E- and P-Selectin:
Differences and Similarities Guide the Development of Novel Selectin Antagonists

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Abstract

Selectins, namely E-, P-, and L-selectin, are carbohydrate-recognizing proteins that mediate the initial step of leukocyte recruitment to sites of inflammation. This vital process can turn deleterious in case of acute and chronic states of inflammation like stroke, reperfusion injury, and psoriasis or rheumatoid arthritis, respectively. In addition, cancer cells have been shown to exploit this selectin mediated pathway to metastasize. Blocking of the selectins is consequently considered a promising therapeutic approach.

The tetrasaccharide sialyl Lewis^x (sLe^x) was identified as the minimum binding epitope of all three selectins and became the lead structure for various drug discovery programs. SLe^x itself suffers from the typical downsides of carbohydrate leads, namely complex structure and synthesis, and poor pharmacokinetic and pharmacodynamic properties, which impede the development of selectin antagonists. The rational design of antagonists is furthermore hampered by the lack of information on the thermodynamics of the selectin-ligand interactions. However, these information are of vital importance for successful lead optimization.

Inter alia, this thesis addresses these major issues in the design of glycomimetic selectin antagonists.

- A fast and efficient synthetic route to the D-Glc*N*Ac mimic (1*R*,2*R*,3*S*)-3-methylcyclohexane-1,2-diol was developed, which allows the multigram scale synthesis of this key intermediate (chapter 2.2.).
- Similarities and differences between E- and P-selectin were exploited to develop less polar, structurally simplified P- and E-selectin antagonists with increased binding affinity compared to sLe^x (chapter 2.3.).
- A series of glycomimetic amides and sulfonamides was developed to target a hitherto unexplored binding pocket of P-selectin. The structurally simplified, and non-charged mimetics exhibited up to threefold higher binding affinities than sLe^x (chapter 2.4.).
- A synthetic route to derivatives of 2,2-dialkyl-2-*O*-glycosyl glycolic acid was developed and the resulting glycomimetics were tested as E-selectin antagonists (chapter 2.5).
- Thermodynamic binding parameters of sLe^x and E-selectin antagonists were analyzed. It was found that a combination of reduced polarity of weakly binding residues and enhanced pre-organization is the key to overcome enthalpy entropy compensation (chapter 2.6.).

- Literature known antagonists were synthesized to evaluate their potential in established in-house assays (chapter 2.7.).

Abbreviations

AcOH	Acetic acid
aq.	Aqueous
Ar	Aryl
ax	Axial
bb	Backbone
cat.	catalytic amount
CR	Complement regulatory-like domains
CRD	Carbohydrate recognition domain
CSA	Camphor sulfonic acid
Cy	Cyclohexyl
d	Days
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
DCE	1,2-Dichlorethane
DDQ	2,3-Dichloro-5,6-dicyano-1,4-benzoquinone
DIC	*N,N*'-Diisopropylcarbodiimide
DMAP	4-Dimethylaminopyridine
DME	Dimethoxyethane
DMF	*N,N*-Dimethylformamide
DMSO	Dimethylsulfoxide
DMTST	Dimethyl(methylthio)sulfonium triflates
dppf	1,1′-Bis(diphenylphosphino) ferrocene
DTBMP	2,6-Di-tert-butyl-4-methylpyridine
DTBP	2,6-Di-tert-butylpyridine
ee	Enantiomeric excess
EGF	Epidermal growth factor
eq	Equivalent
ESL-1	E-selectin ligand 1
FBDD	Fragment based drug discovery
Fuc	Fucose
Gal	Galactose
Glc*N*Ac	*N*-Acetylglucosamine
Glc	Glucose
Gly-CAM-1	Glycosylation-dependent cell adhesion molecule-1
h	Hour(s)
HAc	Acetic acid
HBtU	*O*-(Benzotriazol-1-yl)-*N*,*N*,*N*',*N*'-tetramethyluronium-hexafluoro-phosphate
HEV	High endothelial venules
HOBt	N-Hydroxybenzotriazol
HPLC	High performance / pressure liquid chromatography
IC_{50}	Inhibitory concentration 50%
ICAM-1	Intercellular cell adhesion molecule 1
Ig	Immunglobulin
IL-1	Interleukine-1
IL-8	Interleukine-8
IR	Infrared spectroscopy
kDa	Kilo Dalton
Lac	Lactic acid
LAD	Leukocyte adhesion deficiency
Le^a	Lewis^a
Le^x	Lewis^x
LPS	Lipopolysaccharide
mAb	Monoclonal antibody
MadCAM-1	Mucosal vascular addressin cell adhesion molecule 1
MAN	
MC	Monte-Carlo
MCPBA	Meta chloroperbenzoic acid
MD	Molecular dynamics
min	Minute(s)
mol.	Molecular
MS	Mass spectrometry
mW	Microwave (heating)
NBS	*N*-Brom succinimide
Neu5Ac	*N*-Acetyl neuraminic acid, sialic acid
NHS	*N*-Hydroxysuccinimide
NMR	Nuclear magnetic resonance
NOE	Nuclear Overhauser effect
Nu	Nucleophile
o.n.	Overnight
pdb	Protein data bank
PCC	Pyridinium chlorochromate
PE	Petrol ether
pg	Protecting group
PSGL-1	P-selectin glycoprotein ligand 1
p-TsOH	p-Toluenesulfonic acid
py	Pyridine
rac	Racemic
rIC_{50}	Relative IC_{50}
RT	Room temperature
s	seconds
SAR	Structure-activity relationship
satd.	Saturated
SCR	Short consensus repeats
Sia	Sialic acid
sLe^a	Sialyl Lewis^a
sLe^x	Sialyl Lewis^x
sc	Side chain
SEC	Size exclusion chromatography
SPR	Surface plasmon resonance
SSL	Staphylococcal superantigen-like protein
STD	Saturation transfer difference
TBAB	Tetrabutylammonium bromide
TBAF	Tetrabutylammonium fluoride
TBAHS	Tetrabutylammonium hydrogensulfate
TBS	*tert*-Butyldimethylsilyl
TBSOTf	*tert*-Butyldimethylsilyl triflate

TEMPO	2,2,6,6-Tetramethylpiperidine-1-oxyl
TES	Triethylsilane
Tf	Triflate, (triflouromethanesulfonate)
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
TIS	Triisopropylsilane
TLC	Thin-layer chromatography
TMEDA	*N*,*N*,*N*′,*N*′-Tetramethyl ethylenediamine
TMS	Trimethylsilyl
TMSE	Trimethylsilylethyl
TMSOTf	Trimethylsilyl triflate
TNF-α	Tumor necrosis factor α
Trt	Trityl
Ts	Tosyl
TsCl	Tosyl chloride
VCAM-1	Vascular cell-adhesion molecule 1

Table of Contents

1 Introduction

1.1 Structure and function of selectins

1.1.1 Structure of selectins

The selectins, namely E-, P-, and L-selectin,[a] are cell adhesion molecules that mediate the adhesion of leukocytes and platelets to vascular surfaces. They are classified as C-type lectins, since they recognize carbohydrate ligands in a Ca^{2+}-dependent manner. Overall, selectins share about 50% sequence homology. They are composed of a N-terminal carbohydrate recognition domain (CRD), also called lectin domain, an epidermal growth factor like domain (EGF), several short consensus repeats (SCR), a transmembrane domain and a cytoplasmic tail (Figure 1.1.1).[1]

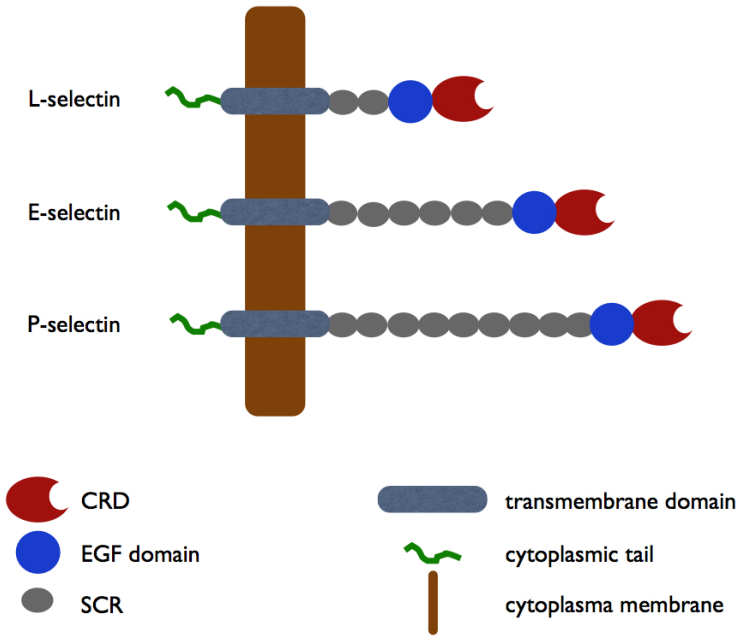

Figure 1.1.1 Schematic presentation of the selectins.

The lectin domain hosts the binding epitope for physiological carbohydrate ligands. It is conformationally stabilized by a Ca^{2+} ion, which is required for recognition.[1] Though the exact function of the EGF domain is not completely clear yet, it is essential for the binding process and is involved in binding either directly, or by allosteric modulation of the CRD.[2] Recent discussions also propose its role in the catch bond behavior of selectins (see section

[a] The selectins have alternative names: E-selectin: CD62E, ELAM-1, LECAM-2; P-selectin: CD62P, LECAM-3; L-selectin: CD62L, LAM-1, LECAM-1.

1.2.2). SCRs serve as a spacer between the lectin domain and the cell membrane to reach through the glycocalix and allow cell-cell interactions. Their number differ between the selectins and different species. Human L-selectin contains two, E-selectin six, and P-selectin nine SCRs.[3] The transmembrane domain anchors the selectins to the cell membrane.[4,5] The final cytoplasmic tail is involved in signal transduction.[6]

1.1.2 Natural selectin ligands

The natural selectin ligands are glycoproteins and glycolipids with typically sialylated and fucosylated glycan epitopes. The tetrasaccharides sialyl Lewis^x (sLe^x) and sialyl Lewis^a (sLe^a) were identified as common carbohydrate motifs recognized by all three selectins (Figure 1.1.2).[7,8] Furthermore, sulfated analogs of sLe^x were reported to act as selectin ligands.[9]

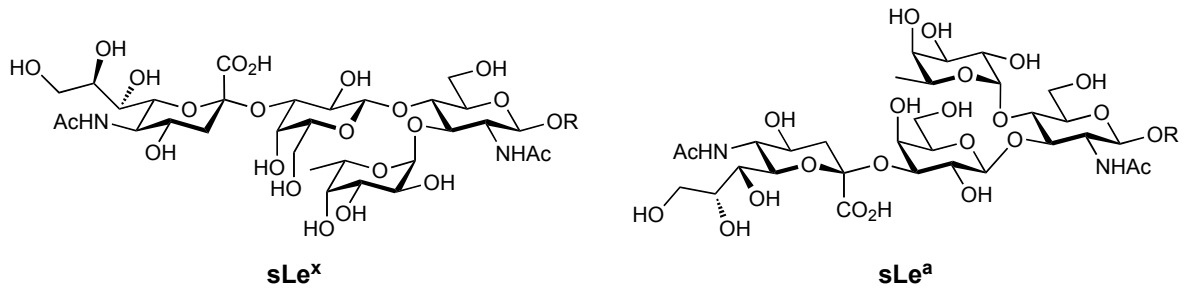

Figure 1.1.2 Common carbohydrate motifs recognized by all three selectins.

Although all selectins share common carbohydrate binding motifs, they differ in their ligand specificity (Figure 1.1.3).

The most important ligand to **P-selectin** is the 250 kDa, homodimeric type-I transmembrane P-selectin glycoprotein ligand 1 (PSGL-1),[10] which also binds to E- and L-selectin, but with different affinity and kinetics.[11] Besides the sLe^x moiety which is essential for recognition, PSGL-1 bears three sulfated tyrosine residues which ensure high binding affinity to P-selectin (see section 1.3.).

Glycoproteins identified as ligands to **L-selectin** comprise MadCAM-1,[a] [12] CD34,[b] [13] endomucin,[14] endoglycan,[15] podocalyxin-like protein,[16] PSGL-1,[15] and the sialo-mucin GlyCAM-1,[c] [17] which is the best characterized L-selectin ligand to date. GlyCAM-1 is

[a] Mucosal vascular addressin cell adhesion molecule 1
[b] Cluster of differentiation molecule 34
[c] Glycosylation-dependent cell adhesion molecule-1

described as regulatory protein involved in the recruitment of lymphocytes into peripheral lymphnodes.[3]

In contrast to P- and L-selectin, **E-selectin** does not require sulfation of ligands.[7,18] E-selectin ligand-1 (ESL-1), is a non-sulfated glycoprotein, which lacks binding to P- and L-selectin.[19] Furthermore, E-selectin binds to PSGL-1 and to carbohydrate structures on L-selectin of human neutrophils.[20]

Besides these glycoproteins, P- and L-selectin, but not E-selectin, recognize various polyanions like sulfatides, fucoidan and heparin. These are bound in a Ca^{2+} independent manner by a second binding site rich in positively charged amino acids (see section 1.3).

