}$ = 9.2 Hz, H-2), 3.62-3.66 (m, 2H, H-4, H-5), 4.30 (d, 1H, $^{3}J_{1,2}$ = 9.2 Hz, H-1).

^{13}C NMR (125 MHz, MeOD) : δ = 15.5 (C-6), 17.1 (-S-CH_2-$\underline{C}H_3$), 25.0 (-S-$\underline{C}H_2$-CH_3), 72.5 (C-2), 73.3 (C-4), 76.5 (C-3), 73.5 (C-5) 87.2 (C-1).

2,3,4-Tri-*O*-benzyl-1-ethylthio-L-fucoside (81)



1_132

1-Ethylthio-L-fucoside (129 mg, 0.62 mmol) (**88**) was dissolved in dry DMF (5 mL) and cooled to 0°C under argon. De-oiled sodium hydride (90 mg, 3.75 mmol) was then added slowly. After 30 min stirring, benzyl bromide (300 µL, 2.53 mmol) was added dropwise. After 16 h stirring at r.t., the reaction mixture was quenched with MeOH (1 mL), diluted with toluene (20 mL) and washed with water (2x 15 mL) and brine (10 mL). The organic layer was then dried

on Na_2SO_4 and concentrated. Chromatography on silica gel with petrol ether and ethyl acetate 8:1 as eluent afforded **81** (237 mg, 80%) as an off-white solid.

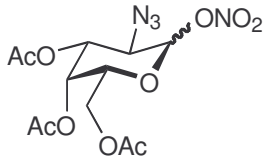
α-anomer : ^{1}H NMR (500 MHz, $CDCl_3$) : δ = 1.13 (d, 3H, $^3J_{5,6}$ = 6.5 Hz, H-6), 1.27 (t, 3H, ^3J = 7.4 Hz, S-CH_2-C$\underline{H}_3$), 2.48 (dq, 1H, ^3J = 7.4 Hz, ^2J = 12.9 Hz, S-CH$\underline{H}$-CH_3), 2.57 (dq, 1H, ^3J = 7.3 Hz, ^2J = 12.9 Hz, S-C$\underline{H}$H-CH_3), 3.64 (d, 1H, $^3J_{3,4}$ = 2.8 Hz, H-4), 3.79 (dd, 1H, $^3J_{3,4}$ = 2.8 Hz, $^3J_{2,3}$ = 9.9 Hz, H-3), 4.19 (q, 1H, $^3J_{5,6}$ = 6.5 Hz, H-5), 4.29 (dd, 1H, $^3J_{1,2}$ = 5.5 Hz, $^3J_{2,3}$ = 9.9 Hz, H-2), 4.65 (A of AB, 1H, $^2J_{A,B}$ = 11.6 Hz, C$\underline{H}$-Ph), 4.69 (A of AB, 1H, $^2J_{A,B}$ = 11.8 Hz, C$\underline{H}$-Ph), 4.71 (A of AB, 1H, $^2J_{A,B}$ = 11.9 Hz, C$\underline{H}$-Ph), 4.76 (B of AB, 1H, $^2J_{A,B}$ = 11.8 Hz, C$\underline{H}$-Ph), 4.86 (B of AB, 1H, $^2J_{A,B}$ = 11.9 Hz, C$\underline{H}$-Ph), 4.98 (B of AB, 1H, $^2J_{A,B}$ = 11.6 Hz, C$\underline{H}$-Ph), 5.46 (d, 1H, $^3J_{1,2}$ = 5.5 Hz, H-1), 7.27-7.41 (m, 15H, Ar-H).

^{13}C NMR (125 MHz, $CDCl_3$) : δ = 14.9 (S-CH_2-$\underline{C}H_3$), 16.6 (C-6), 23.7 (S-$\underline{C}H_2$-CH_3), 66.7 (C-5), 72.4 ($\underline{C}H_2$-Ph), 73.5 ($\underline{C}H_2$-Ph), 74.9 ($\underline{C}H_2$-Ph), 76.1 (C-2), 77.7 (C-4), 79.9 (C-3), 83.4 (C-1), 127.5, 127.6, 127.9, 128.0, 128.2, 128.3, 128.4 (15C, Ar-$\underline{C}$H), 138.3, 138.6, 138.9 (3C, Ar-C quat.).

β-anomer : ^{1}H NMR (500 MHz, $CDCl_3$) : δ = 1.19 (d, 3H, $^3J_{5,6}$ = 6.4 Hz, H-6), 1.29 (t, 3H, ^3J = 7.4 Hz, S-CH_2-C$\underline{H}_3$), 2.66-2.80 (m, 2H, S-C$\underline{H}_2$-CH_3), 3.46 (q, 1H, $^3J_{5,6}$ = 6.2 Hz, H-5), 3.55 (dd, 1H, $^3J_{3,4}$ = 2.4 Hz, $^3J_{2,3}$ = 9.3 Hz, H-3), 3.60 (d, 1H, $^3J_{3,4}$ = 2.4 Hz, H-4), 3.81 (t, 1H, $^3J_{1,2}$ = $^3J_{2,3}$ = 9.5 Hz, H-2), 4.38 (d, 1H, $^3J_{1,2}$ = 9.6 Hz, H-1), 4.68 (A of AB, 1H, $^2J_{A,B}$ = 11.8 Hz, C$\underline{H}$-Ph), 4.72 (A of AB, 1H, $^2J_{A,B}$ = 11.9 Hz, C$\underline{H}$-Ph), 4.76 (B of AB, 1H, $^2J_{A,B}$ = 11.9 Hz, C$\underline{H}$-Ph), 4.79 (A of AB, 1H, $^2J_{A,B}$ = 10.2 Hz, C$\underline{H}$-Ph), 4.88 (B of AB, 1H, $^2J_{A,B}$ = 10.2 Hz, C$\underline{H}$-Ph), 4.98 (B of AB, 1H, $^2J_{A,B}$ = 11.8 Hz, C$\underline{H}$-Ph), 7.24-7.40 (m, 15H, Ar-H).

^{13}C NMR (125 MHz, $CDCl_3$) : δ = 15.0 (S-CH_2-$\underline{C}H_3$), 17.2 (C-6), 24.6 (S-$\underline{C}H_2$-CH_3), 72.8 ($\underline{C}H_2$-Ph), 74.5 (2C, C-5, $\underline{C}H_2$-Ph) 75.7 ($\underline{C}H_2$-Ph), 76.4 (C-4), 78.3 (C-2), 84.4 (C-3), 84.9 (C-1), 127.5, 127.6, 127.7, 128.1, 128.2, 128.3, 128.4 (15C, Ar-$\underline{C}$H), 138.3, 138.4, 138.6 (3C, Ar-C quat.).

3,4,6-Tri-*O*-acetyl-2-azido-2-deoxy-D-galactosyl nitrate (90).



(1_197)

A mixture of cerium ammonium nitrate (3.53 g, 6.44 mmol) and sodium azide (0.21 g, 3.23 mmol) was cooled to –20°C under argon. 3,4,6-Tri-*O*-acetyl-D-galactal (**89**) (585 mg, 2.15 mmol) was dissolved in dry MeCN (20 mL) and added to the solids. After stirring for 18 h at –20°C, cold diethyl ether and water (50 mL each) were added to the reaction mixture. The organic layer was separated, washed with water (2x30 mL), dried with Na_2SO_4 and concentrated. Chromatography on silica gel (petrol ether / ethyl acetate 5:2) yielded **90** (428 mg, 53%, α/β 1.5:1) as a yellow oil.

(1_202a, 25/08/05)

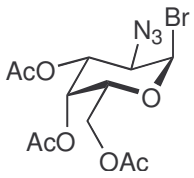
α-anomer : ^{1}H NMR (500 MHz, $CDCl_3$) : δ = 2.03, 2.07, 2.17 (3s, 9H, C$\underline{H}_3$C=O), 4.09-4.13 (m, 3H, H-2, H-6, H-6'), 4.36 (td, 1H $^3J_{4,5}$ = 0.9 Hz, $^3J_{5,6}$ = 6.6 Hz, H-5), 5.24 (dd, 1H, $^3J_{3,4}$ = 3.2 Hz, $^3J_{2,3}$ = 11.3 Hz, H-3), 5.49 (dd, 1H, $^3J_{4,5}$ = 0.9 Hz, $^3J_{3,4}$ = 2.5 Hz, H-4), 6.33 (d, 1H, $^3J_{1,2}$ = 4.1 Hz, H-1).

^{13}C NMR (125 MHz, $CDCl_3$) : δ = 20.5, 20.6 (3C, 3 C$\underline{H}_3$C=O), 55.9 (C-2), 60.9 (C-6), 66.6 (C-4), 68.6 (C-3), 69.5 (C-5), 96.9 (C-1), 169.5 (3C, 3 $CH_3\underline{C}$=O).

β-anomer : ^{1}H NMR (500 MHz, $CDCl_3$) : δ = 2.04, 2.07, 2.17 (3s, 9H, C$\underline{H}_3$C=O), 3.82 (dd, 1H, $^3J_{1,2}$ = 8.7 Hz, $^3J_{2,3}$ = 10.6 Hz, H-2), 4.05 (td, 1H $^3J_{4,5}$ = 0.9 Hz, $^3J_{5,6}$ = 6.6 Hz, H-5), 4.09-4.13 (m, 2H, H-6, H-6'), 4.95 (dd, 1H, $^3J_{3,4}$ = 3.3 Hz, $^3J_{2,3}$ = 10.6 Hz, H-3), 5.38 (dd, 1H, $^3J_{4,5}$ = 0.9 Hz, $^3J_{3,4}$ = 2.8 Hz, H-4), 5.57 (d, 1H, $^3J_{1,2}$ = 8.7 Hz, H-1).

^{13}C NMR (125 MHz, $CDCl_3$) : δ = 20.5, 20.6 (3C, 3 C$\underline{H}_3$C=O), 57.5 (C-2), 60.9 (C-6), 65.8 (C-4), 71.7 (C-3), 71.9 (C-5), 98.1 (C-1), 169.5 (3C, 3 $CH_3\underline{C}$=O).

3,4,6-Tri-*O*-acetyl-2-azido-2-deoxy-α-D-galactosyl bromide (91).



(1_197)

Compound **90** (428 mg, 1.14 mmol) was dissolved in dry MeCN (20 mL) at r.t. under argon. Dry LiBr (500 mg, 5.76 mmol) was added, and the reaction mixture was stirred at r.t. for 15 h. Then cold toluene and water (50 mL each) were added to the reaction mixture. The organic layer was separated, washed with water (2x 30 mL), dried with Na_2SO_4 and concentrated to yield **91** (421 mg, 94%) as a light brown viscous oil. The compound was used in the next step without further purification.

(1_202b, 30/08/05)

^{1}H NMR (500 MHz, $CDCl_3$) : δ = 2.05, 2.06, 2.15 (3s, 9H, 3 C$\underline{H}_3$C=O), 3.98 (dd, 1H $^3J_{1,2}$ = 3.7 Hz, $^3J_{2,3}$ = 10.7 Hz, H-2), 4.10 (dd, 1H, $^3J_{5,6}$ = 6.6 Hz, $^2J_{6,6'}$ = 11.4 Hz, H-6), 4.17 (dd, 1H, $^3J_{5,6'}$ = 6.6 Hz, $^2J_{6,6'}$ = 11.4 Hz, H-6'), 4.47 (t, 1H, $^3J_{5,6}$ = 6.6 Hz, H-5), 5.34 (dd, 1H, $^3J_{3,4}$ = 1.8 Hz, $^3J_{2,3}$ = 10.7 Hz, H-3), 5.50 (d, 1H, $^3J_{3,4}$ = 1.8 Hz, H-4), 6.47 (d, 1H, $^3J_{1,2}$ = 3.7 Hz, H-1).

^{13}C NMR (125 MHz, $CDCl_3$) : δ = 20.5, 20.6 (3C, 3 $\underline{C}H_3$C=O), 58.6 (C-2), 60.7 (C-6), 66.6 (C-4), 69.8 (C-3), 71.4 (C-5), 88.9 (C-1), 169.5, 169.7, 170.3 (3C, 3 $CH_3\underline{C}$=O).

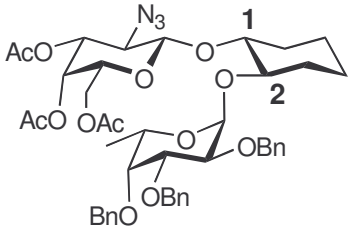
Silica gel-supported silver silicate Ag_2SiO_3.

(1_189)

Silver nitrate (430 mg, 2.53 mmol) was dissolved in water (2.5 mL), and silica gel 60 (380 mg, Fluka) was suspended into the solution. A suspension of sodium trisilicate (1.23 g, 5.08 mmol) in water (2.5 mL) was added dropwise with vigorous stirring. The mixture was then heated to 70°C for 30 min. After decantation, the supernatant was removed, and the solid residue suspended again in water (5 mL) and filtered. The residue was again suspended in a hot (70°C) aqueous solution of silver nitrate (10%, 2 mL) and stirred for 30 min. After cooling to r.t., acetone was added, the suspension was filtered, and subsequently washed with acetone and toluene.

The resulting solid was dried, first in Rotavap, then at 70°C in high vacuum, yielding the promoter (640 mg, estimated silver load : 2.2 mmol/g) as a light-brown powder.

(*1R,2R*)-1-*O*-(3,4,6-Tri-*O*-acetyl-2-azido-2-deoxy-β-D-galactosyl)-2-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucosyl)-cyclohexane-1,2-diol (92).



(1_160)

Compound **84** (355 mg, 0.68 mmol) was dissolved in dry DCM (10 mL) under argon. Silica supported silver silicate (0.85 g) and activated powdered molecular sieves 3 Å (0.4 g) were suspended in the solution, then bromide **91** (288 mg, 0.73 mmol) in dry DCM (10 mL) was added dropwise. After stirring for 185 h at r.t., the mixture was filtered through Celite, and the Celite washed with DCM. The filtrate was washed with water (2x10 mL), dried with Na_2SO_4 and concentrated. Chromatography on silica gel (toluene / ethyl acetate 6:1 to 4:1, with 0.1% Et_3N) yielded **92** (305 mg, 54%) as a yellowish, amorphous solid.

(1_238)

$[\alpha]_D$ = -52.1 (c = 13.8, $CHCl_3$).

(1_234, 31/03/06)

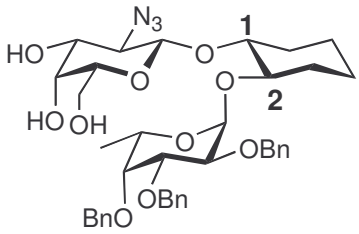
^{1}H NMR (500 MHz, $CDCl_3$) : δ = 1.12 (d, 3H, $^3J_{5,6}$ = 6.5 Hz, Fuc-H6), 1.26-1.72 (m, 8H, diol-H3-H6), 1.92, 2.02, 2.04 (3s, 9H, 3 C$\underline{H}_3$C=O), 3.47 (dd, 1H, $^3J_{1,2}$ = 7.9 Hz, $^3J_{2,3}$ = 10.9 Hz, Gal-H2), 3.63 (m, 1H, diol-H2), 3.65 (d, 1H, $^3J_{3,4}$ = 2.4 Hz, Fuc-H4), 3.74 (m, 1H, diol-H1), 3.78 (t, $^3J_{5,6}$ = 7.3 Hz, Gal-H5) 3.96 (dd, 1H, $^3J_{3,4}$ = 2.4 Hz, $^3J_{2,3}$ = 10.1 Hz, Fuc-H3), 4.00 (dd, 1H, $^3J_{5,6}$ = 5.9 Hz, $^2J_{6,6'}$ = 11.0 Hz, Gal-H6), 4.04-4.08 (m, 2H, Fuc-H2, Gal-H6'), 4.42 (d, 1H, $^3J_{1,2}$ = 7.9 Hz, Gal-H1), 4.51 (q, 1H, $^3J_{5,6}$ = 6.5 Hz, Fuc-H5), 4.69-4.73 (m, 3H, Gal-H3, 2 C$\underline{H}$-Ph), 4.78 (A of AB, 1H, $^2J_{A,B}$ = 11.6 Hz, C$\underline{H}$-Ph), 4.80 (B of AB, 1H, $^2J_{A,B}$ = 11.9 Hz, C$\underline{H}$-Ph), 4.89 (B of AB, 1H, $^2J_{A,B}$ = 11.6 Hz, C$\underline{H}$-Ph), 4.91 (d, 1H, $^3J_{1,2}$ = 3.7 Hz Fuc-H1), 4.99 (B of AB, 1H, $^2J_{A,B}$ = 11.9 Hz, C$\underline{H}$-Ph), 7.17-7.44 (m, 15H, 15 Ar-H).

^{13}C NMR (125 MHz, $CDCl_3$) : δ = 16.9 (Fuc-C6), 20.5, 20.6, 20.7 (3C, 3 $\underline{C}H_3$C=O), 23.0, 29.0, 29.3 (4C, diol), 60.7 (Fuc-C2), 61.3 (Gal-C2), 65.9 (Fuc-C5), 66.2 (GalC-4), 70.2 (Gal-C5), 70.8 (Gal-C3), 73.1, 73.2, 74.3 (3C, 3 $\underline{C}H_2$-Ph), 76.0 (diol-C2), 76.8 (Gal-C6), 77.0 (Fuc-C2), 77.3 (Fuc-C4), 79.5 (diol-C1), 80.0 (Fuc-C3), 95.1 (Fuc-C1), 100.2 (Gal-C1), 127.3-129.0 (9 peaks, 15C, 15 Ar-$\underline{C}$H), 138.7, 139.1 (3C, 3 quat. Ar-C)

(1_234)

Elemental analysis : Calculated for $C_{45}H_{55}N_3O_{13}$: C, 63.89% ; H, 6.55% ; N, 4.97%. Found : C, 63.79% ; H, 6.70% ; N, 4.91%.

(*1R,2R*)-1-*O*-(2-Azido-2-deoxy-β-D-galactosyl)-2-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucosyl)-cyclohexane-1,2-diol (93).



(1_167)

Compound **92** (300 mg, 0.35 mmol) was dissolved in methanol / water (1:1 10 mL), and potassium carbonate (5 mg, 36 µmol) was added. After stirring for 41 h at r.t., the solvents were evaporated. Chromatography on silica gel (dichloromethane / methanol gradient 98:2 to 90:10, with 0.1% Et_3N) yielded **93** (195 mg, 76%) as an off-white foam.

(1_235)

$[\alpha]_D$ = -95.4 (c = 0.52, MeOH).

(1_195, 09/08/05)

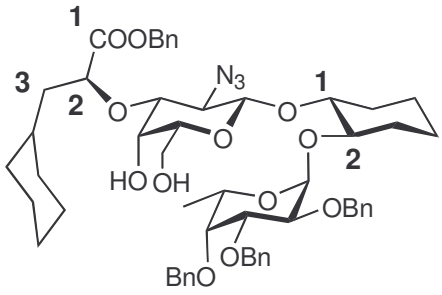
^{1}H NMR (500 MHz, $CDCl_3$) : δ = 1.13 (d, 3H, $^3J_{5,6}$ = 6.4 Hz, Fuc-H6), 1.20-1.72 (m, 8H, diol-H3-H6), 3.39-3.43 (m, 3H, Gal-H2, Gal-H3, Gal-H5), 3.60-3.65 (m, 1H, diol-H2), 3.73 (s, 1H, Fuc-H3), 3.74-3.81 (m, 2H, Gal-H6, diol-H1), 3.86 (dd, 1H, $^2J_{6,6'}$ = 12.3 Hz, $^3J_{5,6'}$ = 5.2 Hz, Gal-H6'), 3.94 (d, 1H, $^3J_{3,4}$ = 2.9 Hz, Gal-H4), 4.02-4.08 (m, 2H, Fuc-H2, Fuc-H4), 4.40 (d, 1H, $^3J_{1,2}$ = 7.7 Hz, Gal-H1), 4.44 (q, 1H, $^3J_{5,6}$ = 6.4 Hz, Fuc-H5), 4.64 (A of AB, 1H, $^2J_{A,B}$ = 11.6 Hz, C$\underline{H}_2$-Ph), 4.65 (A of AB, 1H, $^2J_{A,B}$ = 11.5 Hz, C$\underline{H}_2$-Ph), 4.75 (A of AB, 1H, $^2J_{A,B}$ = 11.6 Hz, C$\underline{H}_2$-Ph), 4.76 (B of AB, 1H, $^2J_{A,B}$ = 11.5 Hz, C$\underline{H}_2$-Ph), 4.80 (B of AB, 1H, $^2J_{A,B}$ = 11.6 Hz, C$\underline{H}_2$-Ph), 4.97 (B of AB, 1H, $^2J_{A,B}$ = 11.6 Hz, C$\underline{H}_2$-Ph), 5.02 (d, 1H, $^3J_{1,2}$ = 3.2 Hz, Fuc-H1), 7.24-7.43 (m, 15H, Ar-H).

^{13}C NMR (125 MHz, $CDCl_3$) : δ = 16.6 (Fuc-C6), 63.8 (Gal-C6), 66.1 (Fuc-C5), 69.3 (Gal-C4), 72.0 (Gal-C3), 73.3, 72.8 (2C, 2 C$\underline{H}_2$-Ph), 74.0 (Gal-C5), 74.9 (2C, diol-C2, C$\underline{H}_2$-Ph), 76.5 (Fuc-C2), 78.1 (2C, Fuc-C3, diol-C1), 79,6 (Fuc-C4), 93.6 (Fuc-C1), 99.4 (Gal-C1).

(1_235)

Elemental analysis : Calculated for $C_{39}H_{49}N_3O_{10}$: C, 65.08% ; H, 6.86% ; N, 5.84%. Found : C, 65.11% ; H, 7.02% ; N, 5.64%.

(*1R,2R*)-1-*O*-{3-*O*-[(Benzyl (*2S*)-3-cyclo-hexyl-propionate)-2-yl]-2-azido-2-deoxy-β-D-galactosyl}-2-*O*-(2,3,4-tri-O-benzyl-α-L-fucosyl)-cyclohexane-1,2-diol (96).



(1_200)

Compound **93** (910 mg, 1.30 mmol), and dibutyltin oxide (350 mg, 1.40 mmol) were dissolved in dry methanol (50 mL) under argon, and activated powdered molecular sieves 3Å (1.0 g) was suspended in the solution. The mixture was refluxed for 18 h, cooled, filtered, and concentrated. The resulting solid was taken up in dry DME (20 mL) under argon, cesium fluoride (250 mg, 1.60 mmol) was added, and the reaction mixture was stirred for 1 h. Then a solution of **95** in DME (15 mL) was added dropwise over 3 h. After stirring for 20 h at r.t., the reaction was quenched with an aqueous KF/KH_2PO_4 solution (10%, 20 mL). The organic layer was separated, washed with water (2x 20 mL), dried with Na_2SO_4 and concentrated. Chromatography on silica gel (toluene / ethyl acetate 4:1 to 2:1, with 0.1% Et_3N) yielded **96** (850 mg, 71%) as a yellowish foam.

(1_200)

$[\alpha]_D$ = - 102.8 (c = 0.985, MeOH).

(1_200, 25/08/05)

^{1}H NMR (500 MHz, $CDCl_3$) : δ = 0.84-0.98 (m, 3H, cyclohexyl), 1.14 (d, 3H, $^3J_{5,6}$ = 6.5 Hz, Fuc-H6), 1.17-1.80 (m, 18H, Lac-H3, cyclohexyl, diol-H3-H6), 2.98 (dd, 1H, $^3J_{3,4}$ = 3.1 Hz, $^3J_{2,3}$ = 10.0 Hz, Gal-H3), 3.28 (dd, 1H, $^3J_{5,6'}$ = 3.0 Hz, $^3J_{5,6}$ = 7.5 Hz, Gal-H5), 3.46 (dd, 1H, $^3J_{1,2}$ = 8.0 Hz, $^3J_{2,3}$ = 10.0 Hz, Gal-H2), 3.54 (dd, 1H, $^3J_{6,6'}$ = 12.3 Hz, $^3J_{5,6}$ = 3.0 Hz, Gal-H6), 3.58 (d, 1H, $^3J_{3,4}$ = 3.1 Hz, Gal-H4), 3.62 (td, 1H, $^3J_{1,2}$ = 4.3 Hz, $^3J_{2,3}$ = 9.3 Hz, diol-H2), 3.71 (td, 1H, $^3J_{1,2}$ = 4.3 Hz, $^3J_{1,6}$ = 9.4 Hz, diol-H1), 3.78 (d, 1H, $^3J_{4,3}$ = 1.5 Hz, Fuc-H4), 3.89 (dd, 1H, $^2J_{6,6'}$ = 12.3 Hz, $^3J_{5,6'}$ = 7.5 Hz, Gal-H6'), 4.02-4.10 (m, 3H, Fuc-H2, Fuc-H3, Lac-H2), 4.30 (d, 1H, $^3J_{1,2}$ =8.0 Hz, Gal-H1), 4.54 (q, 1H, $^3J_{5,6}$ = 6.5 Hz, Fuc-H5), 4.63-4.97 (m, 6H, C$\underline{H}_2$-Ph), 4.98 (s, 1H, Fuc-H1), 5.17 (A of AB, 1H, $^2J_{A,B}$ = 12.0 Hz, C$\underline{H}_2$-Ph), 5.20 (B of AB, 1H, $^2J_{A,B}$ = 12.0 Hz, C$\underline{H}_2$-Ph), 7.26-7.43 (m, 20H, Ar-H).

(1_200, 25/08/05)

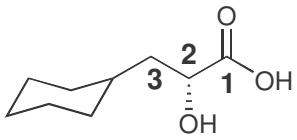
^{13}C NMR (125 MHz, $CDCl_3$) : δ = 16.6 (Fuc-C6), 23.3, 26.2, 26.3, 26.5, 29.0, 29.9, 32.2, 33.3, 33.4, 34.0, 41.0 (11C, Lac-C3, cyclohexyl C, diol-C3-C6), 62.8 (Gal-C6), 63.3 (Gal-C2), 66.1 (Fuc-C5), 66.7 (Gal-C4), 67.4 (C$\underline{H}_2$-Ph), 72.8 (C$\underline{H}_2$-Ph), 73.2 (C$\underline{H}_2$-Ph), 74.3

(Gal-C5), 75.0 ($\underline{C}H_2$-Ph), 75,1 (diol-C2), 76.4 (Fuc-C2), 77.6 (Fuc-C3), 78.1 (Fuc-C4), 78.8 (diol-C1), 79.7 (Lac-C2), 82,2 (Gal-C3), 94.1 (Fuc-C1), 100.0 (Gal-C1), 127.7-129.2 (20C, Ar-$\underline{C}$H), 135.2, 138.9, 139.2, 139.6 (4C, quat. Ar-C), 148.8 (Lac-C1).

(1_200)

Elemental analysis : Calculated for $C_{55}H_{69}N_3O_{12}$: C, 68.52% ; H, 7.21% ; N, 4.36%. Found : C, 68.38% ; H, 7.12% ; N, 4.24%.

(*2R*)-3-Cyclohexyl-2-hydroxypropionic acid (98).



(1_079)

Phenyllactic acid (3.52 g, 21.2 mmol) (**97**) was dissolved under argon in a mixture of water (40 mL), dioxane (20 mL) and acetic acid (10 mL). Rhodium on alumina (5%, 0.35 g) was hydrogenated at 4 bar for 72 h in a Paar shaker. The reaction mixture was filtered through Celite and concentrated. After co-evaporation with toluene, **98** (3.41 g, 93%) was obtained as an off-white solid.

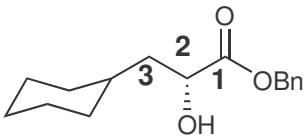
(1_079)

^{1}H NMR (500 MHz, MeOD) : δ = 0.93-1.01 (m, 2H, H-7), 1.19-1.33 (m, 3H, cyclohexyl), 1.56-1.73 (m, 7H, cyclohexyl, H-3), 1.87 (broad s, 1H, H-4), 4.16 (m, 1H, H-2).

(SM)

^{13}C NMR (125 MHz, $CDCl_3$) : δ = 26.0, 26.2, 26.4, 32.2, 33.9 (6C, $\underline{C}_6H_{11}$), 41.9 (C-3), 68.6 (C-2), 185.8 (C-1).

Benzyl (*2R*)-3-cyclohexyl-2-hydroxypropionate (99).