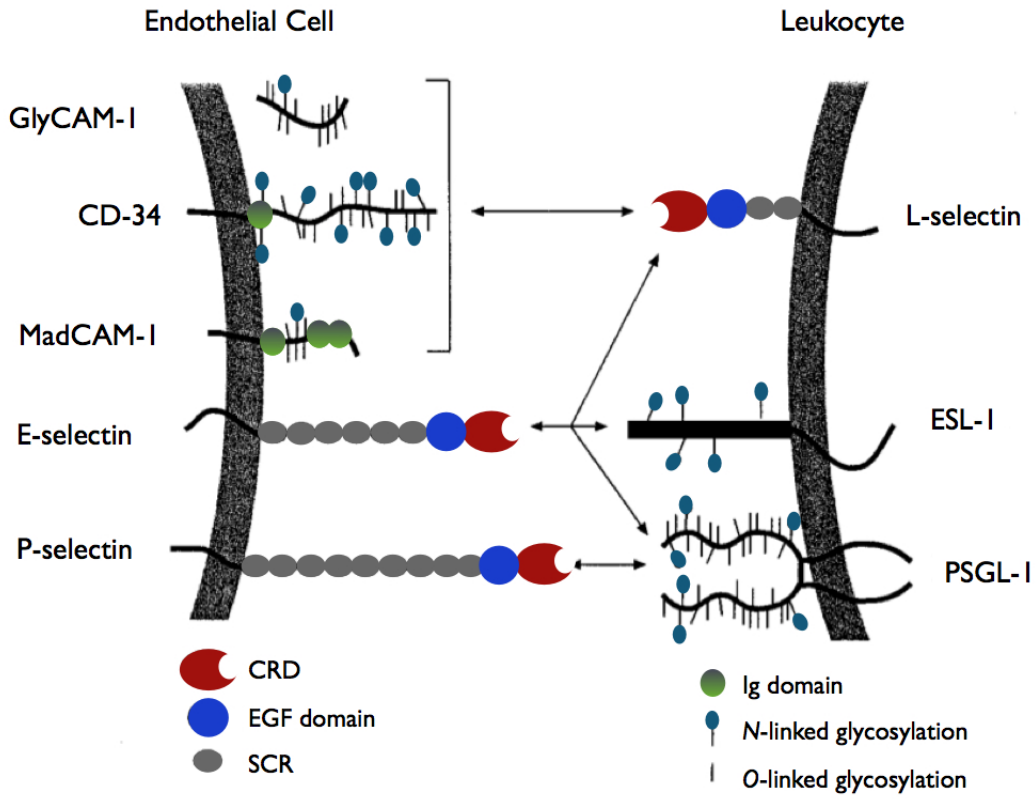

Figure 1.1.3 The selectins and their natural glycoprotein ligands (adapted from [3]).

1.1.3 Physiological role of selectins

Inflammation is a vital defense and protection mechanism in case of tissue injury and invasion of pathogens, which is characterized by the invasion of leukocytes from the bloodstream to the site of inflammation. Selectins are key players of this multistep process, which is described by the inflammatory cascade (Figure 1.1.4).[21]

Upon an **inflammatory stimulus**, pro-inflammatory mediators are released which stimulate endothelial cells of post-capillary venules to display E- and P-selectin on their surface.

Circulating leukocytes interact with the endothelium *via* selectin ligands like PSGL-1 and ESL-1, and are slowed down. The resulting **rolling** of leukocytes along the endothelial layer allows for the cytokine mediated activation of β2-integrins on the leukocytes.[22] Integrins interact with endothelial ligands from the IgG superfamily like ICAM-1[a] and VCAM-1[b], which leads to **firm adhesion** and finally to **extravasation** and migration of the leukocytes to the site of inflammation.[22]

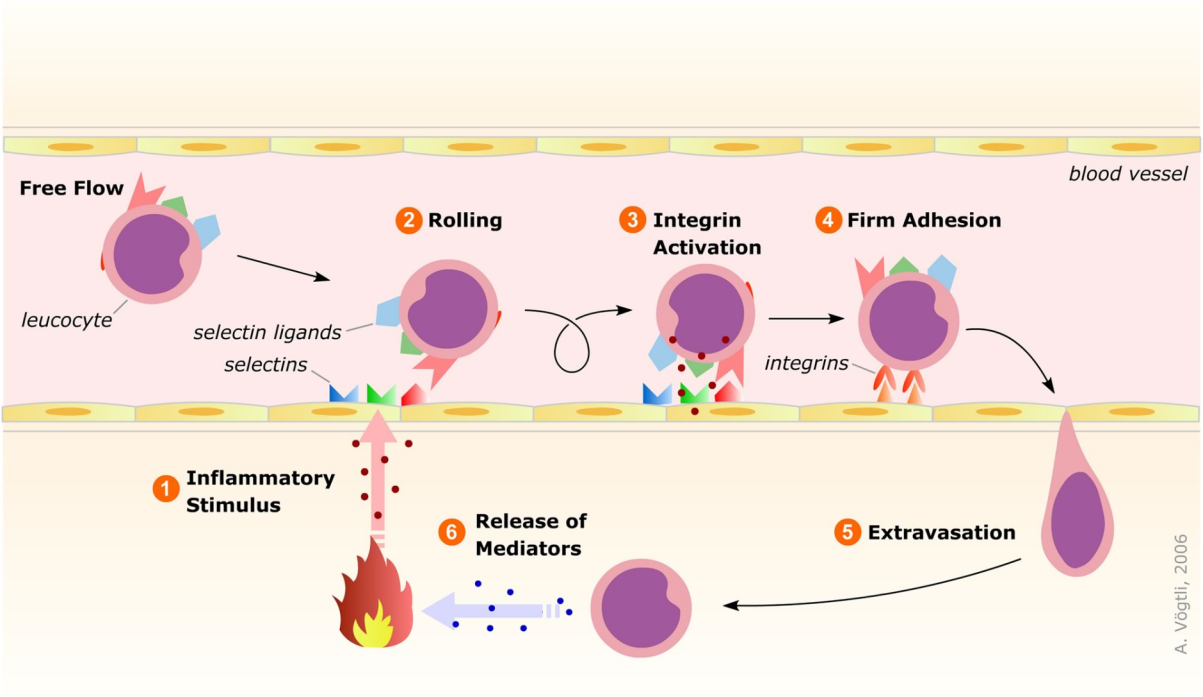

Figure 1.1.4 Schematic presentation of the inflammatory cascade (by courtesy of A. Vögtli).

Though all three selectins have been shown to mediate rolling of leukocytes,[3] they differ in their function and in their spatial and temporal expression.[21,23]

L-selectin is constitutively expressed on most **l**eukocytes and primarily serves as lymphocyte homing receptor, *i.e.* it mediates the attachment of lymphocytes to high endothelial venules (HEV) of peripheral lymph nodes, and thereby allows lymphocyte recirculation.[23] Besides, L-selectin is involved in the "secondary tethering" of free-flowing leukocytes to already adherent ones, which allows the recruitment of further leukocytes to sites of inflammation. This process is mediated by the L-selectin-PSGL-1 interaction.[24]

E-selectin is exclusively expressed on stimulated **e**ndothelial cells after *de novo* synthesis, three to four hours after activation. Its expression is stimulated by transcription factors such

[a] Intercellular cell adhesion molecule 1
[b] Vascular cell adhesion molecule 1

as tumor necrosis factor α (TNF-α), interleukin-1 (IL-1) and lipopolysaccharides (LPS).[25] It mediates slow rolling of leukocytes, which is the prerequisite for further activation of leukocytes by chemo-attractants.[26]

P-selectin is constitutively stored in α-granules of **p**latelets and Weibel-Palade bodies of endothelial cells. Upon stimulation by thrombin, histamine, or other agonists, it is translocated to the cell surface within minutes.[27] In addition, LPS, TNF-α, and IL-1 can induce *de novo* synthesis of P-selectin, which leads to its expression on the cell surface two to four hours later.[28] P-selectin is thought to initiate the capturing of leukocytes and to mediate the fast rolling of leukocytes.

The physiological relevance of selectins has been confirmed by several knockout experiments. L-selectin deficient mice show reduced leukocyte rolling and defects in lymphocyte homing.[29] Mice deficient in P-selectin[30] and mice deficient in P- as well as E-selectin[31] exhibit elevated levels of neutrophils in the blood. Even more, P- and / or E-selectin deficient mice suffered from increased mortality after infection with *Streptococcus pneumoniae*.[32] The relevance of selectins for the human immune system was first evidenced by cases of the leukocyte adhesion deficiency syndrome type 2 (LAD-2). This rare genetic disorder is caused by a mutation in the GDP-fucose transporter gene, leading to a lack of fucosylation in glycoconjugates, which goes along with reduced rolling of leukocytes. LAD-2 is characterized by recurrent infections, mental retardation, and the Bombay Blood group.[33]

1.1.4 Pathophysiological role of selectins

Besides their fundamental role in physiological processes, selectins are also involved in a plethora of severe disease states. In these cases, the actions of leukocytes are misdirected, as they no longer only fight bacteria and decompose damaged tissue, but also cause injury and breakdown of healthy cells.[34] A hallmark of many acute and chronic inflammatory diseases is the excessive recruitment of leukocytes to inflamed tissue. Examples of these diseases include asthma,[35] rheumatoid arthritis,[36] reperfusion injury,[37] and host versus graft disease[38]. Using antibodies, recombinant selectin counter-receptors or low molecular weight antagonists, selectins have been evaluated as promising pharmacological targets to tackle theses diseases.[39]

Furthermore, there is growing evidence that cancer cells, coated with sLe^x and sLe^a exploit the selectin mediated inflammatory pathway to escape the bloodstream and metastasize.[40,41]

The pathophysiological role of selectins and their relevance as pharmacological targets is covered by several excellent reviews: [34,39,41-43]

1.2 Binding properties of selectins

1.2.1 Kinetic properties

Given the physiological role of selectins, namely the transient tethering of fast flowing leukocytes to endothelial cells, relatively weak binding and fast binding kinetics are to be expected. Indeed, surface plasmon resonance (SPR) experiments revealed fast association and dissociation kinetics for all three selectins.

For the interaction of E-selectin and ESL-1, Vestweber and coworkers found a dissociation rate constant k_{off} of 4.6 s^{-1} and calculated an association rate constant k_{on} of 7.4 $\cdot 10^4$ $M^{-1}s^{-1}$. The dissociation constant K_D was determined to be 62 μM. Given the temperature independence of the binding affinity, they concluded that the interaction between ESL-1 and E-selectin must be entropy driven.[44] With a K_D of 0.3 μM, the P-selectin-PSGL-1 interaction displays the highest affinity of the measured selectin-ligand interactions. Association was very fast with a k_{on} of 4.4 $\cdot 10^6$ $M^{-1}s^{-1}$. The k_{off} was 1.4 s^{-1}.[45] The kinetics of the L-selectin-GlyCAM-1 interaction were too rapid for precise measurements. Nevertheless, it was shown that the k_{off} was $\geq 10\ s^{-1}$ and the k_{on} was $\geq 10^5\ M^{-1}s^{-1}$, with a K_D of 108 μM.[46]

The lower k_{on} of the E-selectin-ESL-1 interaction compared to the k_{on} of the P-selectin-PSGL-1 interaction points to different roles of the two selectins. It is assumed that P-selectin mediates the initial capturing of leukocytes, while E-selectin probably serves to further strengthen the contact with the endothelium.[44] This reasoning is supported by the relatively poor ability of E-selectin to capture free-flowing leukocytes.[23]

1.2.2 Catch bond behavior of selectins

It has been observed that rolling of all three selectins requires a minimum shear threshold to become efficient.[47] With increasing shear, higher numbers of leukocytes tether and roll until a peak is reached beyond which the rolling of leukocytes decreases again. This observation was rationalized by increased formation of bonds between leukocytes and endothelial cells with increasing shear[48] and a catch bond behavior of selectins. In contrast to slip bonds, catch bonds initially get stronger with increasing tensile force before they break, a finding that was demonstrated for the P-selectin/PSGL-1 interaction using atomic force microscopy.[49] Two models have been developed to explain this counterintuitive binding behavior, an allosteric model and a sliding rebinding model.

The allosteric model proposes a single binding site that can adopt a high affinity and a low affinity state which can be modulated allosterically by the lectin-EGF interface.[50] This model is based on the two different conformations of P-selectin observed in crystal structures. Apo P-selectin (pdb code 1g1q) and P-selectin soaked with sLe^x (1g1r) favor a bent conformation while P-selectin cocrystallized with a PSGL-1 fragment (1g1s) adopts an extended conformation.[51] These two conformations do not only differ in the orientation of the EGF domain relative to the lectin domain, but also in the lectin domain itself (Figure 1.2.1; more detailed information is given in section 1.2.4).

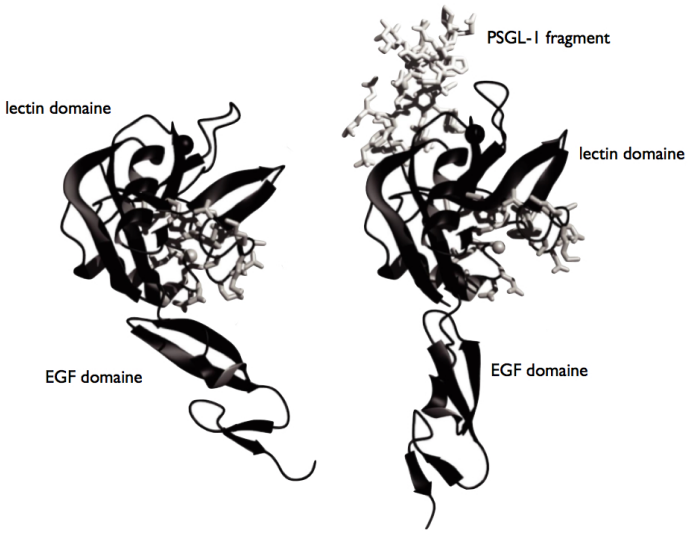

Figure 1.2.1 Bent conformation of apo-P-selectin (left) and extended conformation of P-selectin cocrystallized with a PSGL-1 fragment (right). Figure adapted from [50]

Binding studies with a glycan wedge at the interface of the lectin- and the EGF-domain, which stabilizes the extended conformation of P-selectin, revealed a 5 fold increased affinity towards PSGL-1.[52] The same effect on binding affinity was observed upon mutation of an amino acid in the lectin domain intended to favor the extended, high affinity conformation.[50] The authors concluded that the high and low affinity state are in an equilibrium, which is shifted to the high affinity state upon directional force.[50]

In contrast, in the sliding rebinding model, ligand binding does not induce a conformational change in the lectin domain. Instead, it is proposed that force favors the extended conformation which enables alignment of ligand and selectin parallel to the applied force, and thereby allows the ligand to slide along the selectin surface from one binding site to the next.[53]

Although more data is needed to verify the allosteric model, allosteric modulation of selectin affinity might become a powerful tool for the design of selectin antagonists that overcome the typical drawbacks of competitive selectin antagonists.

1.2.3 Binding of sLe^x to E- and P-selectin

SLe^x was identified as the minimum binding epitope of all three selectins[54] and consequently was the lead for most small molecule selectin antagonists developed to date.[55] Towards both E- and P-selectin, it exhibits only weak binding of 0.3 to 1.1 mM and 7 to 9 mM, respectively.[56]

Initial structure activity relationship (SAR) studies mainly focused on E-selectin and allowed for the identification of the pharmacophoric groups described in Figure 1.2.2. Various studies suggested that the D-Glc*N*Ac moiety is not directly involved in binding,[57] but rather acts as a spacer ensuring the right spatial orientation of L-fucose (L-Fuc) relative to D-galactose (D-Gal).[58] The saturation transfer difference NMR (STD-NMR) pattern of sLe^x on E-selectin agreed with the identified pharmacophoric groups, giving strong signals for groups involved in binding and only weak signals for protons of *N*-acetyl-D-glucosamine (D-Glc*N*Ac) and sialic acid.[59]

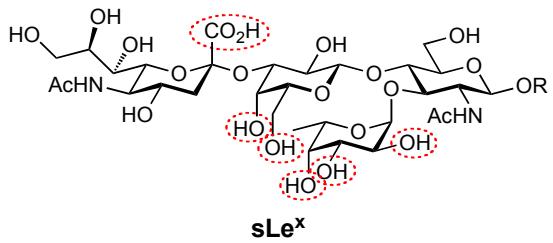

Figure 1.2.2 Pharmacophoric groups of sLe^x identified by SAR studies: hydroxyl of L-fucose,[51,60,61] hydroxyl groups in 4- and 6- position of D-galactose,[62-64] and the carboxylic acid residue of sialic acid[61].

Only limited SAR data is available for the P-selectin-sLe^x interaction. Brandley *et al.* reported that the 3-hydroxyl group of L-Fuc is essential, while either the 2- or the 4-hydroxyl group can be removed without loss in affinity. He further reported, that the carboxylate of sialic acid could be replaced by different charged groups resulting in comparable affinities.[61] Hasegawa and coworkers studied the binding of various deoxy sLe^x gangliosides to P-selectin and found, that the 6-hydroxyl group of D-Gal is essential, while the 4-hydroxyl group of D-Gal was dispensable.[64]

The conformation of sLe^x bound to E- and P-selectin, and consequently the pharmacophore of sLe^x, was deduced from several NMR studies.[56,65,66] The conformation of sLe^x bound to E-selectin found by Scheffler *et al.*[65,67] was confirmed subsequently by the crystal structures of E- and P-selectin in complex with sLe^x.[51] These crystal structures, solved by Somers and Camphausen in 2000, were also in excellent agreement with the pharmacophoric groups identified earlier and finally gave a precise picture of the interactions at molecular level (Figure 1.2.3 and Figure 1.2.4).

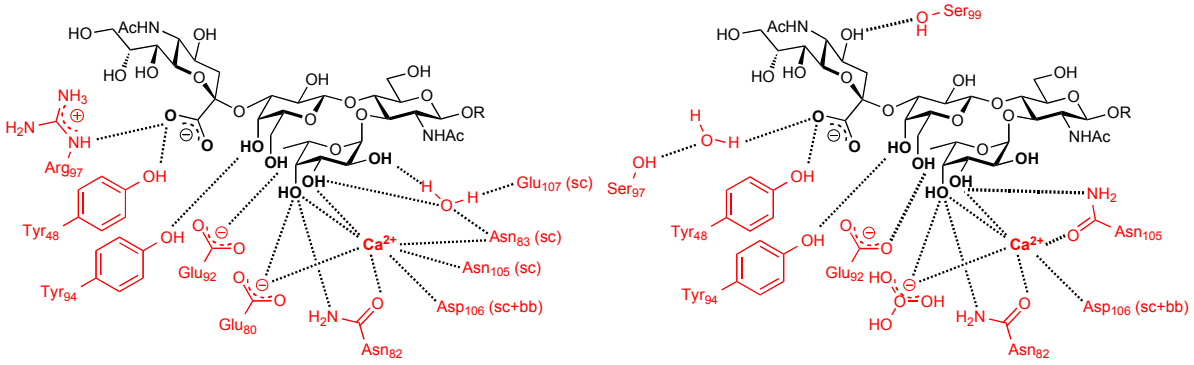

Figure 1.2.3 Schematic presentation of interactions between sLe^x and E-selectin (left) and sLe^x and P-selectin (right) as observed in the crystal structures.[51] sc = side chain; bb = backbone.

The contacts observed in the crystal structures can be summarized as follows.

E-selectin: The hydroxyl groups 3 and 4 of L-Fuc directly coordinate to Ca^{2+} and are involved in further hydrogen bonding with protein side chains coordinating to Ca^{2+}. The hydroxyl group 2 of L-Fuc forms water mediated hydrogen bonds to the side chains of Asn83 and Glu107. The hydroxyl groups 4 and 6 of D-Gal bind to the side chains of Tyr94 and Glu92, respectively. The carboxylate of the sialic acid moiety forms a hydrogen bond to the side chain of Tyr48 and a salt bridge to the side chain of Arg97. The guanidinium moiety of Arg97 furthermore binds to the oxygen of the glycosidic bond between D-Gal and sialic acid.

P-selectin: The hydroxyl groups 3 and 4 of L-Fuc directly coordinate to Ca^{2+} and are involved in further hydrogen bonding with protein side chains coordinating to Ca^{2+}. In contrast to E-selectin, Asn83 does not coordinate to Ca^{2+}, and does not mediate the hydrogen bond network between water, Glu107, and L-Fuc. Identically to E-selectin, the hydroxyl groups 4 and 6 of D-Gal bind to the side chains of Tyr94 and Glu92, respectively. One of the major differences between the two proteins is the mutation of Arg97 in E-selectin to Ser97 in P-selectin, which goes along with the loss of a charge-charge interaction and a favorable hydrogen bond. It is assumed that this mutation is one of the major reasons for the approximately tenfold lower binding affinity to P-selectin compared to E-selectin.

Furthermore, a hydrogen bond between Ser99 and the 4-hydroxyl group of sialic acid is postulated for the P-selectin-sLe^x complex.

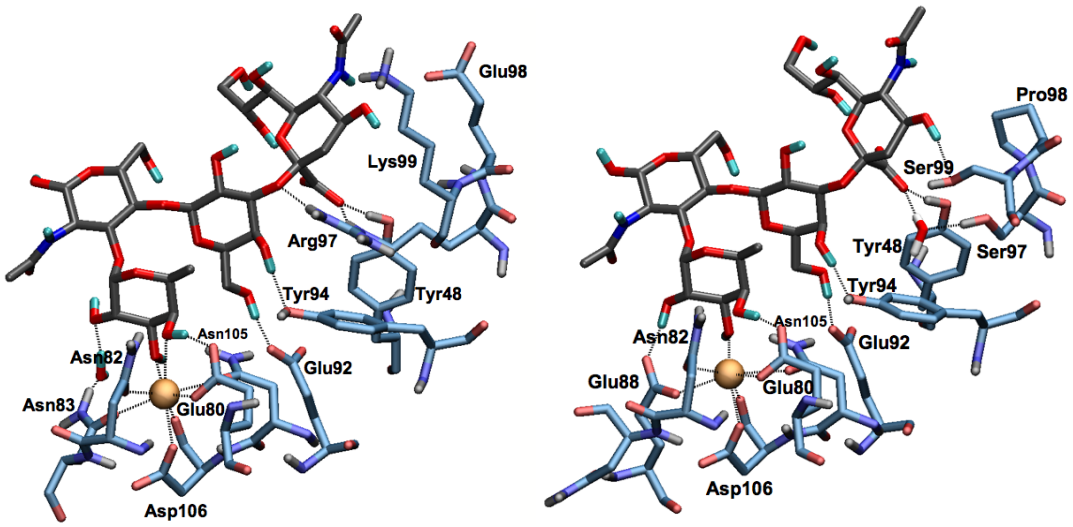

Figure 1.2.4 SLe^x bound to E-selectin (left) and sLe^x bound to P-selectin (right).[51]

Overall, the core of sLe^x (Gal(β1-4)[Fuc(α1-3)]Glc*N*Ac) is coordinated almost identically by both proteins, which can be rationalized by the highly conserved amino acid sequence in this part of the binding site (Figure 1.2.4).[68] The high degree of similarity is also reflected in the nearly identical conformation of sLe^x bound to P- and E-selectin. Differences, however, exist in the protein epitopes near sialic acid. The side chains of Arg97, Glu98, Lys99, and Asp100 in E-selectin form a rather wide pocket, which is stabilized by the guanidine moiety of Arg97 stacking against Tyr94 and interacting with Asp100. Ser97, Pro98, Ser99, and Ala100 of P-selectin in contrast form a binding pocket stabilized by a highly ordered H-bond network.

Comment on the crystal structures:

Apo-E-selectin had already been crystallized by Graves *et al.* in 1994.[69] The crystal structure solved by Somers *et al.* is nearly identical with this earlier one.[51] It is important to note that the sLe^x-P-selectin complex and the sLe^x-E-selectin complex were both formed by soaking sLe^x into preformed crystals of E- and P-selectin.

1.2.4 Binding of PSGL-1 to P-selectin

The physiological ligand to P-selectin, PSGL-1 binds to P-selectin with a K_D of approximately 0.3 μM,[45] which is about 20 000 fold stronger than the binding affinity of sLe^x alone.[56] While various publications point out the importance of tyrosine sulfation for high affinity binding,[70] Somers *et al.* found that non-sulfated PSGL-1 binds already 200 fold

stronger than sLe^x,[51] suggesting that the protein part of PSGL-1 significantly contributes to the increase in binding affinity.

A highly truncated form of PSGL-1, SGP-3, was used for crystallization with P-selectin, since PSGL-1 itself was considered too complex for co-crystallisation.[51] The SGP-3 construct comprises the 19 N-terminal aminoacids of mature PSGL-1 including sLe^x modified Thr16 and the three tyrosine sulfates (Tys6, Tys8, Tys11) essential for high affinity binding. In SPR experiments with SGP-3 and P-selectin kinetics almost identical to the one of a soluble recombinant form of PSGL-1 were observed, providing evidence that SGP-3 is a functional mimetic of full-length PSGL-1. [51]

The crystal structure of SGP-3 and P-selectin revealed a combination of hydrophobic and electrostatic interactions for the protein part of SGP-3 and P-selectin. Tyrosine sulfates were found to bind in a region of positive electrostatic potential, while the sLe^x part bound to the same epitope as in the sLe^x-P-selectin complex. Nevertheless, striking differences were found when comparing the overall conformations of P-selectin bound to SGP-3 with the ones of apo-P-selectin and sLe^x-P-selectin (Figure 1.2.5 and Figure 1.2.6).

Firstly, the loop formed by Asn83 to Asp89 is moved near the Ca^{2+} site upon binding of PSGL-1, and thereby allows additional interactions. Glu107 now forms a hydrogen bond to the 2-hydroxyl group of L-Fuc, and Glu88 simultaneously coordinates Ca^{2+} and binds to L-Fuc (Figure 1.2.5). Furthermore, Arg85 is now involved in hydrogen bonds to Tys10 and Pro14 of SGP-3. Secondly, the Arg54-Glu74 loop is moved. Thirdly, the orientation of the lectin domain relative to the EGF domain is changed. This last observation supports the allosteric model for the catch bond behavior of selectins (see section 1.2.2).[50] The authors concluded that P-selectin exists in two conformational states, a high-affinity state and a low affinity state.[51] However, one should take into consideration that the observed conformational changes might be an artifact caused by the crystallisation conditions.[51]

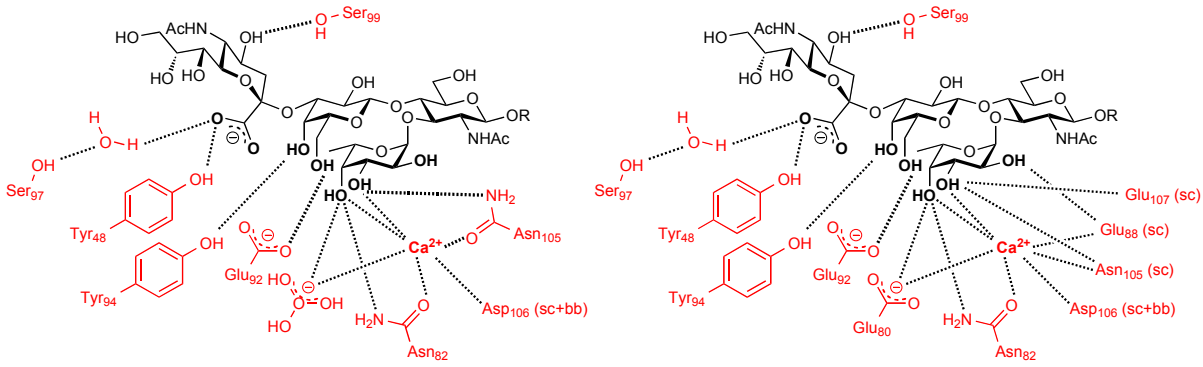

Figure 1.2.5 Schematic representation of the interactions between sLe^x and P-selectin (left) and sLe^x-SGP-3 and P-selectin (right) as observed in the crystal structures.[51] sc = side chain; bb = backbone.