(1_084)

(*2R*)-3-Cyclohexyl-2-hydroxypropionic acid (**98**) (3.40 g, 19.8 mmol) was dissolved in methanol (20 mL), then cesium carbonate (7.10 g, 21.8 mmol) in water (20 mL) was added. The solution was stirred for 1 h at r.t.. The solvents were evaporated, and the residue co-evaporated with toluene (20 mL). After drying for 3 h in high vacuum, the residue was suspended in dry DMF (20 mL), and benzyl bromide (2.60 mL, 21.9 mmol) was added. After stirring for 20 h, the solution was diluted with toluene (50 mL) and washed with water (3x 50 mL). The combined aqueous layers were washed with toluene (2x 50 mL), and combined organic

layer was washed with brine (1x 50 mL), dried with Na_2SO_4 and concentrated. Chromatography on silica gel (petrol ether / ethyl acetate 4:1) yielded **99** (3.0 g, 58%) as a white, amorphous solid.

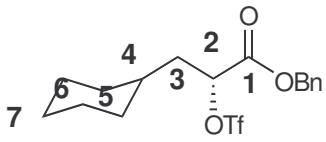
(1_084)

^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.85-0.96 (m, 2H, H-7), 1.09-1.27 (m, 3H, cyclohexyl), 1.50-1.71 (m, 7H, cyclohexyl, H-3), 1.81 (m, 1H, H-4), 4.28 (dd, 1H, ^{3}J = 3.9 Hz, ^{3}J = 9.0 Hz, H-2), 5.21 (s, 2H, C$\underline{H}_2$-Ph), 7.35-7.38 (m, 5H, Ar-H).

(SM)

^{13}C NMR (125 MHz, $CDCl_3$) : δ = 26.0, 26.3, 32.3, 33.7, 33.9 (6C, cyclohexyl), 42.1 (C-3), 67.3 ($\underline{C}H_2$-Ph), 68.6 (C-2), 128.4, 128.6, 128.7 (5C, Ar-CH), 135.2 (Ar-C quat.), 175.9 (C-1).

Benzyl (*2R*)-3-cyclohexyl-2-trifluoromethylsulfonyl-oxy-propionate (95)



(1_168)

Alcohol **99** (315 mg, 1.2 mmol) was dissolved in dry DCM (10 mL) and cooled to –20°C under argon. 2,6-Di-*tert*-butylpyridine (350 µL, 1.6 mmol) was added, followed by dropwise addition of trifluoromethanesulfonic anhydride (240 µL, 1.5 mmol). The reaction mixture was stirred for 3h while heating slowly to 0°C. Cold DCM and 1M aqueous KH_2PO_4 (10 mL each) were added to the reaction mixture. The organic layer was separated and washed with water (2x 10 mL), dried with Na_2SO_4 and concentrated. The crude material was filtered quickly on a short silica gel column, (petrol ether / ethyl acetate 20:1), yielding triflate **95** (330 mg, 70%) as a yellowish oil.

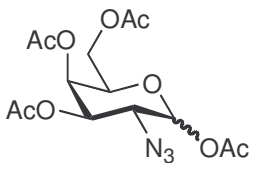
(SM)

^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.85-1.94 (m, 13H, H-3, cyclohexyl), 5.17 (m, 1H, H-2), 5.25 (s, 2H, C$\underline{H}_2$-Ph), 7.31-7.40 (m, 5H, Ar-H).

(SM)

^{13}C NMR (125 MHz, $CDCl_3$) : δ = 25.7, 26.0, 26.1, 32.0, 33.2, 33.4 (cyclohexyl), 39.2 (C-3), 68.2 (2C, C-2, $\underline{C}H_2$-Ph), 81.9 ($\underline{C}F_3$), 128.6, 128.7, 128.9, 134.4 (5C, Ar-CH), 134.4 (Ar-C quat.), 167.6 (C-1).

1,3,4,6-Tetra-*O*-acetyl-2-azido-2-deoxy-D-galactose (101)



(1_166)

Sodium azide (595 mg, 9.20 mmol) was dissolved in water (1.5 mL), cooled to 0°C, and DCM (2.5 mL) was added to the solution. Triflic anhydride (300 µL, 1.82 mmol) was then slowly added under vigorous stirring. After stirring 2h at 0°C, the aqueous layer was separated and washed with DCM (2x 1 mL). The combined organic layers, containing triflyl azide (TfN_3) were finally washed with a saturated aqueous $NaHCO_3$ solution and used in the following without further purification.

K_2CO_3 (192 mg, 1.39 mmol) and $CuSO_4$ (cat.) were added to a solution of 2-amino-2-deoxy-galactose hydrochloride (**100**, 200 mg, 0.93 mmol) in water (3 mL) and MeOH (6 mL), followed by the TfN_3 in DCM solution. Some drops of MeOH were added to homogeneity. After stirring for 16h at r.t., the reaction was quenched with diisopropylamine (200 µL), and solvents were evaporated to dryness.

The residue was taken up in pyridine and acetic anhydride was added slowly. After 16h stirring, the reaction mixture was diluted with ethyl acetate (30 mL), washed with a saturated aqueous $NaHCO_3$ solution (3x20 mL), and with water (2x 20 mL). The organic layer was then dried with Na_2SO_4 and concentrated. Chromatography on silica gel (petrol ether / ethyl acetate 4:1) yielded **101** (3.0 g, 58%) as a white, crystalline solid.

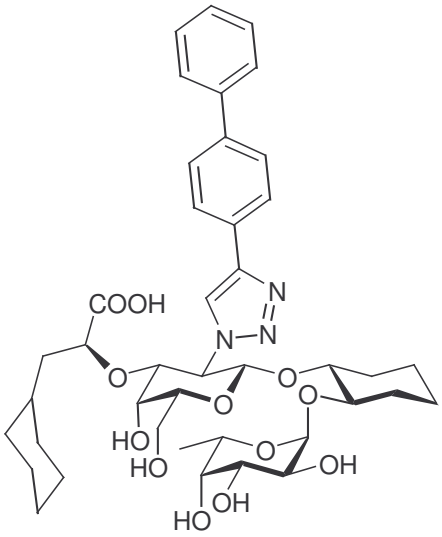
(1_004, 29/09/03)

α-anomer : ^{1}H NMR (500 MHz, $CDCl_3$) : δ = 2.03, 2.08, 2.17, 2.18 (4s, 12H, 4 C$\underline{H}_3$C=O), 3.94 (dd, 1H $^3J_{1,2}$ = 3.6 Hz, $^3J_{2,3}$ = 11.1 Hz, H-2), 4.06 (dd, 1H, $^3J_{5,6}$ = 6.2 Hz, $^2J_{6,6'}$ = 10.7 Hz, H-6), 4.10 (dd, 1H, $^3J_{5,6}$ = 6.3 Hz, $^2J_{6,6'}$ = 10.7 Hz, H-6'), 4.28 (dt, 1H, $^3J_{4,5}$ = 1.3 Hz, $^3J_{5,6}$ = 6.3 Hz, H-5), 5.31 (dd, 1H, $^3J_{3,4}$ = 3.2 Hz, $^3J_{2,3}$ = 11.1 Hz, H-3), 5.48 (dd, 1H, $^3J_{4,5}$ = 1.3 Hz, $^3J_{3,4}$ = 3.2 Hz, H-4), 6.32 (d, 1H, $^3J_{1,2}$ = 3.6 Hz, H-1).

(1_017, 06/12/04)

β-anomer : ^{1}H NMR (500 MHz, $CDCl_3$) : δ = 2.04, 2.07, 2.17, 2.21 (4s, 12H, 4 C$\underline{H}_3$C=O), 3.84 (dd, 1H $^3J_{1,2}$ = 8.5 Hz, $^3J_{2,3}$ = 10.8 Hz, H-2), 4.00 (t, 1H, $^3J_{5,6}$ = 6.6 Hz, H-5), 4.10 (dd, 1H, $^3J_{5,6}$ = 6.1 Hz, $^2J_{6,6'}$ = 11.7 Hz, H-6), 4.15 (dd, 1H, $^3J_{5,6'}$ = 6.9 Hz, $^2J_{6,6'}$ = 11.4 Hz, H-6'), 4.89 (dd, 1H, $^3J_{3,4}$ = 3.3 Hz, $^3J_{2,3}$ = 10.8 Hz, H-3), 5.38 (dd, 1H, $^3J_{4,5}$ = 0.6 Hz, $^3J_{3,4}$ = 3.2 Hz, H-4), 5.54 (d, 1H, $^3J_{1,2}$ = 8.5 Hz, H-1).

(*1R*,*2R*)-1-O-{2-(4-biphenyl-1,2,3-triazol-1-yl)-3-[((*2S*)-3-cyclohexylpropionic acid)-2-O-yl]-2-deoxy-β-D-galactosyl]}-2-O-(α-L-fucosyl)-cyclohexane-1,2-diol sodium salt (103a).



Azide **96** (20 mg, 21 µmol) and 4-ethynylbiphenyl (8 mg, 44 µmol) were dissolved in MeOH (2 mL). Argon was bubbled into the solution, in order to remove oxygen. An aqueous copper sulfate solution (1M, 20 µL) was then added, followed by aqueous sodium ascorbate (1M, 30 µL). After stirring for 2 d at r.t., ethyl acetate (5 mL) and water (2 mL) were added. The organic layer was washed with water (2x5 mL), dried with Na_2SO_4 and concentrated. Chromatography on silica gel (toluene/ethyl acetate 2:1, with 0.1% Et_3N) afforded a mixture of the protected triazole and remaining **96** (7:3, 24 mg), which was used in the next step without further purification.

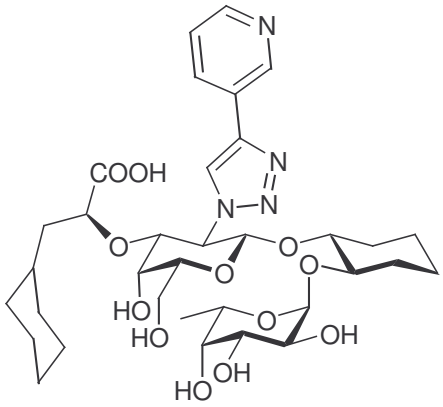
The crude mixture was dissolved in dioxane (2 mL) under argon. 10% palladium on charcoal (10 mg) was added and the suspension hydrogenated at 4 bar for 2 d in a Parr shaker. The reaction mixture was filtered through Celite, concentrated and purified by preparative LC-MS. After lyophilization, **103a** (10.0 mg, 60%) was obtained as a white powder.

^{1}H NMR (500 MHz, CD_3OD) : δ = 0.56-0.80 (m, 3H, cyclohexyl), 0.82-0.96 (m, 3H, 1 diol-H6, 2 cyclohexyl), 1.00-1.10 (m, 3H, 1 diol-H4, 1 diol-H5, 1 cyclohexyl), 1.27 (d, 3H, $^3J_{5,6}$ = 6.6 Hz, Fuc-H6), 1.24-1.39 (m, 7H, 1 diol-H3, 1 diol-H5, 5 cyclohexyl), 1.43-1.45 (m, 2H, Lac-H3), 1.57-1.59 (m, 2H, 1 diol-H4, 1 diol-H6), 1.88-1.91 (m, 1H, 1 diol-H3), 3.33 (td, 1H, $^3J_{1,2}$ = 4.0 Hz, $^3J_{2,3}$ = 8.5 Hz, diol-H2), 3.66 (td, 1H, $^3J_{1,2}$ = 4.0 Hz, $^3J_{1,6}$ = 8.5 Hz, diol-H1), 3.71 (dd, 1H, $^3J_{1,2}$ = 3.8 Hz, $^3J_{2,3}$ = 10.1 Hz, Fuc-H2), 3.73-3.75 (m, 2H, Fuc-H4, Gal-H5), 3.79 (dd, 1H, $^3J_{5,6}$ = 5.2 Hz, $^2J_{6,6'}$ = 11.2 Hz, Gal-H6), 3.83-3.87 (m, 1H, Gal-H6'), 3.87 (dd, 1H, $^3J_{3,4}$ = 3.4 Hz, $^3J_{2,3}$ = 10.1 Hz, Fuc-H3), 3.96 (dd, 1H, $^3J_{2,3}$ = 3.2 Hz, $^3J_{2,3'}$ = 9.5 Hz Lac-H2), 4.07 (d, 1H, $^3J_{3,4}$ = 2.6 Hz, Gal-H4), 4.22 (dd, 1H, $^3J_{3,4}$ = 2.6 Hz, $^3J_{2,3}$ = 10.6 Hz, Gal-H3), 4.55-4.61 (m, 2H, Fuc-H5, Gal-H2), 4.82 (d, 1H, $^3J_{1,2}$ = 3.8

Hz Fuc-H1), 5.08 (d, 1H, $^3J_{1,2}$ = 8.3 Hz, Gal-H1), 7.35, 7.46, 7.67, 7.72, 7.95 (m, 9H, Ar-H), 8.48 (s, 1H, C_2N_3H) ;

^{13}C NMR (125 MHz, CD_3OD) : δ = 16.7 (Fuc-C6), 23.7, 26.5, 26.8, 27.3, 29.3, 29.9, 32.9, 33.9, 35.1, 41.6 (11 C, Lac-C3, cyclohexyl C, diol-C3-C6), 62.6 (Gal-C6), 64.7 (Gal-C4), 67.4 (2C, Fuc-C5, Gal-C2), 70.0 (Fuc-C2), 71.6 (Gal-C5), 73.8 (Fuc-C3), 76.7 (Fuc-C4), 79.0 (diol-C2), 79.5 (Lac-C2), 79.8 (diol-C1), 82.8 (Gal-C3), 97.3 (Fuc-C1), 100.3 (Gal-C1), 125.2, 127.1, 127.9, 128.5, 128.6, 129.9 (10C, 9 Ar-CH, Tzl-CH).

(*1R,2R*)-1-O-{2-[4-(pyridin-3-yl)-1,2,3-triazol-1-yl]-3-[((*2S*)-3-cyclohexyl-propionic acid)-2-O-yl]-2-deoxy-β-D-galactosyl}-2-O-(α-L-fucosyl)-cyclohexane-1,2-diol sodium salt (103b).



Azide **96** (20 mg, 21 µmol) and 3-ethynylpyridine (7 mg, 42 µmol) were dissolved in MeOH (2 mL). Argon was bubbled into the solution, in order to remove oxygen. An aqueous copper sulfate solution (1M, 20 µL) was then added, followed by aqueous sodium ascorbate (1M, 30 µL). After stirring for 2 d at r.t., ethyl acetate (5 mL) and water (2 mL) were added. The organic layer was washed with water (2x5 mL), dried with Na_2SO_4 and concentrated. Chromatography on silica gel (toluene/ethyl acetate 2:1, with 0.1% Et_3N) afforded a mixture of the protected triazole and remaining **96** (8:2, 19 mg), which was used in the next step without further purification.