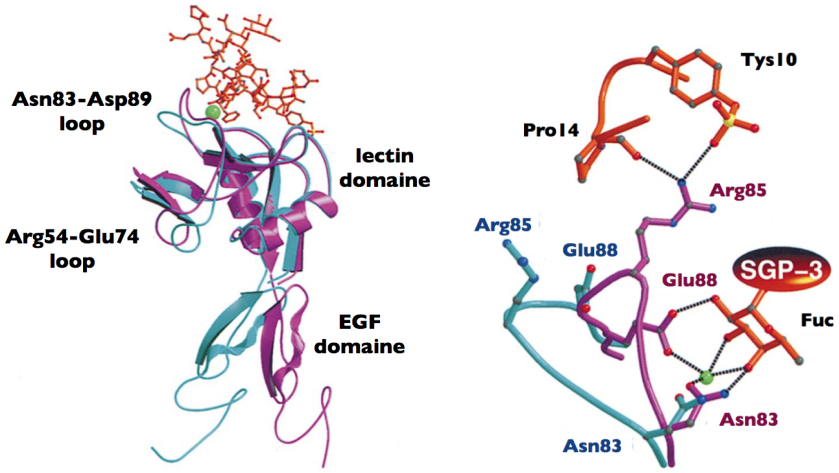

Figure 1.2.6 Left: superposition of apo-P-selectin (blue) and P-selectin in complex with SGP-3 (purple and orange). Right: zoom into the shift of the Asn83-Asp89 loop. Adapted from Somers *et al.*[51]

Comment on the crystal structures:

In their publication describing the crystal structures of E- and P-selectin, Somers and Camphausen claim that the movement of the loop defined by Asn83 to Asp89 enables Asn83 to coordinate to Ca^{2+} and L-Fuc simultaneously (Figure 1.2.6). A closer look at the crystal structure submitted at the RCSB protein data bank (1g1s) in contrast reveals that Asn83 does not form this claimed interaction, but rather stabilizes the loop *via* a hydrogen bond to the backbone of Glu88 (Figure 1.2.7).

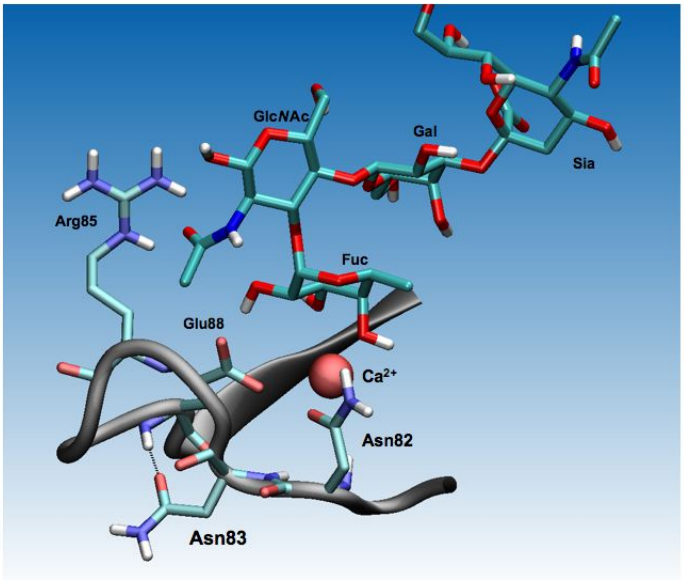

Figure 1.2.7 Conformation and interaction of Asn83 as observed in the crystal structure 1g1s.

1.3 E- and P-selectin: differences, similarities and implications for the design of P-selectin antagonists

A review on selectin antagonists was published in CHIMIA. The main focus of this article is set on P-selectin antagonists and how their development is influenced by structural differences and similarities between E- and P-selectin.

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E- and P-Selectin: Differences, Similarities and Implications for the Design of P-Selectin Antagonists

Florian P. C. Binder§* and Beat Ernst

§SCS Metrohm Prize for best oral presentation

Abstract: Selectins form a family of Ca^{2+}-dependent carbohydrate binding proteins that mediate the initial step of leukocyte recruitment in the inflammatory process. Blocking of selectins is therefore considered a promising therapeutic approach to treat acute and chronic inflammatory diseases which are caused by excessive extravasation of leukocytes. This mini-review highlights the major structural differences between E- and P-selectin and summarizes the resulting strategies for the design of selectin antagonists.

Keywords: Antagonist · Glycomimetics · PSGL-1 · Selectin · Sialyl Lewis^X

1. Background

The selectins, namely E-, P-, and L-selectin, form a family of Ca^{2+}-dependent lectins. Upon an inflammatory stimulus, E- and P-selectin are upregulated on endothelial cells (E-selectin, P-selectin) and platelets (P-selectin). They mediate the rolling of leukocytes on the endothelial surface, which is followed by firm adhesion *via* the interaction of integrins with members of the super IgG family and transmigration to the site of inflammation.[1] This process, forming a vital defense mechanism in the event of injuries or infections, can turn deleterious in numerous diseases with an inflammatory component like stroke, reperfusion injury, psoriasis or rheumatoid arthritis.[2] Furthermore, selectins are involved in tumor metastasis.[3] Therefore, blocking the interaction of selectins with their physiological ligands has been recognized as a promising therapeutic approach for the therapy of these diseases. Consequently, various concepts have been applied to disrupt the selectin-mediated cell–cell interaction, either by antagonizing the receptor, by modulating selectin expression, or by cleaving selectin ligands.[4] This mini-review gives an overview on E- and P-selectin antagonists and elucidates how their design is guided by differences and similarities between E- and P-selectin.

2. E- and P-Selectin: Differences and Similarities

The tetrasaccharide sialyl Lewis^x (sLe^x, Fig. 1) was identified as the minimal carbohydrate binding epitope recognized by all three selectins.[8] The carbohydrate recognition domain (CRD) of E- and P-selectin, which is characterized by a Ca^{2+} ion essential for sLe^x binding, is almost identical.[9,10] Consequently, similar conformations of sLe^x bound to P- and E-selectin were found.[10,11] Besides the CRD for sLe^x, P-selectin offers a second, structurally and operationally distinct binding site not present on E-selectin.[12] This second site is rich in positively charged amino acid side chains and therefore allows binding of a broad range of negatively charged biopolymers (section 4). The natural ligand of P-selectin, P-selectin glycoprotein ligand 1 (PSGL-1), binds to both sites, the CRD and the polyanion binding site, simultaneously.[10] Its binding epitopes include sLe^x, which is essential for recognition,[13] and three sulfated tyrosines (Tyr 46, 48, 51), which are vital for high binding affinity (Fig. 2).[10,14,15] When these two binding epitopes are combined, a synergistic effect[12] leads to an approximately 10'000-fold improved binding of PSGL-1 compared to sLe^x.[11,16]

The existence of a second binding site on P-selectin obviously has major implica-

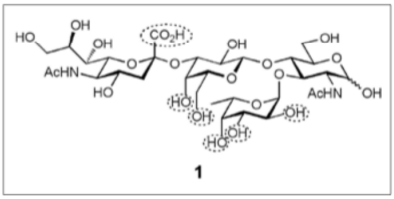

Fig. 1. The tetrasaccharide sialyl Lewis^x (**1**) and its pharmacophoric groups: hydroxyl groups in 2-, 3-, and 4- position of fucose,[5,6] hydroxyl groups in 4- and 6- position of galactose[7] and the carboxylic acid residue of sialic acid.[5]

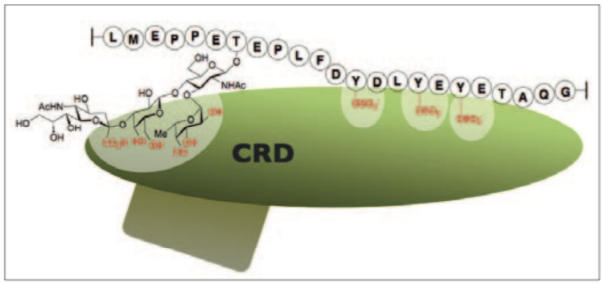

Fig. 2. Interaction between P-selectin and PSGL-1.

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tions on the design of P-selectin antagonists. According to the addressed binding sites, P-selectin antagonists are divided into three groups: mimetics of sLe^x (section 3), polyanions (section 4), and mimetics of PSGL-1 (section 5).

3. Mimetics of sLe^x

Since sLe^x is the common binding epitope recognized by all three selectins, it has served as starting point for numerous drug discovery programs. The tetrasaccharide itself suffers from the typical downsides of carbohydrates, which are the poor pharmacokinetic and pharmacodynamic properties as well as complex synthesis, and therefore ultimately failed as drug candidate.[17] In consequence, efforts were primarily directed to reduce the carbohydrate character while enhancing affinity and conserving the pharmacophore (Fig. 1).

The search for high affinity-antagonists followed two major strategies. In a first approach, the carbohydrate moieties were systematically replaced with mimetics containing the relevant pharmacophoric groups. In a second approach, antagonists were rationally designed based on the pharmacophore of sLe^x. Furthermore, database screening with the 3D pharmacophore of sLe^x and high throughput screening yielded additional leads. Since the resulting antagonists up to 2002 are covered by several excellent reviews,[17,18] only two representative examples of that period are discussed here. Following the first strategy, a group at Ciba and later at Novartis replaced the sialic acid moiety of sLe^x by *S*-cyclohexyl lactic acid and the *N*-acetyl glucosamine unit with *R,R*-1,2-cyclohexanediol. The resulting lead CGP69669A (**2**)[19] was further developed by Glycomimetics Inc. to the pan-selectin antagonist GMI-1070 (**3**)[20] which is now in Phase II clinical trials for the treatment of sickle cell crisis (Fig. 3). Kogan *et al.* applied the second strategy to design a series of biphenyl-based inhibitors mimicking the hydroxyl groups of L-fucose with D-mannose and the carboxylic acid residue of sialic acid.[21] TBC265 (**4**, Fig. 4) shows affinity for E-, P-, and L-selectin. Its dimer TBC1269 (**5**)[22] is currently in Phase II clinical trials for treatment of asthma and psoriasis.

Recently published selectin antagonists exemplify a clear trend to compounds lacking any carbohydrate moiety. Kranich *et al.* used TBC1269 (**5**) as template for the rational design of a series of nonglycosidic, nonpeptidic, polyphenolic pan-selectin antagonists, *e.g.* **6** in Fig. 5.[23] For the best representatives, low micromolar affinities in a static cell free assay and significant inhibition of HL-60 cell attachment to selectins under flow conditions are reported. Although initially designed to mimic sLe^x, the authors also consider the possibility of alternative binding modes. Based on a pharmacophore model originally developed by the Kondo group,[24] Ulbrich *et al.* recently developed pan-selectin inhibitors with two carboxylates and a hydrophobic tail (*e.g.* **7**, Fig. 5).[25] They propose that one carboxylate coordinates to Ca^{2+} and the second one forms a salt bridge to Arg97 of E-selectin or Lys99 of P-selectin. The long alkyl chains are considered to interact with hydrophobic regions of the selectins. Although the authors claim the absence of micelles at concentrations equal to the IC_{50}, the antagonists obviously resemble detergents. Therefore, multivalency due to micelle formation or simply unspecific lipophilic interactions might be the reason for their affinity. This hypothesis is supported by the fact that total loss of affinity was reported for compounds lacking the hydrophobic tail. The groups of Kaila and Girard independently reported on quinic acid derivatives mimicking sLe^x, *e.g.* **8**[26] and **9**[27] (Fig. 5). Their hypothesis that quinic acid mimics fucose and coordinates to Ca^{2+} was confirmed by a crystal structure of quinic acid in complex with E-selectin.[26] Kaila and coworkers found only low affinities for their compounds in a surface plasmon resonance assay. However, two of their compounds efficiently blocked leukocyte rolling *in vivo* (*e.g.* **8**, Fig. 5). Girard *et al.* reported IC_{50} values in the millimolar range in a cell-based competitive binding assay for their P- and E-selectin antagonists (*e.g.* **9**, Fig. 5).[27] Researchers at Wyeth identified quinoline salicylic acids as promising P-selectin antagonists *via* high-throughput screening.[28] The lead compound PSI-697 (**10**)[29] is currently in Phase I clinical trials and the follow-up PSI-421 (**11**)[30] has reached the predevelopment stage. Finally, Schön *et al.* reported on the macrolide efomycine M (**12**) as a potent selectin antagonist mimicking sLe^x.[31] Though the exact binding mode is still being discussed controversially,[32] it is interesting to note that efomycine M significantly reduced leukocyte rolling *in vivo* and alleviated cutaneous inflammation in two mouse models of psoriasis.

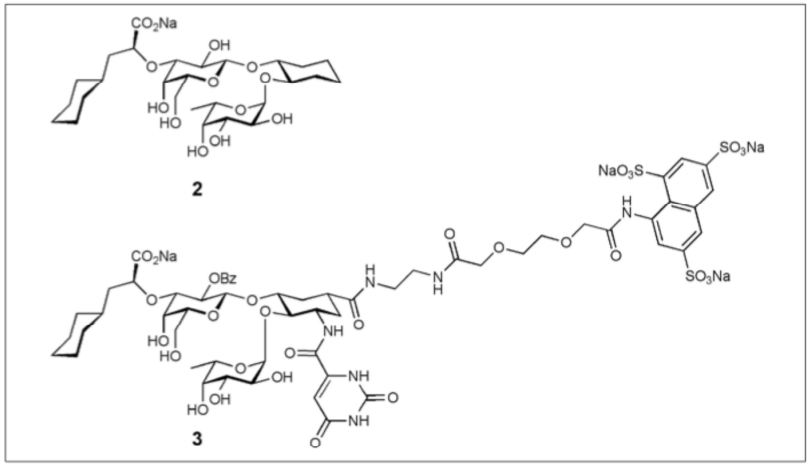

Fig. 3. E-selectin antagonist CGP69669A (**2**) and pan-selectin antagonist GMI-1070 (**3**).

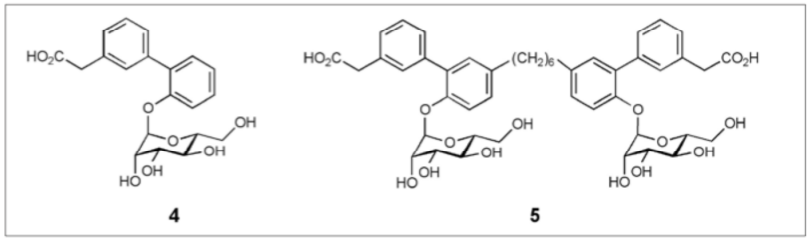

Fig. 4. TBC265 (**4**) and its dimer TBC1269 (**5**).

4. Polyanions

Numerous polyanions like sulfatides,[33] heparin,[34] lipopolysaccharides (LPS),[35] fucoidin, sulfated dextran,[36] chondroitin sulfate,[37] dermatan sulfate,[38] and sulfated hyaluronic acid[39] have been reported to exhibit P-selectin antagonism. The broad range of charged compounds lacking the carbohydrate epitope suggests

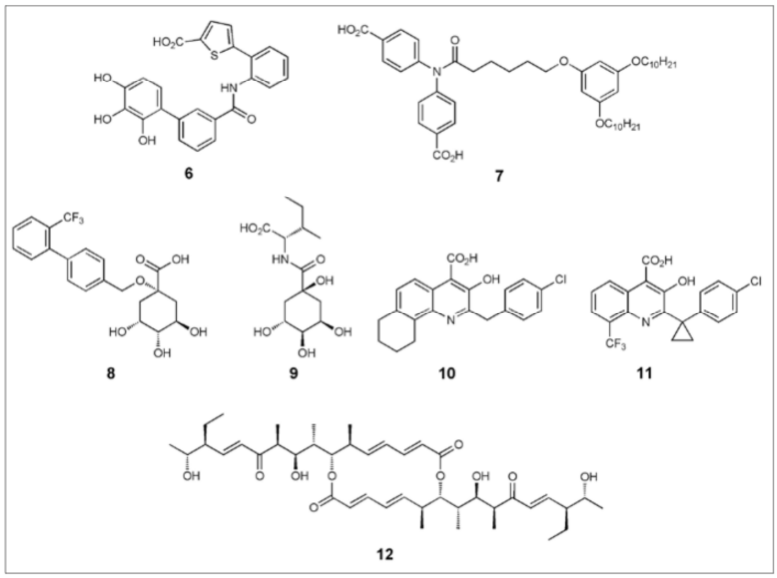

Fig. 5. Representative examples of recently published selectin antagonists.

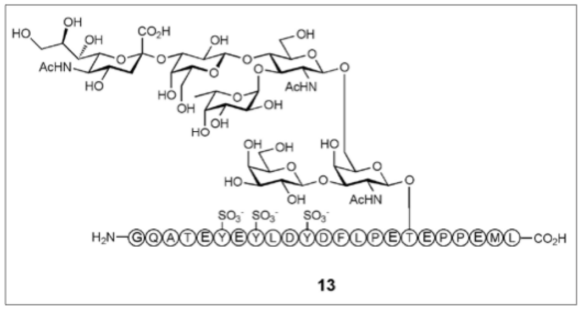

Fig. 6. PSGL-1 fragment GSP-6 (**13**).[46]

unspecific binding *via* charge–charge interactions to the numerous positively charged amino acid side chains in areas adjacent to the CRD. Ca^{2+}-independent binding of LPS, heparin, and sulfatides supports this hypothesis.[34,35,40] Nevertheless, the therapeutic effect of heparin was tested in several clinical trials in selectin associated inflammatory diseases and cancer.[41] Even more, the suppression of P-selectin function by heparin could be correlated to its antimetastatic activity *in vivo*.[42] Current efforts are directed to reduce the anticoagulant effect of heparin, while retaining its anti-inflammatory and antimetastatic potential. Besides natural polyanions, dendritic polyglycerol sulfates[43] and functionalized nanoparticles[44] were developed to block P-selectin *via* electrostatic interactions.

5. PSGL-1 and Mimetics Thereof

PSGL-1 binds to P-selectin with nanomolar affinity (320 nM)[16] and consequently is a promising lead for high affinity P-selectin antagonists. Several groups have been working on chemoenzymatic and chemical routes to synthesize the N-terminal recognition domain of PSGL-1.[45,46] These truncated glycosulfopeptides, *e.g.* GSP-6 (**13**, Fig. 6), comprise sulfated tyrosines and a core 2-based *O*-glycan with sLe^x and exhibit binding affinities similar to PSGL-1.[10,46] Furthermore, a recombinant soluble form of PSGL-1, rPSGL-Ig, was developed and is currently in clinical trials.

Besides these analogues of PSGL-1, mimetics have been designed that combine a carbohydrate and an anionic part, and potentially target both binding sites of P-selectin. GMI-1070 (Fig. 3) for instance comprises a sLe^x mimic combined with a naphtyl sulfonic acid moiety to support binding to all three selectins. Furthermore, to directly mimic PSGL-1, polymers that combine sLe^x and tyrosine sulfate were developed. These polymers revealed synergistic effects compared to polymers containing sLe^x tetrasaccharides or tyrosine sulfates alone.[47] Smaller fragments mimicking the binding epitopes on PSGL-1 have also been assembled successfully on nanoparticles.[44,48] Since monovalent fragments show no significant inhibitory effect on P-selectin binding, the affinity of these nanoparticles was suggested to result from multivalency.

6. Conclusion and Outlook

E- and P-selectin share a high degree of similarity in their CRDs. Numerous antagonists that target these binding sites by mimicking sLe^x have been developed, and the successful performance of several of these antagonists in preclinical and clinical tests convincingly prove that small molecules are indeed suited as selectin antagonists. Finally, small molecules occupying both binding sites of P-selectin potentially offer increased affinity and selectivity and might fill an important gap in the therapy with selectin antagonists.

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1.4 Ligand pre-organization and rational design of E-selectin antagonists

Pre-organization describes the correlation between solution conformation and bioactive, *i.e.* bound conformation of a molecule. The closer the solution conformation resembles the bioactive conformation, the higher is the degree of pre-organization. A high degree of pre-organization is expected to reduce the loss of conformational entropy upon binding and consequently to increase the binding affinity. Carbohydrates are mostly flexible,[71] which is reflected in the unfavorable conformational entropies reported for a broad range of lectin-carbohydrate interactions[71] and accounts for their weak binding affinities. Carver consequently suggested to increase the binding affinity of carbohydrates by increasing their pre-organization.[72] Interestingly, carbohydrate ligands that were pre-organized by covalent means showed no increase in binding affinity, which could be traced back to enthalpy/entropy compensation.[73] Even more, ligands that were covalently pre-organized in the wrong conformation suffered from significant loss in affinity.[74]

In the case of E-selectin antagonists, progress was made by successively replacing carbohydrate moieties with mimics that were tuned to improve the pre-organization of the ligand and thereby its binding affinity.

1.4.1 Pre-organization of sLe^x

SLe^x mediates the recognition of physiological selectin ligands by selectins. As this process takes place under flow conditions, it requires fast binding kinetics and consequently a high degree of pre-organization is expected to be beneficial.

The solution conformation and the conformation of sLe^x bound to E-selectin have extensively been studied by NMR spectroscopy and molecular dynamics (MD) simulations.[5,56,65-67,75] Overall, the data suggest a high degree of pre-organization of the Lewis^X core, while conformational changes of the sialic acid residue upon binding to E-selectin are discussed controversially. However, the conformation of sLe^x bound to E-selectin was identified as one of two low energy solution conformations of sLe^x, and molecular modeling studies predicted a high degree of pre-organization for sLe^x.[76,77] Moreover, recently published crystal structures of the staphylococcal toxins SSL5 and SSL11 revealed that these lectins bind essentially the same conformation of sLe^x as E- and P-selectin,[78] again pointing to a strong similarity between solution conformation and bound conformation of sLe^x.

1.4.2 Rational design of E-selectin antagonists

Carbohydrate leads typically suffer from low affinities, high polarity, and complex structure, which strongly limits their direct application as drugs. A common strategy to overcome these limitations is to eliminate unnecessary polar groups and carbohydrate moiety or to replace them with less polar mimics. [43]

This concept was also applied to the development of E-selectin antagonists based on sLe^x. D-Glc*N*Ac, known to act as a spacer between L-Fuc and D-Gal, was replaced with numerous linkers.[58,79] Ernst and coworkers showed that the affinity of the resulting mimetics correlated with the ability of the linker to pre-organize the Lewis^x core.[58] Conformationally restricted linkers like (*R,R*)-cyclohexane-1,2-diol were better suited than more flexible ones like ethane-1,2-diol. Sialic acid was typically replaced by glycolic acid, lactic acid, or derivatives thereof.[80] Pre-organization also turned out to be essential for the carboxy group of sialic acid, *i.e.* (*S*)-lactic acid derivatives were superior to (*R*)-lactic acid derivatives. Efforts to replace both D-Glc*N*Ac and sialic acid culminated in the substitution with (*R,R*)-cyclohexane-1,2-diol and (*S*)-cyclohexyllactic acid, respectively, resulting in the lead **CGP69669**, which was 15-fold more potent than sLe^x (Figure 1.4.1).[81]

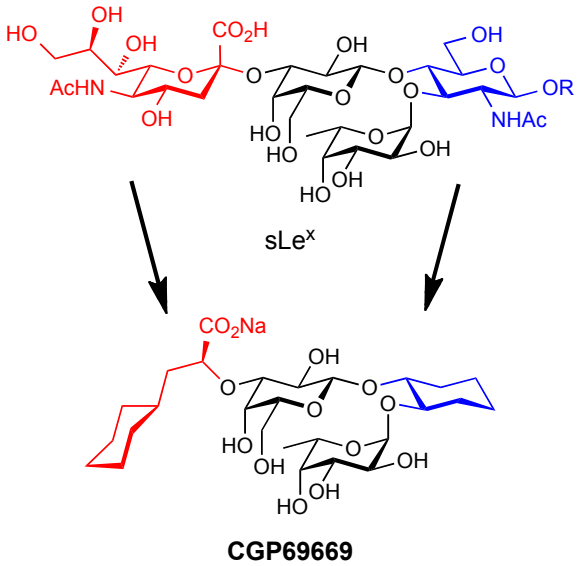


Figure 1.4.1 Systematic replacement of carbohydrate moieties of sLe^x resulted in the lead **CGP69669.**

To easily compare the conformational preferences of different ligands, Ernst and Kolb defined two internal dihedral angles. The core conformation, *i.e.* the orientation of D-Gal relative to L-Fuc, and the acid orientation, *i.e.* the tilting angle of the sialic acid C1-C2 bond relative to the core (Figure 1.4.2). Furthermore, they developed a molecular modeling tool

that allowed to assess the conformational preference of a ligand in solution based on a Monte Carlo (jumping between wells)/stochastic dynamics [MC(JBW)/SD] simulation.[76,77] Calculated conformations were weighed with their probability and plotted in an internal coordinate system (Figure 1.4.3). The conformational preference found for sLe^x closely resembled the conformation determined by tr-NOE NMR,[65,67] which was used to define the bioactive window. Ligands populating this window were predicted to have a high degree of pre-organization and consequently superior binding affinity compared to ligands outside the window. Indeed, it was shown for a set of E-selectin ligands, that the predicted degree of pre-organization correlates with relative affinities measured in bioactivity assays.

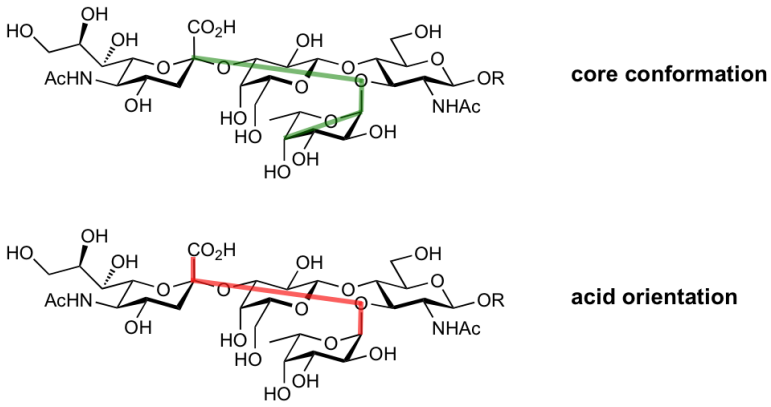


Figure 1.4.2 Graphical representation of the internal dihedral angles that define core orientation and acid conformation.

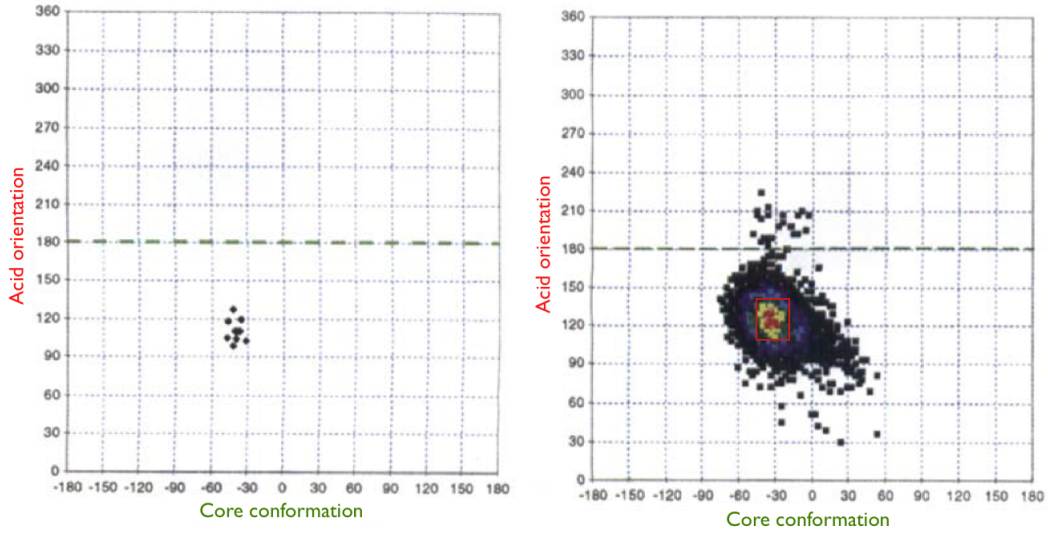
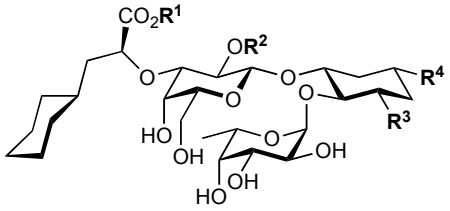


Figure 1.4.3 Left: conformation of sLe^x bound to E-selectin as determined by tr-NOE NMR; right conformational preference calculated for sLe^x: the highest probability for the core conformation and the acid orientation were calculated as -20° to -50° and 110° to 140°, respectively; the red square indicates the bioactive window (adapted from [76]).