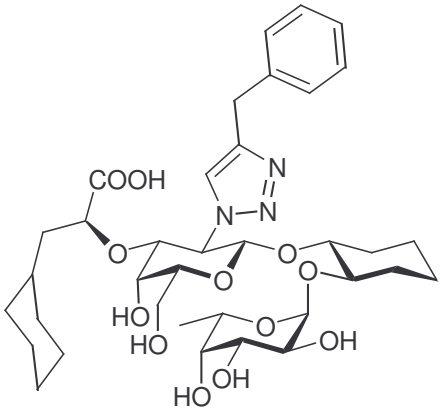
The crude mixture was dissolved in liquid ammonia (7 mL) and kept at –75°C under argon. Sodium (15 mg) was added piecewise. Once the blue color persisted, the reaction was quenched by slowly adding solid ammonium chloride (30 mg). Liquid ammonia was removed by heating to r.t. and the residue was taken up in water/MeOH 1:1 (10 mL), filtered and concentrated. Purification by LC-MS and lyophilization yielded **103b** (6.2 mg, 41%) as a white powder.

^{1}H NMR (500 MHz, CD_3OD) : δ = 0.49-0.80 (m, 5H, 1 diol-H6, 4 cyclohexyl), 0.89-1.02 (m, 2H, cyclohexyl), 1.02-1.13 (m, 2H, 1 diol-H4, 1 diol-H5), 1.27 (d, 3H, $^3J_{5,6}$ = 6.6 Hz,

FucH-6), 1.20-1.41 (m, 8H, 1 diol-H3, 1 diol-H6, 5 cyclohexyl), 1.45-1.47 (m, 2H, Lac-H3), 1.58-1.60 (m, 2H, 1 diol-H4, 1 diol-H6), 1.89-1.92 (m, 1H, 1 diol-H3), 3.33 (td, 1H, $^3J_{1,2}$ = 3.9 Hz, $^3J_{2,3}$ = 8.6 Hz, diol-H2), 3.67 (td, 1H, $^3J_{1,2}$ = 3.9 Hz, $^3J_{1,6}$ = 8.6 Hz, diol-H1), 3.71 (dd, 1H, $^3J_{1,2}$ = 3.8 Hz, $^3J_{2,3}$ = 10.1 Hz, Fuc-H2), 3.75 (d, 1H, $^3J_{3,4}$ = 3.2 Hz, Fuc-H4), 3.80 (dd, 1H, $^3J_{5,6}$ = 4.9 Hz, $^2J_{6,6'}$ = 11.3 Hz, Gal-H6), 3.85 (dd, 1H, $^3J_{5,6'}$ = 7.0 Hz, $^2J_{6,6'}$ = 11.3 Hz, Gal-H6'), 3.88 (dd, 1H, $^3J_{3,4}$ = 3.2 Hz, $^3J_{2,3}$ 10.1 Hz, Fuc-H3), 4.05 (s, 1H, Gal-H4), 4.17 (d, 1H, $^3J_{2,3}$ = 9.8 Hz, Gal-H3), 4.59 (m, 2H, Fuc-H5, Gal-H2), 4.82 (d, 1H, $^3J_{1,2}$ = 3.8 Hz, Fuc-H1), 5.10 (d, 1H, $^3J_{1,2}$ = 8.2 Hz, Gal-H1), 7.69, 8.20, 8.37, 8,39, 8.63 (m, 6H, C_6H_5N, C_2N_3H) ;

^{13}C NMR (125 MHz, MeOD) : δ = 16.7 (Fuc-C6), 23.8, 26.7, 27.0, 27.3, 29.5, 30.1, 32.9, 34.0, 35.2, 42.9 (11C, Lac-C3, cyclohexyl C, diol-C3-C6), 62.7 (Gal-C6), 64.8 (Gal-C2), 66.6 (Gal-C4), 67.4 (Fuc-C5), 69.9 (Fuc-C2), 71.5 (Gal-C5), 73.8 (Fuc-C3), 76.2 (Fuc-C4), 76.6 (Diol-C2), 79.8 (diol-C1), 82.9 (Gal-C3), 97.2 (Fuc-C1), 100.1 (Gal-C1).

(*1R,2R*)-1-O-{2-(4-benzyl-1,2,3-triazol-1-yl)-3-[((*2S*)-3-cyclohexylpropionic acid)-2-O-yl]-2-deoxy-β-D-galactosyl}-2-O-(α-L-fucosyl)-cyclohexane-1,2-diol sodium salt (103c).



Azide **96** (20 mg, 21 µmol) and 3-phenyl-1-propyne (5 µL, 46 µmol) were dissolved in MeOH (2 mL). Argon was bubbled into the solution, in order to remove oxygen. An aqueous copper sulfate solution (1M, 20 µL) was then added, followed by aqueous sodium ascorbate (1M, 30 µL). After stirring for 2 d at r.t., ethyl acetate (5 mL) and water (2 mL) were added. The organic layer was washed with water (2x5 mL), dried with Na_2SO_4 and concentrated. Chromatography on silica gel (toluene/ethyl acetate 2:1, with 0.1% Et_3N) afforded a mixture of the protected triazole and remaining **96** (8:2, 22 mg), which was used in the next step without further purification.

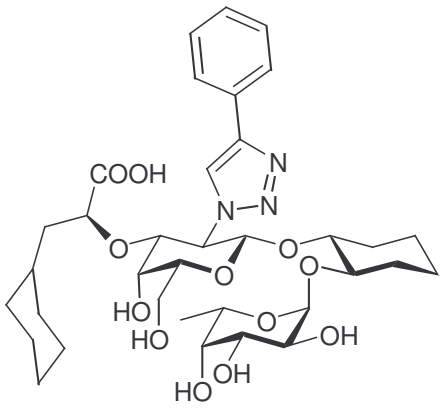
Crude mixture was dissolved in dioxane (2 mL) under argon. 10% palladium on charcoal (10 mg) was added and the suspension hydrogenated at 4 bar for 2 d in a Parr shaker. The reaction mixture was filtered through Celite, concentrated and purified by

preparative LC-MS. After lyophilization, **103c** (10.2 mg, 66%) was obtained as a white powder.

^{1}H NMR (500 MHz, CD_3OD) : δ = 0.52-0.79 (m, 4H, 1 diol-H6, 3 cyclohexyl), 0.98-1.16 (m, 5H, 1 diol-H4, 1 diol-H5, 3 cyclohexyl), 1.20 (d, 3H, $^3J_{5,6}$ = 6.6 Hz, Fuc-H6), 1.23 1.60 (m, 11H, Lac-H3, 4 diol-H3-H6, 5 cyclohexyl), 1.82-1.85 (m, 1H, diol-H3), 3.33 (m, 1H, diol-H2), 3.52 (td, 1H, $^3J_{1,2}$ = 3.9 Hz, $^3J_{1,6}$ = 8.5 Hz, diol-H1), 3.66 (t, 1H, $^3J_{5,6}$ = 6.1 Hz, Gal-H5), 3.69 (m, 2H, Fuc-H2, Fuc-H4), 3.75 (dd, 1H, $^3J_{5,6}$ = 5.3 Hz, $^2J_{6,6'}$ = 11.2 Hz, Gal-H6), 3.80 (dd, 1H, $^3J_{5,6'}$ = 6.7 Hz, $^2J_{6,6'}$ = 11.2 Hz, Gal-H6'), 3.83 (dd, 1H, $^3J_{3,4}$ = 3.3 Hz, $^3J_{2,3}$ = 10.1 Hz, Fuc-H3), 3.93 (t, 1H, $^3J_{2,3}$ = 6.4 Hz, Lac-H2), 4.07 (s, 2H, $\underline{C}H_2$-Ph), 4.04 (d, 1H, $^3J_{3,4}$ = 2.8 Hz, Gal-H4), 4.21 (dd, 1H, $^3J_{3,4}$ = 2.8 Hz, $^3J_{2,3}$ = 10.6 Hz, Gal-H3), 4.44 (dd, 1H, $^3J_{1,2}$ = 8.4 Hz, $^3J_{2,3}$ = 10.6 Hz, Gal-H2), 4.51 (q, 1H, $^3J_{5,6}$ = 6.6 Hz, Fuc-H5), 4.79 (d, 1H, $^3J_{1,2}$ = 3.9 Hz, Fuc-H1), 4.85 (d, 1H, $^3J_{1,2}$ = 8.4 Hz, Gal-H1), 7.20, 7.27-7.28 (m, 5H, Ar-H), 7.74 (s, 1H, $C_2N_3\underline{H}$) ;

^{13}C NMR (125 MHz, MeOD) : δ = 16.4 (Fuc-C6), 23.6, 26.6, 27.0, 32.6, 33.8, 34.9, 42.3 (11C, Lac-C3, cyclohexyl C, diol-C3-C6), 32.9 ($\underline{C}H_2$-Ph), 62.3 (Gal-C6), 64.4 (Gal-C2), 66.6 (Gal-C4), 67.2 (Fuc-C5), 69.8 (Fuc-C2), 71.4 (2C, Fuc-C4, Fuc-C3), 73.6 (Gal-C5), 75.8 (diol-C2), 76.4 (Lac-C2), 77.7 (diol-C1), 82.1 (Gal-C3), 97.0 (Fuc-C1), 100.2 (Gal-C1), 125.9, 127.4, 129.5 (6C, 5 Ar-CH, Tzl-H), 140.3, 147.7 (2C, Ar-C quat., Tzl-C quat.).

(*1R,2R*)-1-O-{2-(4-phenyl-1,2,3-triazol-1-yl)-3-[((*2S*)-3-cyclohexylpropionic acid)-2-O-yl]-2-deoxy-β-D-galactosyl}-2-O-(α-L-fucosyl)-cyclohexane-1,2-diol sodium salt (103d).



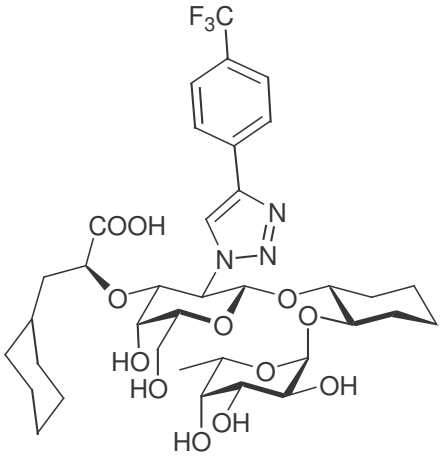
Azide **96** (20 mg, 21 µmol) and phenylacetylene (5 µL, 44 µmol) were dissolved in MeOH (2 mL). Argon was bubbled into the solution, in order to remove oxygen. An aqueous copper sulfate solution (1M, 20 µL) was then added, followed by aqueous sodium ascorbate (1M, 30 µL). After stirring for 2 d at r.t., ethyl acetate (5 mL) and water (2 mL) were added. The

organic layer was washed with water (2x5 mL), dried with Na_2SO_4 and concentrated. Chromatography on silica gel (toluene/ethyl acetate 2:1, with 0.1% Et_3N) afforded a mixture of the protected triazole and remaining **96** (7:3, 20.4 mg), which was used in the next step without further purification.

The crude mixture was dissolved in dioxane (2 mL) under argon. 10% palladium on charcoal (10 mg) was added and the suspension hydrogenated at 4 bar for 2 d in a Parr shaker. The reaction mixture was filtered through Celite, concentrated and purified by preparative LC-MS. After lyophilization, **103d** (8.2 mg, 54%) was obtained as a white powder.

^{1}H NMR (500 MHz, CD_3OD) : δ = 0.48-0.85 (m, 4H, diol, 3 cyclohexyl), 0.87-1.12 (m, 6H, 3 diol, 3 cyclohexyl), 1.23 (d, 3H, $^3J_{5,6}$ = 6.6 Hz, Fuc-H6), 1.22-1.27 (m, 6H, diol, 5 cyclohexyl) 1.46 (m, 2H, Lac-H3), 1.58-1.61 (m, 2H, diol), 1.85-1.88 (m, 1H, diol), 3.32 (td, 1H, $^3J_{1,2}$ = 3.9 Hz, $^3J_{2,3}$ = 8.5 Hz, diol-H2), 3.65 (td, 1H, $^3J_{1,2}$ = 3.9 Hz, $^3J_{1,6}$ = 8.6 Hz, diol-H1), 3.69-3.73 (m, 3H, Fuc-H2, Fuc-H4, Gal-H5), 3.78 (dd, 1H, $^3J_{5,6}$ = 5.1 Hz, $^2J_{6,6'}$ = 11.2 Hz, Gal-H6), 3.83 (dd, 1H, $^3J_{5,6'}$ = 6.5 Hz, $^2J_{6,6'}$ = 11.2 Hz, Gal-H6'), 3.85 (dd, 1H, $^3J_{3,4}$ = 3.1 Hz, $^3J_{2,3}$ = 10.0 Hz, Fuc-H3), 3.90 (dd, 1H, $^3J_{2,3}$ = 3.5 Hz, $^3J_{2,3'}$ = 9.5 Hz Lac-H2), 4.05 (d, 1H, $^3J_{3,4}$ = 2.5 Hz, Gal-H4), 4.19 (dd, 1H, $^3J_{3,4}$ = 2.5 Hz, $^3J_{2,3}$ = 10.3 Hz, Gal-H3), 4.55-4.60 (m, 2H, Fuc-H5, Gal-H2), 4.81 (d, 1H, $^3J_{1,2}$ = 3.9 Hz Fuc-H1), 4.97 (d, 1H, $^3J_{1,2}$ = 8.2 Hz, Gal-H1), 7.34-7.42 (m, 4H, Ar-H), 7.89 (m, 2H, Tzl-H, Ar-H) ; ^{13}C NMR (125 MHz, CD_3OD) : δ = 16.6 (Fuc-C6), 23.8, 26.7, 26.9, 27.3, 29.3, 30.0, 32.8, 33.8, 35.0, 42.0 (11 C, Lac-C3, cyclohexyl C, diol-C3-C6), 62.7 (Gal-C6), 65.0 (Gal-C4), 67.4 (2C, Fuc-C5, Gal-C2), 70.1 (Fuc-C2), 71.6 (Gal-C5), 73.8 (Fuc-C3), 76.5 (Fuc-C4), 76.4 (diol-C2), 79.6 (Lac-C2), 79.8 (diol-C1), 82.7 (Gal-C3), 97.3 (Fuc-C1), 100.3 (Gal-C1), 125.2, 127.3, 128.5, (6C, Ar-CH, Tzl-CH), 140.2, 145.6 (2C, Ar-C-quat., Tzl-C quat.).