Based on the lead CGP69669, numerous E-selectin antagonists have been synthesized. To date, cyclohexyllactic acid was identified as the best mimic of sialic acid,[82,83] and several attempts to improve the affinity *via* additional lipophilic interactions at the sLe^x binding site failed.[63,83,84] However, it was found that various substituents in 2-position of D-Gal enhance the binding affinity (Table 1.1).[85,86] STD NMR experiments indicated direct contact of these substituents to the protein,[86] while the crystal structure of sLe^x bound to E-selectin suggested no contact at all. To unambiguously identify the binding mode of these antagonists, a crystal structure is required.

Significant improvements were made by enhancing the pre-organization of the core and thereby the affinity of E-selectin antagonists.[58,87] Most importantly, it was found that D-Glc*N*Ac mimics bearing substituents vicinal to L-Fuc enhance the pre-organization *via* steric compression of the core.[58] Ernst, Wagner, and Schwizer systematically studied the influence of various alkyl groups and identified (1*R*,2*R*,3*S*)-3-methyl-1,2-cyclohexanediol and (1*R*,2*R*,3*S*)-3-ethyl-1,2-cyclohexanediol as superior mimics of D-Glc*N*Ac (Table 1.1).[87] A beneficial effect was also reported for substituents at the former ring oxygen position of D-Glc*N*Ac. Since these should not directly bind to E-selectin according to the crystal structure of sLe^x bound to E-selectin, stabilization of the chair conformation was discussed.[87]

Table 1.1 SAR studies of E-selectin antagonists.[84]



Compound	R^1	R^2	R^3	R^4	rIC_{50} [a)]
CGP69669	Na	H	H	H	0.080
BW408-0 DS4115	H	H	Me	H	0.013
DS226h	Na	H	Et	H	0.009
LT2_036	H	Bz	H	H	0.040
GMI 1077 DS226a	Na	Bz	Me	H	0.005
DS226e	Na	Bz	Et	H	0.007
DS226b	Na	Bz	*n*Bu	H	0.009
DS226c	Na	Bz	*c*Pr	H	0.032
DS244	H	Bz	Me	CO_2Me	0.002

a) rIC_{50} values are referenced to sLe^x (IC_{50} = 1 mM, rIC_{50} = 1.0)

Finally, Jonas Egger and Céline Weckerle used a fragment based drug discovery (FBDD) approach to identify nanomolar E-selectin antagonists.[86,88]

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2 Results and discussion

2.1 General strategy for the synthesis of sLe^x, sLe^x mimetics and key building blocks

Depending on the target molecule and site of modification, different synthetic strategies were applied. In general, synthetic routes were designed convergent to require a minimum number of building blocks and steps, as well as to introduce modifications at the latest stage possible. The following section gives an overview on synthetic strategies and key building blocks used throughout this work. The synthesis of building blocks and final compounds is covered by the corresponding chapters.

Nomenclature: Each compound number is composed of the chapter number and a consecutive number (left). Compounds that appear in paper draft sections are given simple consecutive numbers in this section (middle). If paper draft compounds are referred to in another chapter, they are complemented by the chapter number and marked with an asterisk (right). A formula index is provided in chapter 4.

2.3
└ compound number
└ chapter

3
└ compound number

2.3*
└ compound number
└ chapter

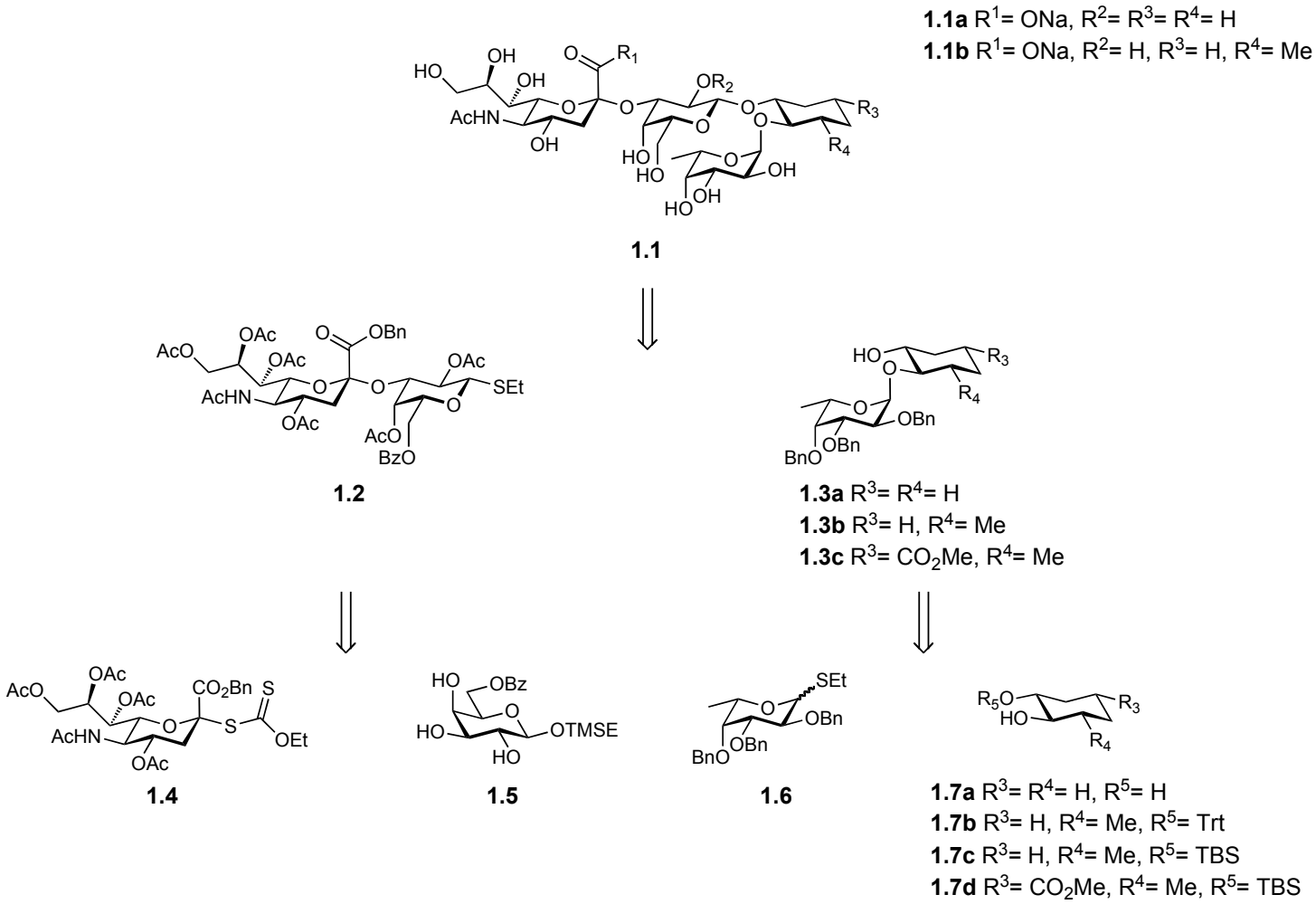
2.1.1 Synthesis of sLe^x and sLe^x mimetics with sialic acid or cyclohexyllactic acid

For the synthesis of sLe^x mimetics with sialic acid (Scheme 2.1.1) or cyclohexyllactic acid (Scheme 2.1.2), as well as for the synthesis of sLe^x (Scheme 2.1.3), a 2+2 strategy was applied. This strategy is ideally suited to introduce different derivatives and mimics of D-Glc*N*Ac with a minimum number of steps and building blocks.

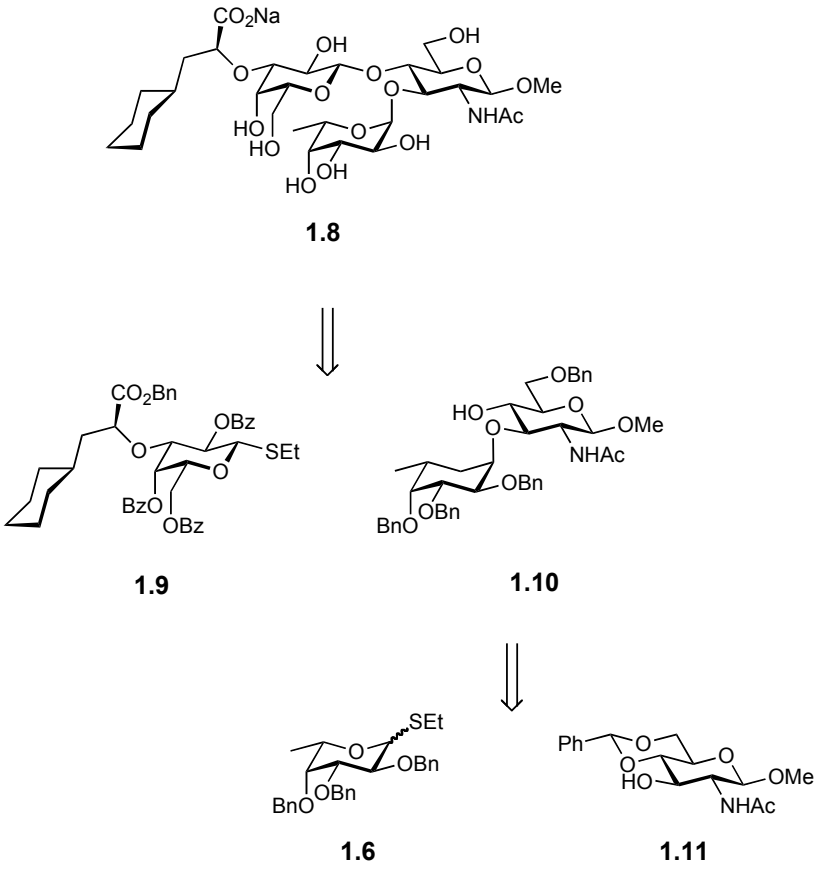
SLe^x mimetics with sialic acid were dissected into sialylgalactoside **1.2** and pseudodisaccharides **1.3a-c** (Scheme 2.1.1). Disaccharide **1.2** was further cleaved into sialic acid donor **1.4** and galactoside **1.5**, while the pseudodisaccharides were dissected into thiofucoside **1.6** and the four D-Glc*N*Ac mimics **1.7a-d**.

SLe^x mimetic **1.8** was synthesized form galactoside **1.9** and the disaccharides **1.10**. The latter ones were available from thiofucoside **1.6** and the corresponding *N*-acetyl-D-glucosamine building block **1.11.**

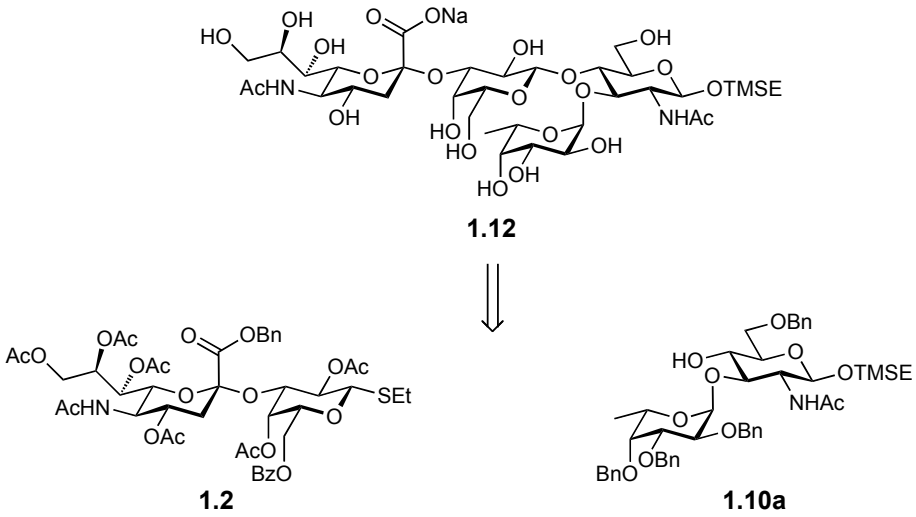
Trimethylsilylethyl (TMSE) protected sLe^x (**1.12**) was dissected into donor **1.2** and acceptor **1.10a** (Scheme 2.1.3).



Scheme 2.1.1 Retrosynthesis of sLe^x mimetics with sialic acid.