(*1R*,*2R*)-1-O-{2-[4-(4-trifluoromethylphenyl)-1,2,3-triazol-1-yl]-3-[((*2S*)-3-cyclohexylpropionic acid)-2-O-yl]-2-deoxy-β-D-galactosyl}-2-O-(α-L-fucosyl)-cyclohexane-1,2-diol sodium salt (103e).



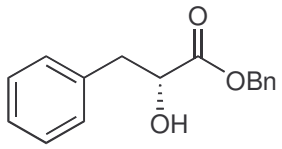
Azide **96** (20 mg, 21 µmol) and 4-ethynyl-α,α,α-trifluorotoluene (7 µL, 43 µmol) were dissolved in MeOH (2 mL). Argon was bubbled into the solution, in order to remove oxygen. An aqueous copper sulfate solution (1M, 20 µL) was then added, followed by aqueous sodium ascorbate (1M, 30 µL). After stirring for 2 d at r.t., ethyl acetate (5 mL) and water (2 mL) were added. The organic layer was washed with water (2x5 mL), dried with Na_2SO_4 and concentrated. Chromatography on silica gel (toluene/ethyl acetate 2:1, with 0.1% Et_3N) afforded a mixture of the protected triazole and remaining **96** (7:3, 22 mg), which was used in the next step without further purification.

The crude mixture was dissolved in dioxane (2 mL) under argon. 10% palladium on charcoal (10 mg) was added and the suspension hydrogenated at 4 bar for 2 d in a Parr shaker. The reaction mixture was filtered through Celite, concentrated and purified by preparative LC-MS. After lyophilization, **103e** (10.3 mg, 62%) was obtained as a white powder.

^{1}H NMR (500 MHz, CD_3OD) : δ = 0.56-0.95 (m, 6H, 2 diol, 4 cyclohexyl), 0.99-1.13 (m, 4H, 2 diol, 2 cyclohexyl), 1.26 (d, 3H, $^3J_{5,6}$ = 6.5 Hz, Fuc-H6), 1.21-1.26 (m, 6H, diol, 5 cyclohexyl) 1.44 (m, 2H, Lac-H3), 1.57-1.60 (m, 2H, diol), 1.85-1.90 (m, 1H, 1 diol), 3.33 (m, 1H, diol-H2), 3.66 (m, 1H, diol-H1), 3.69 (dd, 1H, $^3J_{1,2}$ = 3.8 Hz, $^3J_{2,3}$ = 10.0 Hz, Fuc-H2), 3.72 (t, 1H, $^3J_{5,6}$ = 6.0 Hz, Gal-H5), 3.75 (d, 1H, $^3J_{3,4}$ = 3.2 Hz, Fuc-H4), 3.79 (dd, 1H, $^3J_{5,6}$ = 5.1 Hz, $^2J_{6,6'}$ = 11.3 Hz, Gal-H6), 3.85 (dd, 1H, $^3J_{5,6'}$ = 6.8 Hz, $^2J_{6,6'}$ = 11.3 Hz, Gal-H6'), 3.87 (dd, 1H, $^3J_{3,4}$ = 3.4 Hz, $^3J_{2,3}$ = 10.0 Hz, Fuc-H3), 3.96 (dd, 1H, $^3J_{2,3}$ = 3.2 Hz, $^3J_{2,3'}$ = 9.4 Hz Lac-H2), 4.07 (d, 1H, $^3J_{3,4}$ = 2.6 Hz, Gal-H4), 4.21 (dd, 1H, $^3J_{3,4}$ = 2.6 Hz, $^3J_{2,3}$ = 10.2 Hz, Gal-H3), 4.56-4.60 (m, 2H, Fuc-H5, Gal-H2), 4.81 (d, 1H, $^3J_{1,2}$ = 3.8 Hz Fuc-H1), 5.09 (d, 1H, $^3J_{1,2}$ = 8.3 Hz, Gal-H1), 7.67 (m, 2H, Ar-H), 7.89 (s, 1H, Tzl-H), 7.94 (m, 2H, Ar-H) ; ^{13}C NMR (125 MHz, CD_3OD) : δ = 16.7 (Fuc-C6), 23.7, 26.6, 26.8,

27.3, 29.4, 29.9, 32.9, 33.9, 35.1, 41.8 (11 C, Lac-C3, cyclohexyl C, diol-C3-C6), 62.7 (Gal-C6), 64.8 (Gal-C4), 67.0 (Gal-C2), 67.3 (Fuc-C5), 69.9 (Fuc-C2), 71.6 (Gal-C5), 73.9 (Fuc-C3), 76.3 (2C, Fuc-C4, diol-C2), 79.3 (Lac-C2), 79.5 (diol-C1), 82.9 (Gal-C3), 97.1 (Fuc-C1), 100.1 (Gal-C1), 124.9, 125.2, 127.3, 128.4, (5C, Ar-CH, Tzl-CH), 139.8, 143.2 (3C, 2 Ar-C-quat., Tzl-C quat.).

Benzyl (*2R*)-3-phenyl-2-hydroxypropionate (106)



(2_015)

D(+)-phenyllactic acid (**97**) (500 mg, 3.0 mmol) was dissolved in dry DMF (10 mL) containing activated powdered molecular sieve 3 Å (500 mg), then cesium carbonate (1.96 g, 6.0 mmol) was added. After the solution was stirred for 1 h at r.t., benzyl bromide (400 µL, 3.4 mmol) was added dropwise over 4 h. The mixture was then stirred 1 h more, filtered on Celite, diluted with ethyl acetate (20 mL) and washed with water (3x 20 mL). The combined aqueous layers were washed with ethyl acetate (2x 20 mL), and combined organic layer was washed with brine (1x 50 mL), dried with Na_2SO_4 and concentrated. Chromatography on silica gel (petrol ether / ethyl acetate 4:1) yielded **106** (680 mg, 88%) as a yellowish oil.

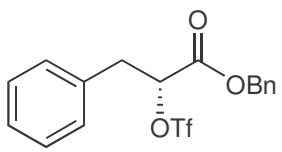
(2_011, 03/02/06)

^{1}H NMR (500 MHz, $CDCl_3$): δ = 2.75 (d, 1H, ^3J = 6.4 Hz, OH), 3.00 (dd, 1H, $^3J_{2,3}$ = 6.4 Hz, ^2J = 13.9 Hz, H-3), 3.15 (dd, 1H, $^3J_{2,3}$ = 4.4 Hz, ^2J = 13.9, H-3'), 4.52 (m, 1H, H-2), 5.21 (AB system, 2H, CH_2-Ph), 7.16-7.41 (m, 10H, Ar-H).

(2_011, 03/02/06)

^{13}C NMR (125 MHz, $CDCl_3$) : δ = 40.5 (C-3), 67.5 (CH_2-Ph), 71.2 (C-2), 126.9, 128.4, 128.7, 129.5 (10C, Ar-CH), 135.0, 136.1 (2C, Ar-C quat.), 174.0 (C-1).

Benzyl (*2R*)-3-phenyl-2-trifluoromethylsulfonyl-oxy-propionate (107)



(2_016)

Alcohol **106** (682 mg, 2.7 mmol) was dissolved in dry DCE (30 mL) and cooled to –20 °C under argon. Pyridine (280 µL, 3.5 mmol) was added, followed by dropwise addition of trifluoromethanesulfonic anhydride (500 µL, 3.0 mmol).

The reaction mixture was stirred for 2h while heating slowly to -5°C. Reaction was quenched using ice cold 1M aqueous KH_2PO_4 (20 mL). The organic layer was separated and washed with ice cold water (2x 20 mL), dried with Na_2SO_4 and concentrated. The crude material was filtered quickly on a short silica gel column, (petrol ether / ethyl acetate 20:1), yielding triflate **107** (870 mg, 84%) as a yellowish oil.

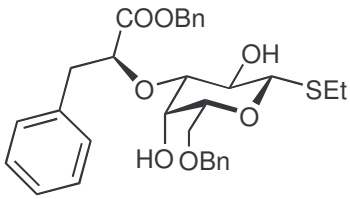
(2_012, 07/02/06)

^{1}H NMR (500 MHz, $CDCl_3$): δ = 3.18 (dd, 1H, $^3J_{2,3}$ = 8.4 Hz, ^2J = 14.6, H-3), 3.31 (dd, 1H, $^3J_{2,3}$ = 4.2 Hz, ^2J = 14.6, H-3'), 5.21 (AB system, 2H, C$\underline{H}_2$-Ph), 5.25 (m, 1H, $^3J_{2,3}$ = 4.2 Hz, $^3J_{2,3'}$ = 8.4 Hz, H-2), 7.13-7.37 (m, 10H, Ar-H).

(2_012, 07/02/06)

^{13}C NMR (125 MHz, $CDCl_3$) : δ = 38.2 (C-3), 68.4 (C$\underline{H}_2$-Ph), 83.8 (C-2), 127.9, 128.6, 128.7, 128.8, 129.5 (10C, Ar-CH), 133.2, 134.2 (2C, Ar-C quat.), 166.5 (C-1).

Thioethyl 6-*O*-benzyl-3-*O*-[(benzyl (*2S*)-3-phenyl-propionate)-2-yl]-β-D-galactoside (109).



(2_017)

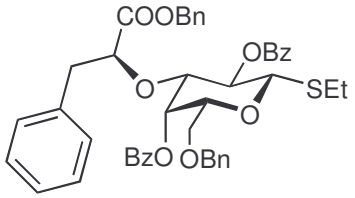
Thioethyl 6-O-benzyl-β-D-galactoside **108** (309 mg, 0.99 mmol), and dibutyltin oxide (280 mg, 1.12 mmol) were dissolved in dry methanol (30 mL) under argon, and activated powdered molecular sieves 3Å (500 mg) was suspended in the solution. The mixture was refluxed for 18 h, cooled, filtered, and concentrated. The resulting solid was taken up in dry DME (15 mL) under argon, cesium fluoride (250 mg, 1.65 mmol) was added, and the reaction mixture was stirred for 30 min. Then a solution of **107** in DME (5 mL) was added dropwise over 3 h. After stirring for 21 h at r.t., the reaction was quenched with an aqueous KF/KH_2PO_4 solution (10%, 20 mL). The organic layer was separated, washed with water (2x 20 mL), dried with Na_2SO_4 and concentrated. Chromatography on silica gel (toluene / ethyl acetate 9:1 to 2:1, with 0.1% Et_3N) yielded **109** (197 mg, 36%) as a yellowish oil. Product is mixed with its lactonized forms. It was used in the next step without further purification.

(2_013, 16/02/06, seulement 1H et COSY)

^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.26 (t, 3H, ^3J = 7.5 Hz, -S-CH_2-C$\underline{H}_3$), 2.66-2.74 (m, 2H, -S-C$\underline{H}_2$-CH_3), 3.17-3.27 (m, 3H, Gal-H3, Lac-H3), 3.51 (t, 1H, $^3J_{5,6}$ = 5.3 Hz, Gal-H5), 3.65-3.75 (m, 2H, Gal-H6), 4.06 (broad s, 1H, Gal-H4), 4.30 (d, 1H, $^3J_{1,2}$ = 9.6 Hz, Gal-

H1), 4.45-4.57 (m, 3H, Gal-H2, 2 C$\underline{H}_2$-Ph), 4.83 (dd, 1H, $^3J_{2,3}$ = 4.7 Hz, $^3J_{2,3'}$ = 6.2 Hz, Lac-H2), 7.20-7.35 (m, 15H, Ar-H).

Thioethyl 2,4-dibenzoyl-6-*O*-benzyl-3-*O*-[(benzyl (*2S*)-3-phenyl-propionate)-2-yl]-β-D-galactoside (111).



(2_019)

Thioethyl galactoside **109** (197 mg, 0.36 mmol) was dissolved in dry pyridine (5 mL) under argon. Benzoyl chloride (250 µL, 2.13 mmol) was added slowly to the mixture. After stirring for 16 h at r.t., the solution was diluted with ethyl acetate (30 mL), washed with saturated aqueous $NaHCO_3$ (3x 20 mL) and with water (2x 20 mL), dried on Na_2SO_4 and concentrated. Chromatography on silica gel (petrol ether / toluene / ethyl acetate 5:5:1) yielded **111** (124 mg, 46%) and both lactone forms (2-Gal, 59 mg, 30%, **124a** and 4-Gal, 24 mg, 12%, **124b**).

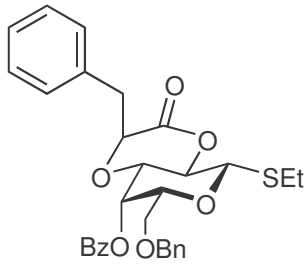
(2_019, 09/03/06)

^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.24 (t, 3H, ^3J = 7.5 Hz, -S-CH$_2$-C$\underline{H}_3$), 2.68-2.82 (m, 4H, 2 Lac-H3, 2 -S-C$\underline{H}_2$-CH$_3$), 3.54-3.62 (m, 2H, Gal-H6), 3.80 (t, 1H, $^3J_{5,6}$ = 5.8 Hz, Gal-H5), 3.86 (dd, 1H, $^3J_{3,4}$ = 2.3 Hz, $^3J_{2,3}$ = 9.5 Hz, Gal-H3), 4.32 (t, 1H, $^3J_{2,3}$ = 6.1 Hz, Lac-H2), 4.45 (A of AB, 1H, $^2J_{A,B}$ = 11.8 Hz, C$\underline{H}_2$-Ph), 4.50-4.53 (m, 2H, Gal-H1, C$\underline{H}_2$-Ph), 5.05 (A of AB, 1H, $^2J_{A,B}$ = 12.0 Hz, C$\underline{H}_2$-Ph), 5.14 (B of AB, 1H, $^2J_{A,B}$ = 12.0 Hz, C$\underline{H}_2$-Ph), 5.54 (t, 1H, $^3J_{1,2}$ = $^3J_{2,3}$ = 9.7 Hz, Gal-H2), 5.90 (broad s, 1H, Gal-H4), 6.65 (d, 2H, J = 7.5 Hz, Ar-H), 6.77 (t, 2H, J = 7.6 Hz, Ar-H), 6.95 (t, 1H, J = 7.4 Hz, Ar-H), 7.22-7.32 (m, 10H, Ar-H), 7.44 (t, 2H, J = 7.7 Hz, Ar-H), 7.51 (t, 2H, J = 7.6 Hz, Ar-H), 7.56-7.64 (m, 2H, Ar-H), 7.95 (d, 2H, J = 7.3 Hz, Ar-H), 8.12 (d, 2H, J = 7.3 Hz, Ar-H).