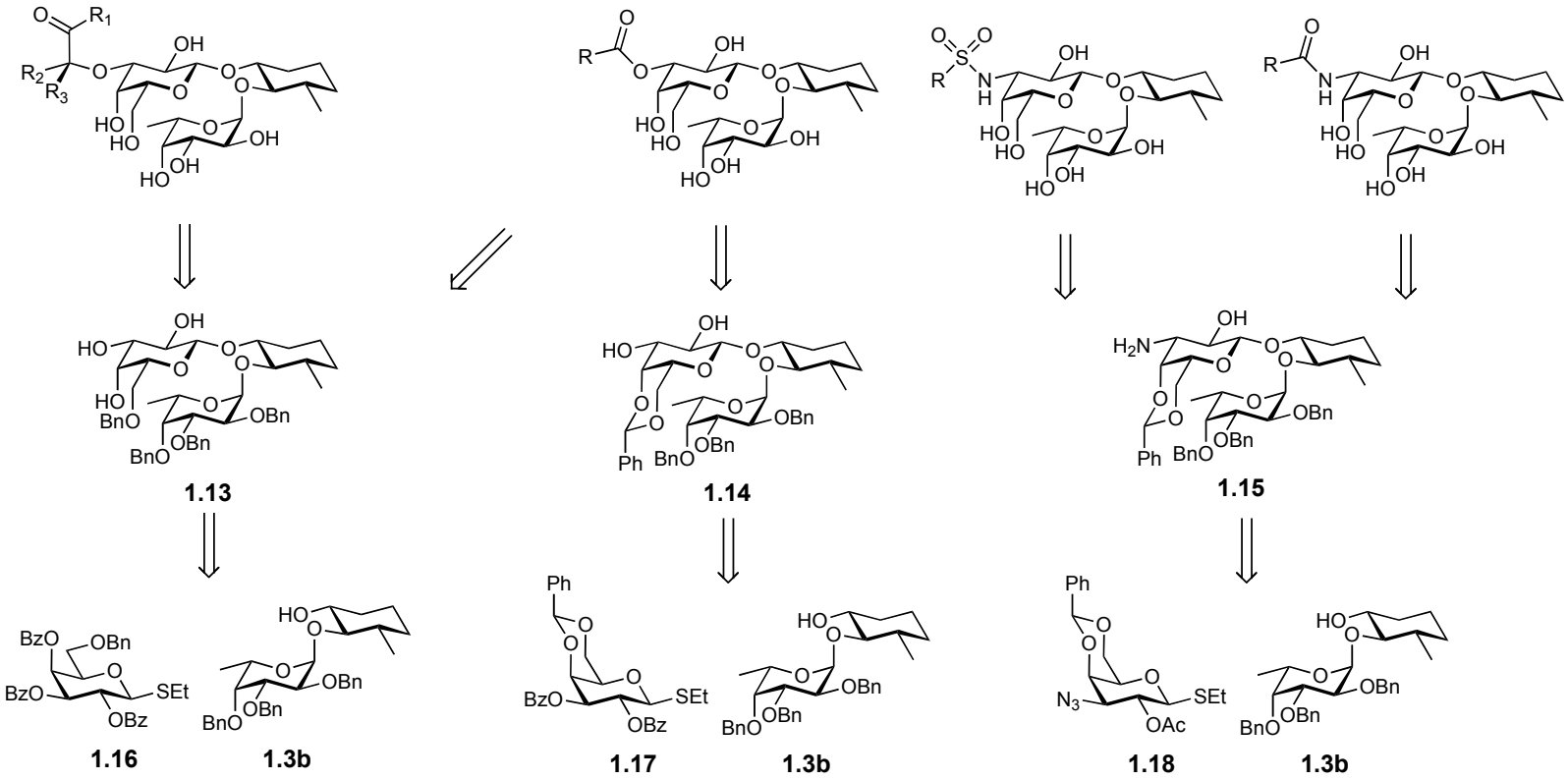
Scheme 2.1.2 Retrosynthesis of a sLe^x mimetic with (*S*)-cyclohexyllactic acid.



Scheme 2.1.3 Retrosynthesis of TMSE-sLe^x **1.12.**

2.1.2 Synthesis of sLe^x mimetics with novel sialic acid mimics

Mimetics of sLe^x with novel sialic acid replacements were derived from one of the three key building blocks **1.13**, **1.14**, or **1.15**, applying a 1+3 strategy (Scheme 2.1.4). These building blocks only differ in the D-galactose part and were obtained from **1.3b** and the corresponding galactosides **1.16**, **1.17** and **1.18**.



Scheme 2.1.4 Retrosynthesis of sLe^x mimetics with novel sialic acid mimics.

2.2 Fast and efficient route to [(1*R*,2*R*,3*S*)-1-hydroxy-3-methyl-cyclohex-2-yl] 2,3,4-tri-*O*-benzyl-α-L-fucopyranoside 1.3b – a key building block in the synthesis of glycomimetic selectin antagonists

2.2.1 Exploratory synthetic route to 1.3b

In his PhD thesis, Daniel Schwizer studied the influence of various substituents in 3-position of D-Glc*N*Ac mimics on the binding affinity of E-selectin antagonists (section 1.4.2.). He found, that a simple methyl group is best suited to improve the affinity. The corresponding building block **1.3b** consequently became the standard for the design of novel selectin antagonists and was therefore needed in gram amounts (Figure 2.2.1).

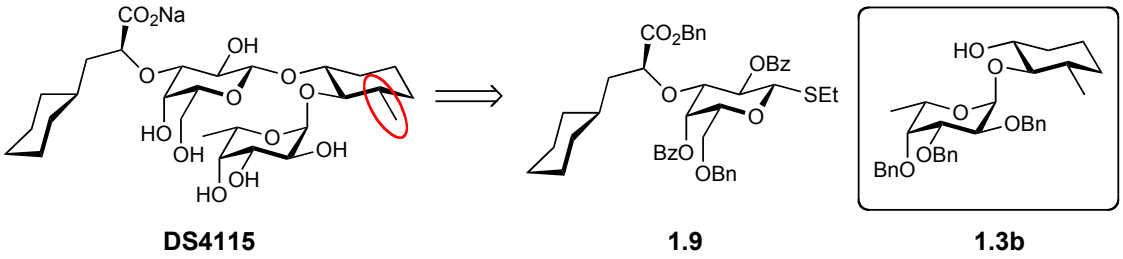
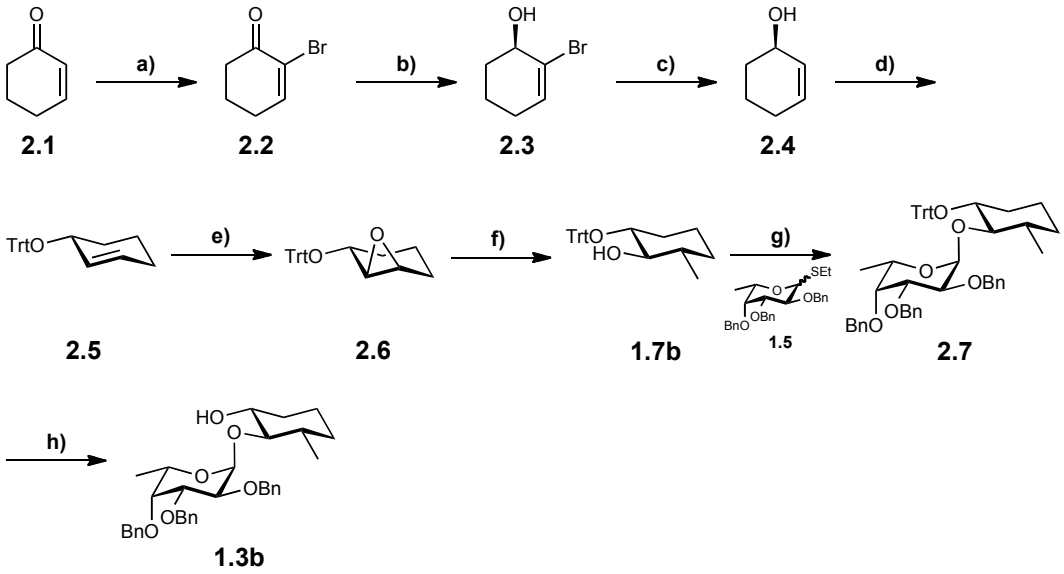


Figure 2.2.1 (1*R*,2*R*,3*S*)-3-methyl-cyclohexane-1,2-diol was identified as potent mimic of D-Glc*N*Ac and made **1.3b** the standard building block for the synthesis of novel selectin antagonists.

The synthetic route originally developed by Daniel Schwizer was designed to introduce substituents at the latest stage possible, in this case *via* epoxide opening (Scheme 2.2.1, step f). Although perfectly suited for the exploration of the best alkyl substituent, the route turned out to be quite unpractical for the large-scale synthesis of disaccharide mimic **1.3b**. Especially the costly enantioselective Corey-Bakshi-Shibata reduction[1] and the low temperature epoxide opening with the higher order cyanocuprate $Me_2Cu(CN)Li_2$[2] impeded the scale up. Besides, the bulky trityl group, which is needed to ensure regio- and stereoselectivity of epoxide formation and opening, hampered effective fucosylation. Steric hindrance and lack of stability under acidic conditions of the trityl group led to various side products and decreased yields substantially. Finally, in larger scale, detritylation with zinc bromide as Lewis acid and triethylsilane as hydride donor led to silylation of **1.3b** and made an additional deprotection step necessary.



Scheme 2.2.1 a) i. Br_2, CH_2Cl_2, 0°C, 2.5 h; ii. Et_3N, CH_2Cl_2, r.t., 2 h (60%); b) (*S*)-α,α-diphenylprolinol, $B(OMe)_3$, BH_3·*N*,*N*-diethylaniline, THF, -10°C to 0°C, 3 h, 93%; c) i. *t*-BuLi, Et_2O, -78°C to -20°C, 3 h; ii. aq. $NaHCO_3$, -20°C to r.t., 1 h (79%); d) Ph_3CCl, CH_2Cl_2, DBU, r.t., 14 h (91%); e) *m*-CPBA, $NaHCO_3$, CH_2Cl_2, 0°C to r.t., 5 h (72%); f) MeLi, CuCN, BF_3·Et_2O, THF, -78°C to -30°C, 2 h (91%); g) i. Br_2, CH_2Cl_2, 0°C, 1h; ii. Et_4NBr, MS 3 Å, CH_2Cl_2, DMF, r.t. 16 h, 30% to 43%; h) $ZnBr_2$, TES, CH_2Cl_2, r.t., 32 h, 66%.

Without the need to introduce different substituents in 3-position of the D-Glc*N*Ac mimic, we were able to develop a more efficient route to **1.3b**, which is described in the following section.

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2.2.2 Fast and efficient synthesis of a carbocyclic D-Glc*N*Ac mimic, a key building block for the synthesis selectin antagonists

Author contributions: F.P.C. Binder: synthetic route, all experiments except determination of enantiomeric excess using chiral HPLC; Dr. E. Francotte (Novartis): determination of the enantiomeric excess using chiral HPLC.

Manuscript

Fast and Efficient Synthesis of a Carbocyclic D-Glc*N*Ac Mimic, a Key Building Block for the Synthesis of Selectin Antagonists

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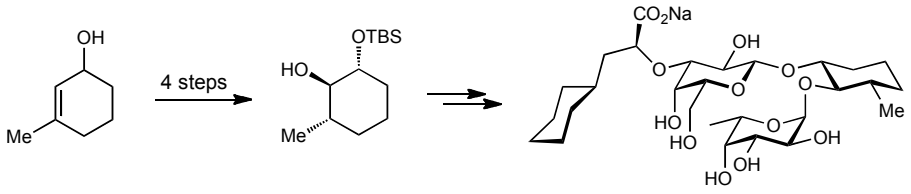
Abstract

For the synthesis of selectin antagonists, a fast and efficient approach to a D-Glc*N*Ac mimic was developed. Starting from racemic seudenol, it permits the gram-scale synthesis of (1*R*,2*R*,3*S*)-1-[(*tert*-butyldimethylsilyl)oxy]-3-methyl-cyclohexan-2-ol in 4 steps with only two chromatographic purifications.



Keywords: Carbohydrate, carbocycle, enzyme, glycomimetic, *N*-acetyl-D-glucosamine

Introduction

Lectins, such as selectins,[1] galectins,[2] or siglecs[3] have gained increasing attention as drug targets. Although valuable leads for the development of new drugs, carbohydrates themselves rarely find therapeutic application, as they typically suffer from complex synthesis and poor pharmacokinetic and pharmacodynamic properties. As a consequence, small molecules mimicking the carbohydrate epitope, *e.g.* the sialidase inhibitor oseltamivir,[4] have been developed to overcome these unfavorable properties. A common strategy for the design of glycomimetics is the substitution of a carbohydrate moiety with a carbocyclic scaffold, which offers increased hydrolytic and metabolic stability and reduced polarity. In addition, the facile synthetic accessibility of the mimetic structures, which as a consequence of the chirality of the parent carbohydrate compound include stereochemical challenges, is of cardinal importance.

In the case of selectin antagonists, stepwise modification of the natural ligand sialyl Lewis^X (sLe^X, **1**, Figure 1) led to the new lead structure CGP69669 (**2**),[5] where *N*-acetyl-D-neuraminic acid (Neu5Ac) was replaced with (*S*)-cyclohexyl lactic acid and the *N*-acetyl-D-glucosamine (D-Glc*N*Ac) unit with (*R*,*R*)-cyclohexane-1,2-diol. When (*R*,*R*)-cyclohexane-1,2-diol, on its part, was replaced by (1*R*,2*R*,3*S*)-3-methylcyclohexane-1,2-diol (→ **3**),[6] affinity could be further improved. The reported synthetic route for D-Glc*N*Ac mimetic **4**[6] allows the introduction of different alkyl substituents R *via* epoxide opening with higher order cyanocuprates. However, this route is not feasible for the gram scale synthesis of **4**. Since larger amounts of building block **4** were required for a broad exploration of its potential for a new class of selectin antagonists, a more convenient synthetic route had to be developed.