(2_019, 09/03/06)

^{13}C NMR (125 MHz, $CDCl_3$) : δ = 14.8 (-S-CH$_2$-$\underline{C}$H$_3$), 24.2 (-S-$\underline{C}$H$_2$-CH$_3$), 38.9 (Lac-C3), 69.0 (2C, Gal-C6, $\underline{C}$H$_2$-Ph), 70.4 (Gal-C4), 70.9 (Gal-C2), 73.7 ($\underline{C}$H$_2$-Ph), 77.4 (Gal-C5), 78.8 (Gal-C3), 80.8 (Lac-C2), 83.9 (Gal-C1), 126.4, 127.7, 127.9, 128.4, 128.6, 128.7, 129.3, 129.5, 129.9, 130.1, 133.2, 135.3, 135.7, 137.9 (30C, Ar-C), 164.8, 165.5, 171.2 (3C, Lac-C1, 2 Ph-($\underline{C}$=O)-O-).

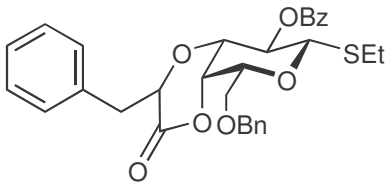
Thioethyl 2,4-dibenzoyl-6-*O*-benzyl-3-*O*-[(benzyl (*2S*)-3-phenyl-propionate)-2-yl]-β-D-galactoside (2-lactone, 124a).



(2_019, 09/03/06)

^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.33 (t, 3H, ^{3}J = 7.5 Hz, -S-CH_2-C$\underline{H}_3$), 2.75-2.83 (m, 2H, -S-C$\underline{H}_2$-CH_3), 3.15 (dd, 1H, $^{3}J_{2,3}$ = 4.6 Hz, ^{2}J = 14.2 Hz, Lac-H3), 3.25 (dd, 1H, $^{3}J_{2,3}$ = 5.6 Hz, ^{2}J = 14.2 Hz, Lac-H3'), 3.37 (dd, 1H, $^{3}J_{3,4}$ = 3.0 Hz, $^{3}J_{2,3}$ = 9.3 Hz, Gal-H3), 3.50-3.54 (m, 2H, Gal-H6), 3.77 (t, 1H, $^{3}J_{5,6}$ = 6.3 Hz, Gal-H5), 4.38-4.42 (m, 2H, Gal-H1, C$\underline{H}_2$-Ph), 4.46-4.49 (m, 2H, Gal-H2, C$\underline{H}_2$-Ph), 4.70 (t, 1H, $^{3}J_{2,3}$ = 4.7 Hz, Lac-H2), 5.71 (broad s, 1H, Gal-H4), 7.15-7.19 (m, 5H, Ar-H), 7.22-7.32 (m, 4H, Ar-H), 7.43-7.47 (m, 2H, Ar-H), 7.57-7.64 (m, 2H, Ar-H), 7.98-8.01 (m, 2H, Ar-H).

Thioethyl 2,4-dibenzoyl-6-*O*-benzyl-3-*O*-[(benzyl (*2S*)-3-phenyl-propionate)-2-yl]-β-D-galactoside (4-lactone, 124b).



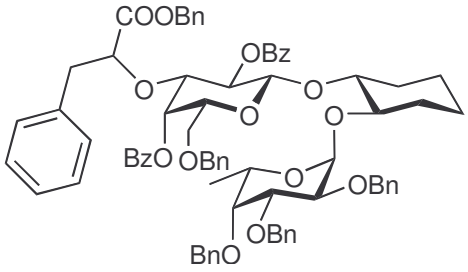
(2_019, 09/03/06)

^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.23 (t, 3H, ^{3}J = 7.5 Hz, -S-CH_2-C$\underline{H}_3$), 2.65-2.80 (m, 2H, 2 -S-C$\underline{H}_2$-CH_3), 2.95 (dd, 1H, $^{3}J_{2,3}$ = 9.3 Hz, ^{2}J = 14.3 Hz, Lac-H3), 3.34 (dd, 1H, $^{3}J_{2,3}$ = 2.4 Hz, ^{2}J = 14.3 Hz, Lac-H3'), 3.77 (dd, 1H, $^{3}J_{5,6}$ = 5.5 Hz, ^{2}J = 9.0 Hz, Gal-H6), 3.83-3.92 (m, 2H, Gal-H5, Gal-H6), 4.23 (dd, 1H, $^{3}J_{3,4}$ = 3.8 Hz, $^{3}J_{2,3}$ = 9.2 Hz, Gal-H3), 4.44 (dd, 1H, $^{3}J_{2,3'}$ = 3.0 Hz, $^{3}J_{2,3}$ = 9.3 Hz, Lac-H2), 4.53 (d, 1H, $^{3}J_{1,2}$ = 10.1 Hz, Gal-H1), 4.54-4.60 (m, 2H, C$\underline{H}_2$-Ph), 4.81 (broad s, 1H, Gal-H4), 5.36 (t, 1H, $^{3}J_{1,2}$ = $^{3}J_{2,3}$ = 9.7 Hz, Gal-H2), 7.04-7.19 (m, ?H, Ar-H), 7.24-7.37 (m, 4H, Ar-H), 7.48 (t, 2H, J = 7.7 Hz, Ar-H), 7.62 (t, 1H, J = 7.4 Hz, Ar-H), 8.07 (d, 2H, J = 7.8 Hz, Ar-H).

(2_019, 09/03/06)

^{13}C NMR (125 MHz, $CDCl_3$) : δ = 14.8 (-S-CH_2-$\underline{C}H_3$), 23.5 (-S-$\underline{C}H_2$-CH_3), 37.3 (Lac-C3), 66.9 (Gal-C6), 69.6 (Gal-C2), 71.7 (Lac-C2), 71.8 (Gal-C4), 73.1 (Gal-C3), 73.8 ($\underline{C}H_2$-Ph), 75.2 (Gal-C5), 83.0 (Gal-C1), 126.6, 127.9, 128.0, 128.2, 128.5, 128.6, 129.6, 129.9, 133.3, 136.9 (18C, Ar-C), 165.4, 169.6 (2C, Lac-C1, Ph-($\underline{C}$=O)-O-).

(*1R,2R*)-1-*O*-{2,4-dibenzoyl-3-*O*-[(Benzyl (*2S*)-3-phenyl-propionate)-2-yl]-β-D-galactosyl}-2-*O*-(2,3,4-tri-O-benzyl-α-L-fucosyl)-cyclohexane-1,2-diol (112).



(2_027)

Compound **111** (124 mg, 0.16 mmol), and (*1R,2R*)-2-*O*-(2,3,4-Tri-*O*-benzyl-α-L-fucosyl)-cyclohexane-1,2-diol (**84**) (141 mg, 0.26 mmol) were dissolved in dry DCM (10 mL) under argon and activated powdered molecular sieve 3 Å (500 mg) was added. After 90 min stirring, dimethyl(methylthio)sulfonium triflate (DMTST, 170 mg, 0.66 mmol) in dry DCM (10 mL) was added. After stirring for 19 h at r.t., the reaction mixture was filtered through Celite, diluted with DCM (20 mL), washed with saturated aqueous $NaHCO_3$ (2x 20 mL) and water (1x 20 mL), dried on Na_2SO_4 and concentrated. Chromatography on silica gel (petrol ether / toluene / ethyl acetate 5:5:1 with 0.1% v/v Et_3N) yielded **112** (103 mg, 52%) as a yellowish oil.

(2_018, 03/03/06)

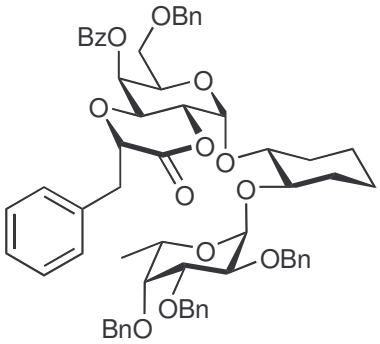
^{1}H NMR (500 MHz, $CDCl_3$) : δ = 1.04 (m, 1H, diol-H6), 1.26 (m, 5H, Fuc-H6, 1 diol-H3, diol-H4), 1.42 (m, diol-H6'), 1.53-1.65 (m, 2H, diol-H3', diol-H5), 1.80-1.90 (m, 2H, diol-H4', diol-H5'), 2.72 (dd, 1H, $^3J_{2,3}$ = 6.5 Hz, ^2J = 13.8 Hz, Lac-H3), 2.81 (dd, 1H, $^3J_{2,3}$ = 5.3 Hz, $^3J_{2,3}$ = 13.8 Hz, Lac-H3'), 3.52-3.55 (m, 3H, 2 Gal-H6, diol-H2), 3.67 (m, 1H, diol-H1), 3.71 (t, 1H, $^3J_{5,6}$ = 5.9 Hz, Gal-H5), 3.81 (broad d, 1H, $^3J_{2,3}$ = 9.8 Hz, Gal-H3), 3.96 (m, 2H, Fuc-H2, Fuc-H3), 4.29 (t, 1H, $^3J_{2,3}$ = 5.9 Hz, Lac-H2), 4.36 (A of AB, 1H, $^2J_{A,B}$ = 11.6 Hz, C$\underline{H}_2$-Ph), 4.43-4.50 (m, 4H, Fuc-H5, 3 C$\underline{H}_2$-Ph), 4.56 (d, 1H, $^3J_{1,2}$ = 8.0 Hz, Gal-H1), 4.61 (A of AB, 1H, $^2J_{A,B}$ = 12.0 Hz, C$\underline{H}_2$-Ph), 4.68 (A of AB, 1H, $^2J_{A,B}$ = 12.7 Hz, C$\underline{H}_2$-Ph), 4.71 (B of AB, 1H, $^2J_{A,B}$ = 12.3 Hz, C$\underline{H}_2$-Ph), 4.84 (B of AB, 1H, $^2J_{A,B}$ = 11.6 Hz, C$\underline{H}_2$-Ph), 4.89 (s, 1H, Fuc-H1), 5.04 (AB, 2H, $^2J_{A,B}$ = 12.0 Hz, C$\underline{H}_2$-Ph), 5.48 (t, 1H, $^3J_{1,2}$ = $^3J_{2,3}$ = 8.9 Hz, Gal-H2), 5.83 (s, 1H, Gal-H4), 6.70 (d, 2H, J = 7.5 Hz, Ar-H), 6.78 (t, 2H, J = 7.6 Hz, Ar-H), 6.94 (t, 1H, J = 7.3 Hz, Ar-H), 7.14-7.32 (m, 25H, Ar-H), 7.44 (m, 4H, Ar-H), 7.57 (m, 2H, Ar-H), 7.96 (d, 2H, J = 7.6 Hz, Ar-H), 8.12 (d, 2H, J = 7.6 Hz, Ar-H).

(2_018, 03/03/06)

^{13}C NMR (125 MHz, $CDCl_3$) : δ = 16.7 (Fuc-C6), 22.7 (diol-C6), 28.3, 29.7 (3C, diol-C3, diol-C4, diol-C5), 38.9 (Lac-C3), 66.3 (Fuc-C5), 66.8 (C$\underline{H}_2$-Ph), 68.8 (Gal-C6), 70.0 (Gal-C4), 72.7 (C$\underline{H}_2$-Ph), 72.9 (C$\underline{H}_2$-Ph), 73.3 (Gal-C2), 73.8 (diol-C1), 74.5 (diol-C2), 74.8

(CH_2-Ph), 76.3 (CH_2-Ph), 78.1 (Fuc-C3), 78.5 (Gal-C3), 78.8 (Fuc-C4), 79.8 (Fuc-C2), 80.7 (Lac-C2), 94.0 (Fuc-C1), 99.3 (Gal-C1), 126.4, 127.1, 127.2, 127.4, 127.6, 127.8, 127.9, 128.0, 128.1, 128.2, 128.4, 128.5, 128.6, 129.3, 129.6, 130.0, 130.1, 133.1, 133.2, 135.3, 135.7, 137.9, 138.8, 139.1 (48C, Ar-C), 164.5, 165.6, 171.1 (3C, Lac-C1, 2 Ph-(C=O)-O-).

(*1R,2R*)-1-*O*-{"lactone?"-β-D-galactosyl}-2-*O*-(2,3,4-tri-O-benzyl-α-L-fucosyl)-cyclohexane-1,2-diol (126).



(2_027)

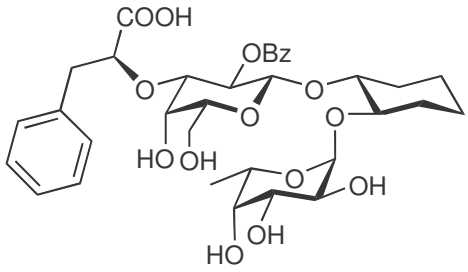
Lactone **124a** (59 mg, 0.11 mmol) is reacted in similar conditions than diester **111**, to yield **126** (26 mg, 23%).

(2_018, 03/03/06)

^{1}H NMR (500 MHz, $CDCl_3$) : δ = 1.06 (m, 1H, diol-H6), 1.17-1.31 (m, 5H, 3 Fuc-H6, diol-H3, diol-H4), 1.65-1.71 (m, 3H, diol-H4, 2 diol-H5), 1.98 (m, 1H, diol-H3'), 2.17 (m, 1H, diol-H6'), 3.05 (dd, 1H, $^3J_{2,3}$ = 6.9 Hz, ^2J = 14.3 Hz, Lac-H3), 3.05 (dm, 1H, ^2J = 14.3 Hz, Lac-H3'), 3.48 (m, 1H, diol-H1), 3.52-3.58 (m, 2H, Gal-H6), 3.63 (m, 1H, diol-H2), 3.93 (dm, 1H, $^3J_{2,3}$ = 10.6 Hz, Gal-H3), 4.05-4.23 (m, 6H, Gal-H2, Gal-H5, Fuc-H2, Fuc-H3, Fuc-H4, Fuc-H5), 4.35 (m, 1H, Lac-H2), 4.42 (A of AB, 1H, $^2J_{A,B}$ = 11.7 Hz, CH_2-Ph), 4.51 (B of AB, 1H, $^2J_{A,B}$ = 11.7 Hz, CH_2-Ph), 4.73 (A of AB, 2H, $^2J_{A,B}$ = 11.4 Hz, 2 CH_2-Ph), 4.83-4.87 (m, 3H, CH_2-Ph), 5.06 (B of AB, 1H, $^2J_{A,B}$ = 11.5 Hz, CH_2-Ph), 5.11 (s, 1H, Fuc-H1), 5.24 (s, 1H, Gal-H1), 5.67 (s, 1H, Gal-H4), 7.13-7.17 (m, 3H, Ar-H), 7.18-7.21 (m, 3H, Ar-H), 7.23-7.31 (m, 9H, Ar-H), 7.33-7.38 (m, 4H, Ar-H), 7.41-7.48 (m, 6H, Ar-H), 7.51-7.55 (m, 2H, Ar-H), 7.66 (m, 1H, Ar-H), 8.03 (m, 2H, Ar-H).

^{13}C NMR (125 MHz, $CDCl_3$) : δ = 16.3 (Fuc-C6), 23.4 (diol-C5), 24.3 (diol-C6), 27.7 (diol-C4), 32.2 (diol-C3), 37.6 (Lac-C3), 66.3 (Fuc-C5), 67.4 (Gal-C3), 68.5 (Gal-C6), 68.7 (Gal-C5), 69.0 (Gal-C4), 70.7 (CH_2-Ph), 72.6 (Fuc-C4), 73.1 (CH_2-Ph), 73.6 (CH_2-Ph), 74.5 (Lac-C2), 74.6 (diol-C1), 75.2 (CH_2-Ph), 76.2 (Gal-C2), 77.8 (Fuc-C3), 80.2 (2C, Fuc-C2, diol-C2), 91.2 (Fuc-C1), 96.9 (Gal-C1), 126.8, 127.0, 127.3, 127.4, 127.5, 127.6, 127.8, 128.0, 128.1, 128.3, 128.4, 129.4, 129.8, 133.4, 136.6, 137.7, 139.1, 139.2, 140.0 (36C, Ar-C), 165.1, 168.7 (2C, Lac-C1, Ph-(C=O)-O-).

(*1R*,*2R*)-1-*O*-{2-benzoyl-3-*O*-[((*2S*)-3-phenyl-propionate)-2-yl]-β-D-galactosyl}-2-*O*-α-L-fucosyl)-cyclohexane-1,2-diol (105).



(2_028-29)

Compound **112** (70 mg, 57 µmol) was dissolved in dioxane (3.0 mL) under argon. 10% palladium on charcoal (20 mg) was added and the suspension hydrogenated at 4 bar for 72 h in a Paar shaker. The reaction mixture was then filtered through Celite and concentrated. The residue was taken up in a sodium methanolate solution (1M in methanol, 1 mL). After 3 h stirring at r.t., it was concentrated and preparative LC-MS isolated **105** (14 mg, 36%) which was then freeze-dried.

$[\alpha]_D$ = - 89.7 (c = 0.685, MeOH).

(2_029, 04/04/06)

^{1}H NMR (500 MHz, CD_3OD) : δ = 0.97 (m, 1H, diol-H6), 1.07 (m, 2H, diol-H4, diol-H5), 1.24 (d, 3H, $^3J_{5,6}$ = 6.6 Hz, Fuc-H6), 1.25 (m, 1H, diol-H3), 1.45 (m, 1H, diol-H5'), 1.57 (m, 1H, diol-H4'), 1.86 (m, 1H, diol-H6'), 1.93 (m, 1H, diol-H3'), 2.84 (dd, 1H, $^3J_{2,3}$ = 7.7 Hz, ^2J = 14.1 Hz, Lac-H3), 2.96 (dd, 1H, $^3J_{2,3}$ = 4.0 Hz, ^2J = 14.1 Hz, Lac-H3'), 3.42 (dt, 1H, $^3J_{1,2}$ = 4.4 Hz, $^3J_{2,3}$ = 9.2 Hz, diol-H2), 3.53 (t, 1H, $^3J_{5,6}$ = 5.9 Hz, Gal-H5), 3.63 (m, 2H, diol-H1, Gal-H3), 3.70 (dd, 1H, $^3J_{3,4}$ = 3.9 Hz, $^3J_{2,3}$ = 10.1 Hz, Fuc-H2), 3.72-3.74 (m, 2H, Gal-H6, Fuc-H4), 3.79 (dd, 1H, $^3J_{5,6}$ = 6.9 Hz, $^2J_{6,6'}$ = 11.4 Hz, Gal-H6'), 3.87 (dd, 1H, $^3J_{3,4}$ = 3.3 Hz, $^3J_{2,3}$ = 10.1 Hz, Fuc-H3), 4.01 (broad s, 1H, Gal-H4), 4.31 (dd, 1H, $^3J_{2,3}$ = 4.0 Hz, $^3J_{2,3'}$ = 7.7 Hz, Lac-H2), 4.56 (q, 1H, $^3J_{5,6}$ = 6.6 Hz, Fuc-H5), 4.59 (d, 1H, $^3J_{1,2}$ = 8.0 Hz, Gal-H1), 4.82 (d, 1H, $^3J_{1,2}$ = 3.9 Hz, Fuc-H1), 5.34 (dd, 1H, $^3J_{1,2}$ = 8.0 Hz, $^3J_{2,3}$ = 9.7 Hz, Gal-H2), 6.82-6.89 (m, 3H, benzyl Ar-H), 6.95-6.98 (m, 2H, benzyl Ar-H), 6.95-6.98 (m, 2H, benzyl Ar-H), 7.47-7.51 (m, 2H, benzoyl Ar-H), 7.64 (m, 1H, benzoyl Ar-H), 7.94-7.97 (m, 2H, benzoyl Ar-H).

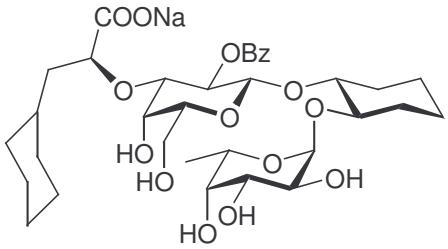
^{13}C NMR (125 MHz, MeOD) : δ = 16.6 (Fuc-C6), 24.0, 24.1 (2C, diol-C4, diol-C5), 29.4, 30.7 (2C, diol-C3, diol-C6), 40.5 (Lac-C3), 62.6 (Gal-C6), 67.5 (Fuc-C5), 67.9 (Gal-C4), 70.0 (Fuc-C2), 71.5 (Fuc-C3), 73.2 (Gal-C2), 73.8 (Fuc-C4), 76.0 (Gal-C5), 76.4 (diol-C2), 79.4 (diol-C1), 81.2 (Lac-C2), 83.1 (Gal-C3), 96.8 (Fuc-C1), 100.4 (Gal-C1),

127.3, 129.0, 129.5, 130.2, 130.8, 131.6, 134.2, 138.1 (12C, Ar-C), 166.8 (benzoyl C=O), 177.1 (Lac-C1).

Elemental analysis : Calculated for $C_{34}H_{44}O_{14}$ (+H_2O): C, 58.78% ; H, 6.67%. Found : C, 59.38% ; H, 6.70%.

(*1R,2R*)-1-*O*-{2-benzoyl-3-*O*-[((*2S*)-3-cyclohexyl-propionate)-2-yl]-β-D-galactosyl}-2-*O*-α-L-fucosyl)-cyclohexane-1,2-diol (105).

(2_036)



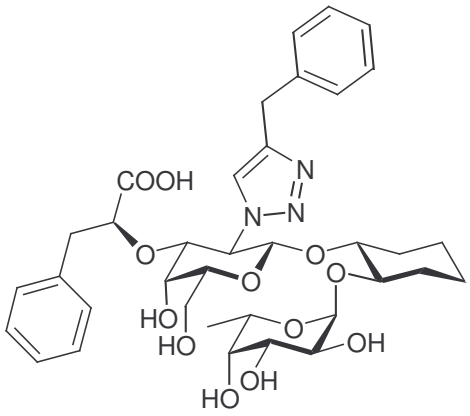
(2_036, 17/08/06)

^{1}H NMR (500 MHz, CD_3OD) : δ = 0.56-0.75 (m, 4H, 1 diol-H6, 3 cyclohexyl), 0.88-1.17 (m, 5H, 1 diol-H4, 1 diol-H5, 3 cyclohexyl), 1.24 (d, 3H, $^3J_{5,6}$ = 6.2 Hz, Fuc-H6), 1.23 1.60 (m, 9H, Lac-H3, 2 diol-H3-H6, 5 cyclohexyl), 1.93-1.96 (m, 2H, diol-H3), 3.44 (dt, 1H, $^3J_{1,2}$ = 4.2 Hz, $^3J_{1,6}$ = 9.2 Hz, diol-H2), 3.58 (t, 1H, $^3J_{5,6}$ = 5.7 Hz, Gal-H5), 3.66 (m, 1H, diol-H1), 3.68-3,74 (m, 4H, Fuc-H2, Fuc-H4, Gal-H3, Gal-H6), 3.81 (dd, 1H, $^3J_{5,6'}$ = 7,0 Hz, $^2J_{6,6'}$ = 11.4 Hz, Gal-H6'), 3.89 (dd, 1H, $^3J_{3,4}$ = 3.2 Hz, $^3J_{2,3}$ = 10.1 Hz, Fuc-H3), 3,98 (s, 1H, Gal-H4), 4,07 (m, 1H, Lac-H2), 4.60 (q, 1H, $^3J_{5,6}$ = 6.2 Hz, Fuc-H5), 4.69 (d, 1H, $^3J_{1,2}$ = 8.0 Hz, Gal-H1), 4.82 (d, 1H, $^3J_{1,2}$ = 3.7 Hz, Fuc-H1), 5.38 (t, 1H, $^3J_{1,2}$ = $^3J_{2,3}$ = 8.9 Hz, Gal-H2), 7.51 (t, 2H, $^3J_{Ar}$ = 7.7 Hz, Ar-H), 7,63 (t, 1H, $^3J_{Ar}$ = 7.4 Hz, Ar-H), 8,09 (d, 1H, $^3J_{Ar}$ = 7.3 Hz, Ar-H) ;

^{13}C NMR (125 MHz, MeOD) : δ = 16.6 (Fuc-C6), 24.1, 24.2, 26.6, 26.8, 27.3, 29.5, 30.9, 33.1, 34.2, 35.1, 42.8 (11C, Lac-C3, cyclohexyl C, diol-C3-C6), 62.6 (Gal-C6), 67.5 (Fuc-C5), 67.9 (Gal-C4), 70.0 (Fuc-C2), 71.5 (2C, Fuc-C4, Fuc-C3), 73.3 (Gal-C2), 73.9 (Fuc-C4),76.1 (diol-C2), 76.5 (Gal-C5), 78.2 (Lac-C2), 79.4 (diol-C1), 83.3 (Gal-C3), 96.8 (Fuc-C1), 100.4 (Gal-C1), 129.7, 130.8, 131.7, 134.4 (6C, Ar-C), 166.9 (acid).

Elemental analysis : Calculated for $C_{34}H_{49}O_{14}Na_1$ (+4H_2O): C, 52.53% ; H, 7.34%. Found : C, 52.60% ; H, 7.30%.

(*1R,2R*)-1-O-{2-(4-benzyl-1,2,3-triazol-1-yl)-3-[((*2S*)-3-benzylpropionic acid)-2-O-yl]-2-deoxy-β-D-galactosyl}-2-O-(α-L-fucosyl)-cyclohexane-1,2-diol sodium salt (103c).



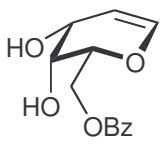
(2_038, 19/08/06)

^{1}H NMR (500 MHz, CD_3OD) : δ = 0.47 (m, 1H, diol-H6), 0.89-1.00 (m, 2H, diol-H4, diol-H5), 1.19 (d, 3H, $^{3}J_{5,6}$ = 6.4 Hz, Fuc-H6), 1.20-1.26 (m, 3H, diol-H3, diol-H5', diol-H4'), 1.53 (m, 1H, diol-H6'), 1.82 (m, 1H, diol-H3'), 2.73 (m, 2H, Lac-H3), 3.27-3.32 (m, 1H, diol-H2), 3.49 (m, 1H, diol-H1), 3.63 (t, 1H, $^{3}J_{5,6}$ = 5.8 Hz, Gal-H5), 3.67-3.70 (m, 2H, Fuc-H2, Fuc-H4), 3.73-3.83 (m, 3H, 2 Gal-H6, Fuc-H3), 3.97-4.01 (m, 3H, Lac-H2, 2 Tzl-C$\underline{H}_2$), 4.13 (s, 1H, Gal-H4), 4.25 (d, 1H, $^{3}J_{2,3}$ = 10.6 Hz, Gal-H3), 4.35 (t, 1H, $^{3}J_{1,2}$ = $^{3}J_{2,3}$ = 9.3 Hz, Gal-H2), 4.50 (q, 1H, $^{3}J_{5,6}$ = 6.4 Hz, Fuc-H5), 4.75-4.78 (m, 2H, Fuc-H1, Gal-H1), 6.84 (d, 2H, $^{3}J_{Ar}$ = 7.0 Hz, Ar-H) 7.12-7.19 (m, 4H, Ar-H), 7.23-7.25 (m, 4H, Ar-H), 7.35 (s, 1H, $C_2N_3\underline{H}$) ;

^{13}C NMR (125 MHz, MeOD) : δ = 16.7 (Fuc-C6), 23.7, 23.8, 29.4, 29.9 (4C, diol-C3-C6), 32.8 (Tzl-$\underline{C}H_2$, 40.1 (Lac-C3), 62.5 (Gal-C6), 64.7 (Gal-C2), 67.2 (Gal-C4), 67.4 (Fuc-C5), 69.9 (Fuc-C2), 71.5 (Fuc-C3), 73.8 (Fuc-C4), 76.2 (Gal-C5), 76.6 (diol-C2), 79.7 (diol-C1), 80.9 (Lac-C2), 81.6 (Gal-C3), 97.2 (Fuc-C1), 100.3 (Gal-C1), 125.8, 127.4, 127.5, 129.1, 129.6, 130.5 (11C, 10 Ar-CH, Tzl-CH), 138.1, 140.6 (2C, Ar-C quat.), 147.8 (Tzl-C quat.), 164.5 (acid), 176.6 (??).

Elemental analysis : Calculated for $C_{36}H_{46}O_{12}Na_1$ (+4H_2O): C, 52.48% ; H, 6.69%. Found : C, 53.23% ; H, 6.66%.

6-*O*-Benzoyl-D-galactal (115)



(1_055)

3,4,6-Tri-O-acetyl-D-galactal (**89**) (248 mg, 0.91 mol) and K_2CO_3 (13 mg, 0.09 mmol) were dissolved in aqueous MeOH (10 mL).

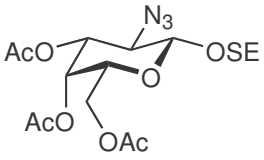
After stirring for 5h at r.t., solvent was evaporated to dryness. The residue was filtered quickly on a short silica gel column, (DCM / MeOH 9:1), yielding the deprotected D-galactal (**113**, 128 mg, 0.88 mmol) as a white solid.

The obtained product was dissolved under argon in a dry mixture of MeCN (6 mL) and Et_3N (2 mL), and cooled to –45°C. A solution of benzoyl cyanide (116 mg, 0.88 mmol) in MeCN (2 mL) was then added dropwise over 2h, and the mixture was let heat slowly to r.t. After 4h stirring, the reaction was quenched with MeOH (1 mL), and solvent was evaporated. The residue was taken up in DCM (15 mL), washed with aqueous HCl 1N (10 mL), aqueous saturated $NaHCO_3$ (10 mL) and with brine (5 mL), dried on Na_2SO_4 and concentrated. Chromatography on silica gel (DCM / MeOH 98:2) yielded **115** (88 mg, 39%) as an off-white solid.

(1_055, 15/03/04)

^{1}H NMR (500 MHz, $CDCl_3$) : δ = 4.00 (s, 1H, H-4), 4.24 (t, 1H, $^3J_{5,6}$ = 6.3 Hz, H-5), 4.44 (s, 1H, H-3), 4.56 (dd, 1H, $^3J_{5,6}$ = 7.1 Hz, $^2J_{6,6'}$ = 11.6 Hz, H-6), 4.68 (dd, 1H, $^3J_{5,6}$ = 5.8 Hz, $^2J_{6,6'}$ = 11.6 Hz, H-6), 4.76 (m, 1H, H-2), 6.33 (d, 1H, $^3J_{1,2}$ = 6.1 Hz, H-1).

3,4,6-Tri-*O*-acetyl-2-azido-2-deoxy-1-trimethylsilylethyl-β-D-galactose (119).



(1_034)

Silica gel supported silver silicate (470 mg) and activated powdered molecular sieve 4 Å (300 mg) were suspended under argon in a solution of trimethylsilylethanol (137 µL, 0.96 mmol) in dry DCM (5 mL). After stirring for 1h at r.t., bromide **91** (252 mg, 0.64 mmol) in DCM (5 mL) was added dropwise. The reaction mixture was stirred further at r.t. over 16h and filtered through Celite. The filtered solution was then washed with aqueous saturated $NaHCO_3$ (2x 10 mL) and water (2x 10 mL), dried on Na_2SO_4 and concentrated. Chromatography on silica gel (petrol ether / EtOAc 4:1) yielded **119** (91 mg, 33%) as a yellowish oil.

(1_034, 16/01/04)

^{1}H NMR (500 MHz, $CDCl_3$) : δ = 1.00-1.12 (m, 2H, -O-CH_2-C$\underline{H}_2$-$SiMe_3$), 2.04, 2.05, 2.15 (3s, 9H, C$\underline{H}_3$C=O), 3.63-3.69 (m, 2H, H-2, -O-C$\underline{H}$H-CH_2-$SiMe_3$), 3.84 (td, 1H, $^3J_{4,5}$ = 0.8 Hz, $^3J_{5,6}$ = 6.8 Hz, H-5), 4.02 (m, 1H, -O-CH$\underline{H}$-CH_2-$SiMe_3$), 4.10 (dd, 1H, $^3J_{5,6}$ = 7.1 Hz, $^2J_{6,6'}$ = 11.3 Hz, H-6), 4.19 (dd, 1H, $^3J_{5,6}$ = 6.5 Hz, $^2J_{6,6'}$ = 11.3 Hz, H-6'), 4.37 (d, 1H, $^3J_{1,2}$

= 8.0 Hz, H-1), 4.77 (dd, 1H, $^3J_{3,4}$ = 3.3 Hz, $^3J_{2,3}$ = 10.9 Hz, H-3), 5.49 (dd, 1H, $^3J_{4,5}$ = 0.8 Hz, $^3J_{3,4}$ = 3.3 Hz, H-4).

2,3,4-Tri-*O*-(*para*-bromobenzyl)-1-ethylthio-β-L-fucoside (120).

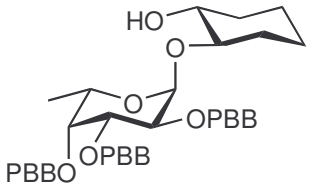
1_132

1-Ethylthio-L-fucoside (129 mg, 0.62 mmol) (**88**) was dissolved in dry DMF (5 mL) and cooled to 0°C under argon. De-oiled sodium hydride (90 mg, 3.75 mmol) was then added slowly. After 30 min stirring, *para*-bromobenzyl bromide (696 mg, 2.78 mmol) was added dropwise. After 16 h stirring at r.t., the reaction mixture was quenched with MeOH (1 mL), diluted with toluene (20 mL) and washed with water (2x 15 mL) and brine (10 mL). The organic layer was then dried on Na_2SO_4 and concentrated. Chromatography on silica gel (petrol ether and ethyl acetate 6:1) afforded **120** (353 mg, 80%) as an off-white solid.

(1_132, 16/11/04)

^{1}H NMR (500 MHz, $CDCl_3$) : δ = 1.23 (d, 3H, $^3J_{5,6}$ = 6.0 Hz, H-6), 1.29 (t, 3H, ^3J = 7.4 Hz, S-CH_2-C$\underline{H}_3$), 2.66-2.80 (m, 2H, S-C$\underline{H}_2$-CH_3), 3.49 (m, 2H, H-3, H-5), 3.58 (broad s, 1H, H-4), 3.74 (t, 1H, $^3J_{1,2}$ = $^3J_{2,3}$ = 9.3 Hz, H-2), 4.37 (d, 1H, $^3J_{1,2}$ = 9.6 Hz, H-1), 4.59-4.64 (m, 3H, C$\underline{H}_2$-Ph), 4.67 (A of AB, 1H, $^2J_{A,B}$ = 10.6 Hz, C$\underline{H}$-Ph), 4.83 (B of AB, 1H, $^2J_{A,B}$ = 10.6 Hz, C$\underline{H}$-Ph), 4.85 (B of AB, 1H, $^2J_{A,B}$ = 12.0 Hz, C$\underline{H}$-Ph), 7.10-7.24 (m, 6H, Ar-H), 7.42-7.45 (m, 6H, Ar-H).

(*1R*,*2R*)-2-*O*-(2,3,4-Tri-*O*-benzyl-α-L-fucosyl)-cyclohexane-1,2-diol (122).



(1_133)

Thioethyl 2,3,4-tri-*O*-(*para*-bromobenzyl)-L-fucoside (**120**) (353 mg, 0.49 mmol) was dissolved in dry DCM (5 mL), and cooled to 0°C under argon. Bromine (50 µL, 0.98 mmol) was added slowly at 0°C, then the mixture was heated slowly to r.t.. After 1 h, the remaining bromine was quenched using cyclohexene (1.0 mL, 30 min stirring at r.t.).

(*1R*,*2R*)-Cyclohexane-1,2-diol (**83**, 115 mg, 0.99 mmol) and tetraethylammonium bromide (208 mg, 0.99 mmol) were dissolved in DMF (2 mL). Activated powdered

molecular sieves 3 Å (200 mg) was suspended in the solution which then was stirred for 30 min under argon at r.t.. Then, the fucosyl bromide (**121**) solution was added dropwise to the suspension.

After stirring for 2h, the reaction mixture was neutralized with pyridine (0.5 mL), diluted with EtOAc (10 mL), and washed with saturated aqueous $NaHCO_3$ solution (2x10 mL) and water (2x10 mL). The organic layer was dried with Na_2SO_4 and concentrated. Chromatography on silica gel (petrol ether / ethyl acetate 1:1, with 0.1% Et_3N) yielded **122** (222 mg, 59%) as a white solid.

(1_133, 25/11/04)

^{1}H NMR (500 MHz, $CDCl_3$) : δ = 1.18 (d, 3H, $^3J_{5,6}$ = 6.5 Hz, Fuc-H6), 1.21-1.26 (m, 4H, diol), 1.57-1.74 (m, 2H, diol), 1.97-2.04 (m, 2H, diol), 3.26 (m, 1H, diol-H1), 3.46 (m, 1H, diol-H2), 3.66 (d, 1H, $^3J_{3,4}$ = 2.8 Hz, Fuc-H4), 3.90 (dd, 1H, $^3J_{3,4}$ = 2.8 Hz, $^3J_{2,3}$ = 10.1 Hz, Fuc-H3), 4.00 (dd, 1H, $^3J_{1,2}$ = 3.8 Hz, $^3J_{2,3}$ = 10.1 Hz, Fuc-H2), 4.09 (q, 1H, $^3J_{5,6}$ = 6.4 Hz, Fuc-H5), 4.57-4.61 (m, 2H, C<u>H</u>$_2$-Ph), 4.63 (A of AB, 1H, $^2J_{A,B}$ = 12.0 Hz, C<u>H</u>-Ph), 4.67 (B of AB, 1H, $^2J_{A,B}$ = 12.1 Hz, C<u>H</u>-Ph), 4.73 (B of AB, 1H, $^2J_{A,B}$ = 12.0 Hz, C<u>H</u>-Ph), 4.85 (B of AB, 1H, $^2J_{A,B}$ = 11.6 Hz, C<u>H</u>-Ph), 5.01 (d, 1H, $^3J_{1,2}$ = 3.8 Hz, Fuc-H1), 7.17-7.20 (m, 6H, Ar-H), 7.42-7.47 (m, 6H, Ar-H).

(1_133 25/11/04)

^{13}C NMR (125 MHz, $CDCl_3$) : δ = 16.7 (Fuc-C6), 24.0, 24.4, 30.1, 32.5 (4C, diol), 67.0 (Fuc-C5), 72.4, 73.3, 74.4 (3C, 3 C<u>H</u>$_2$-Ph), 76.2 (Fuc-C2), 78.2 (diol-C2), 79.0 (2C, Fuc-C3, Fuc-C4), 83.9 (diol-C1), 95.8 (Fuc-C1), 128.9, 129.5, 129.8 (6C, Ar-CH), 131.4, 131.5 (3C, Ar-C<u>C</u>Br), 137.3, 137.4, 137.6 (3C, Ar-C quat.).

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Lionel TSCHOPP
16, boulevard de l'Europe
68100 Mulhouse, FRANCE
Tel. : +33(0)3.89.43.74.98
+33(0)6.76.80.91.66
e-mail : lionel.tschopp@wanadoo.fr

Age 28, born on September 28th, 1978.
French.

Education :

2004-2006 : PhD in Medicinal Chemistry (9th of November 2006) at the University of Basle (CH). Director : Prof. Beat ERNST.
Subject : **Exploring the Binding Site of E-Selectin**.
The E-selectin protein is an actor of the mediation of inflammatory processes. During this doctoral work, a library of tetrasaccharide mimics was built, which are expected to be antagonists of E-selectin. The work consisted of organic synthesis (especially carbohydrate chemistry) as well as the extensive use of analytical tools (among them NMR and LC-MS). A place was also given to teaching, in the form of plenary conferences as well as practical courses towards pharmacy students (Solid phase synthesis of peptides, 400 h).

2002 : Master thesis in Medicinal Chemistry at F. Hoffmann-La Roche Ltd in Basle (CH). Director: Dr Guido Galley.
Subject : **Several aspects of Lead Generation Chemistry. Syntheses Development**.
γ-secretase plays a major role in the occurrence of Alzheimer's disease. Powerful inhibitors are in constant development, and the synthesis of three of them was studied and improved. A particular emphasis was given to Palladium catalysed cross-couplings, based on the Suzuki reaction as well as on Hartwig's work on amines. A good place was given to analytics (especially MS and NMR).

2002 : **Graduation as an Engineer in Chemistry** at the Ecole Nationale Supérieure de Chimie de Mulhouse (ENSCMu).

1999-2002 : 4 years preparation to the diploma of chemical engineer at the ENSCMu, including 12 months traineeship.

1999 : Licence de Chimie-Physique (License degree) specialising in Physical Chemistry

1997-98 : Classes Préparatoires : 2-year intensive course of Physics, Chemistry and Mathematics.

1996 : Baccalauréat S (scientific), specialising in Biology-Geology.

Languages : French : mother language
English / German : proficient (TOEIC : 885)
Arabic : basic knowledge (writing and reading)

Professional experience :

Nov. 2006 : Teacher in physics and chemistry : 4-month fixed term contract in the highschool LEGTAV (lycée d'enseignement général et technique agricole viticole) in Rouffach (68). Students from the 2nde to Tale levels.

2003 : 9 months traineeship at F. Hoffmann-La Roche Ltd in Basle (CH).
Work in gram lab : This specific lab is aimed to synthesize gram scale quantities of molecules for further studies in the various R&D departments. It was a good way to practice on very different reaction types and various techniques (involving gases, catalysis, microwave reactions, protections-deprotections, LC-MS, parallel flash chromatographies, etc.)

Feb. 2001 : 6 months traineeship at Clariant Ltd in Basle (CH).
Subject : **Tests on Reactive Dyes**.
Reactive dyes are a class of dyestuff binding covalently to fibres. Reaction time is therefore a parameter that has a dramatic influence on the final colour of a particular textile. Clariant scientists therefore designed a software predicting the optimal reaction times for a given dye at a given concentration, which predictions were then evaluated in mono-, di- or trichromies.

Aug. 2000 : 6 months traineeship at Novartis Pharma Ltd in Basle (CH).
Subject : **Capillary Electrophoresis applied to Purity Tests**.
In Capillary Electrophoresis (CE), the analysis process is driven mainly by the choice of the suitable electrolyte. However this choice is very broad, considering all the possibilities (aqueous or water-free, pH variation, addition of surfactants, etc.). Using three different formulations of marketed Novartis drugs (Bethamethasone, terbinafine base and Trileptal® 6% syrup), we succeeded to define standard CE conditions that could be applicable with few changes to very different mixtures. The work was done following GMP rules.

1997-2001 : Many jobs as youth leader and treasurer in youth centres.

Other abilities :

First Aid certificate

Full **driving licence**

Good **computer skills** : using software (SciFinder, Isisdraw, Chemdraw, MSOffice, Visual Basic) and programming.

BAFA (youth leader competence) certificate

Sports & Hobbies :

- Archery and cycling (mountain bike).
- Reading (scientific journals, french classics, science-fiction).
- Musician, playing the flute and the guitar.