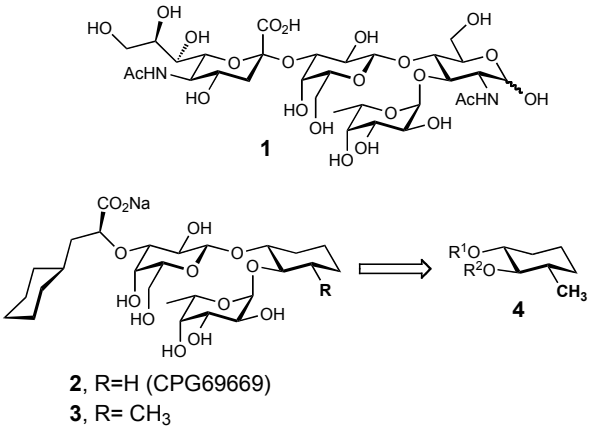
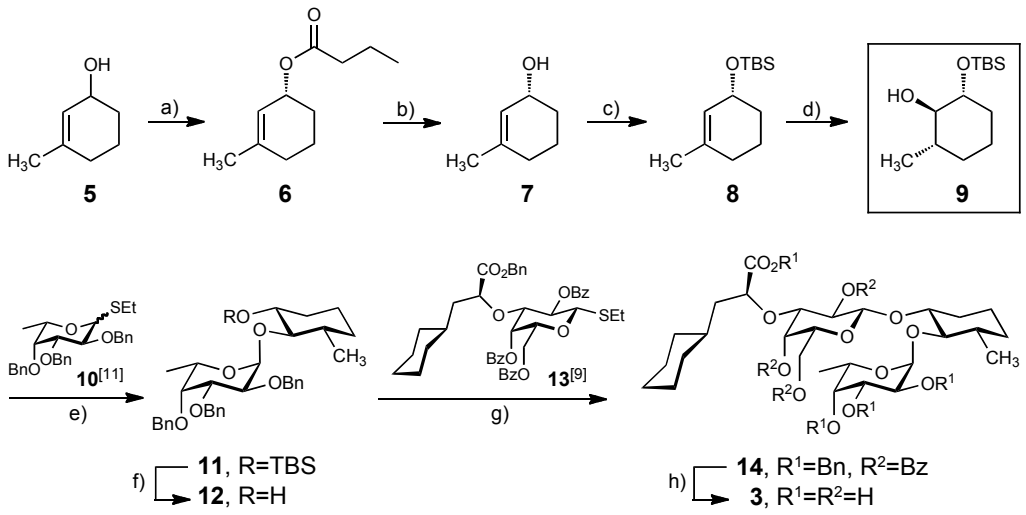


Figure 1. Selectin antagonists **1**-**3**: The natural carbohydrate epitope sLe^X (**1**), selectin antagonists **2** (CGP69669)[5] and **3**[6], and the D-Glc*N*Ac mimetic **4**.

Results and discussion

Starting from commercially available racemic seudenol (3-methyl-2-cyclohexenol, **5**) the stereoselective enzymatic acylation (→ **6**) and the subsequent hydrolysis with aqueous sodium hydroxide leading to enantiomerically pure (*R*)-seudenol (**7**) is described by ter Halle et al.[7] Using optimized conditions for the butanoylation with immobilized *Candida antarctica* lipase C (Novozym 435),[7] we could easily isolate (*R*)-seudenolester (**6**) in 46% yield in 10 g scale.[8] Subsequent saponification afforded (*R*)-seudenol in 84% yield and 97.5% enantiomeric excess (ee) as determined by HPLC using a Chiracel OD-H column. Since the protection group of the hydroxy group in **7** has to be stable under strongly basic and acidic conditions, should not hamper fucosylation by steric bulk, and finally allow cleavage under mild conditions orthogonal to benzyl protecting groups, a *tert*-butyldimethylsilyl (TBS) ether (→ **8**) was chosen. Hydroboration followed by oxidation yielded all-*trans* **9** in 81% over two steps. Starting from racemic seudenol, this short sequence allowed the gram scale synthesis of **9** in 31% overall yield, requiring only two chromatographic purifications.

Fucosylation of **9** under *in situ* anomerisation conditions[9] gave **11**, which was smoothly deprotected with tetrabutylammonium fluoride (TBAF), affording pseudodisaccharide **12**[6] in excellent yield over two steps. Galactosylation with donor **13**[10] promoted by dimethyl(methylthio)sulfonium triflate (DMTST) afforded **14** β-selectively.[6] Debenzylation by hydrogenolysis followed by saponification with lithium hydroxide and ion exchange chromatography finally gave **3**.[6]



Scheme 2. a) Novozyme 435, vinylbutyrate, heptane, 23°C, 200 rpm, 2 h 25 min, 46%; b) aqueous NaOH, MeOH, 0°C, 5 h, 84%; c) TBSCl, imidazol, DMAP, CH_2Cl_2, r.t., 15 h; d) i. BH_3·THF, THF, 0°C to r.t., 2 h; ii. H_2O_2, aqueous NaOH, 0°C to r.t., 1 h, 81% from **7**; e) $CuBr_2$, DTBMP, TBAB, CH_2Cl_2, DMF, MS 4 Å, r.t., 10 h, 87%; f) TBAF, THF, r.t., 20 h, quant. g) DMTST, MS 3Å, CH_2Cl_2, r.t., 43 h, 59%;[6] h) i. Pd/C, H_2, EtOH, cat. AcOH, r.t., ii. LiOH, MeOH/H_2O, r.t., 2 d, iii. Dowex (Na^+), Sephadex-G15, 74%.[6]

Overall, we developed a fast and efficient route to (1*R*,2*R*,3*S*)-1-[(*tert*-butyldimethylsilyl)oxy]-3-methylcyclohexan-2-ol (**9**) starting from racemic seudenol. By subsequent fucosylation and galactosylation, a novel class of selectin antagonists can easily be explored.

Experimental Part

NMR spectra were recorded on a Bruker Avance DMX-500 (500 MHz) spectrometer. The assignment of ^1H and ^{13}C NMR spectra was achieved using 2D methods (COSY, HSQC). Chemical shifts are given in ppm and were assigned in relation to the solvent signals on the δ-scale[11] or to tetramethylsilane (0 ppm) as internal standard. Coupling constants *J* are given in Hertz (Hz). Multiplicities were specified as follows: s (singlet), d (doublet), dd (doublet of a doublet), t (triplet), q (quartet), m (multiplet). Optical rotations were measured using a Perkin-Elmer Polarimeter 341. Electron spray ionization mass spectra (ESI-MS) were obtained on a Waters Micromass ZQ. HRMS analysis were carried out using a Agilent 1100 LC equipped with a photodiode array detector and a Micromass QTOF I equipped with a 4 GHz digital-time converter. The elemental analysis was performed at the Institute of Organic Chemistry at the University of Basel, Switzerland. Reactions were monitored by TLC using glass plates coated with silica gel 60 F_{254} (Merck) and visualized by using UV light and/or by charring with a molybdate solution (a 0.02 M solution of ammonium cerium sulfate dihydrate

and ammonium molybdate tetrahydrate in aqueous 10% H_2SO_4). Column chromatography was performed on silica gel (Fluka, 40-60 mesh). Tetrahydrofurane (THF) was freshly distilled under argon over sodium and benzophenone. Dichloromethane (CH_2Cl_2) was dried by filtration over Al_2O_3 (Fluka, type 5016 A basic). Enantiomeric excess (ee) was determined by HPLC: Agilent 1200 instrument with DAD detection equipped with a Chiralcel OD-H column (250 mm x 4.6 mm); Eluent: hexane/isopropanol (99/1 vol); flow rate 1.0 mL/min; temperature 23°C; detection, signals measured at 210 nm. Typical retention times: 12.9 (*S*)-seudenol, 14.1 (*R*)-seudenol.

(*R*)-3-Methylcyclohex-2-en-1-yl butyrate (6).

Immobilized Novozyme 435 (222 mg, 444 U, EC 232-619-9) was added to a solution of **5** (10.0 g, 89 mmol) and vinyl butyrate (22.6 mL, 20.3 g, 178 mmol) in heptane (90 mL). The mixture was stirred at 23°C and 200 rpm. After 2 h 25 min the mixture was filtered and volatiles were evaporated at 60°C and 10 mbar to give 12 g of a clear oil. Column chromatography on silica (CH_2Cl_2) yielded pure **6** (7.50 g, 41 mmol, 46%).

^{1}H NMR (500.1 MHz, $CDCl_3$): δ 5.44 (m, 1H, H-2), 5.23 (m, 1H, H-1), 2.24 (t, ^3J = 7.4Hz, 2H, COC*H*$_2$CH$_2$CH$_3$), 2.02-1.84 (m, 2H, H-4a, H-4b), 1.81-1.56 (m, 9H, H-5a, H-5b, H6-a, H6-b, -C*H*$_3$, COCH$_2$C*H*$_2$CH$_3$), 0.92 (t, ^3J = 7.4Hz, 3H, COCH$_2$CH$_2$C*H*$_3$); ^{13}C NMR (125.8 MHz, $CDCl_3$): δ 173.5 (*C*OCH$_2$CH$_2$CH$_3$), 141.0 (C-3), 120.2 (C-2), 68.6 (C-1), 36.7 (CO*C*H$_2$CH$_2$CH$_3$), 30.0 (C-4), 28.1 (C-6), 23.8 (-*C*H$_3$), 19.1, 18.7 (2C, C-5, COCH$_2$*C*H$_2$CH$_3$), 13.7 (COCH$_2$CH$_2$*C*H$_3$); $[\alpha]_D$ +168.7 (*c* 9.28, $CHCl_3$); MS (ESI) *m*/*z*: calcd for $C_{11}H_{18}NaO_2^+$ $[M+Na]^+$: 205.12; found: 204.83; elemental analysis calcd (%) for $C_{11}H_{18}O_2$ (182.26): C 72.49, H 9.95; found: C 72.87, H 9.65.

(*R*)-seudenol (7). A solution of NaOH in H_2O (10.3 mL, 4N) was slowly added to a solution of seudenol butyrate **6** (3.50 g, 19 mmol) in MeOH (30 mL) at 0°C and stirred at 0°C for 5 h. The mixture was diluted with H_2O (25 mL) and extracted with CH_2Cl_2 (25 mL + 20 mL + 15 mL). The combined organic layers were washed with brine (25 mL) and dried over Na_2SO_4. Filtration and evaporation of volatiles (200 mbar, 40°C) gave spectroscopically pure (*R*)-seudenol (**7**) (1.81 g, 16 mmol, 84%) as a clear oil, which was directly used in the next step.

$[\alpha]_D$ +91.7 (*c* 0.74, $CHCl_3$); HPLC: 97.5% ee, 98% purity; NMR data were in accordance with literature.[7]

(*R*)-1-[(*tert*-butyldimethylsilyl)oxy]-3-methylcyclohex-2-en (**8**). Imidazol (4.40 g, 65 mmol) was added to a solution of (*R*)-seudenol **7** (3.50 g, 31 mmol), DMAP (cat.), and TBSCl (7.31 g, 48 mmol) in anhydrous CH_2Cl_2 (65 mL) at r.t. under argon. After stirring for 15 h, the reaction mixture was quenched with satd. aqueous $NaHCO_3$ (50 mL) and extracted with CH_2Cl_2 (20 mL). The organic layer was washed with aqueous HCl (20 mL, 0.01 N), satd. aqueous $NaHCO_3$ (20 mL), and brine (20 mL) and dried over Na_2SO_4. Filtration and evaporation of volatiles (200 mbar, 40°C) gave the TBS ether **8** as clear oil.

(1*R*,2*R*,3*S*)-1-[(*tert*-butyldimethylsilyl)oxy]-3-methylcyclohexan-2-ol (**9**). A solution of BH_3·THF (60 mL, 1M in THF) was slowly added to a solution of the crude TBS ether (**8**) in anhydrous THF (60 mL) under argon at 0°C. After stirring for 2 h at r.t., the reaction mixture was cooled to 0°C again and aqueous NaOH (180 mL, 3N) followed by aqueous H_2O_2 (180 mL, 30%) were slowly added *via* dropping funnel (**CAUTION:** strong gas development). The mixture was stirred at 0°C for 1 h, subsequently acidified to pH 3 by slow addition of 10% aqueous HCl *via* dropping funnel (**CAUTION:** strong gas development) and extracted with CH_2Cl_2 (2 · 300 mL). The extracts were dried over Na_2SO_4, filtered, concentrated (100 mbar, 40°C) and purified by column chromatography (PE/Et_2O 98.5/1.5) to yield pure **9** (6.20 g, 25 mmol, 81%) as clear oil.

^{1}H NMR (500.1 MHz, $CDCl_3$): δ 3.34 (m, 1H, H-1), 2.92 (dd, ^3J = 8.5 Hz, 10.0 Hz, 1H, H-2), 2.47 (s, 1H, OH), 1.81 (m, 1H, H-6a), 1.63-1.56 (m, 2H, H-4a, H-5a), 1.41 (m, 1H, H-3), 1.34-1.99 (m, 2H, H-5b, H-6b), 1.04-0.92 (m, 4H, H-4b, -CH_3), 0.91-0.83 (m, 9H, SiC(CH_3)$_3$), 0.07 (s, 3H, SiCH_3), 0.05 (s, 3H, SiCH_3); ^{13}C NMR (125.8 MHz, $CDCl_3$): δ 81.0 (C-2), 77.0 (C-1), 37.0 (C-3), 33.4, 33.9 (2C, C-4, C-6), 25.9 (3C, SiC(*C*H_3)$_3$), 23.6 (C-5), 18.5 (-*C*H_3), 18.1 (Si*C*(CH$_3$)$_3$), -3.9, -4.6 (Si*C*H_3); $[\alpha]_D$ - 13.7 (*c* 3.14, $CHCl_3$); HR-MS (ESI) *m*/*z*: calcd for $C_{13}H_{28}NaO_2Si^+$ $[M+Na]^+$: 267.1751; found: 267.1752.

[(1*R*,2*R*,3*S*)-1-((*tert*-Butyldimethylsilyl)oxy)-3-methyl-cyclohex-2-yl] 2,3,4-tri-*O*-benzyl-α-L-fucopyranoside (**11**). Ethylthio fucoside **10**[12] (3.90 g, 8.15 mmol) and TBAB (4.00 g, 12.41 mmol) were dried at high vacuum overnight. Powdered activated molecular sieves 4 Å (5.0 g), compound **9** (1.00 g, 4.09 mmol), 2,6-di-tert-butyl-4-methylpyridine (2.50 g, 12.17 mmol), anhydrous CH_2Cl_2 (35 ml) and DMF (5 ml) were added and the mixture was stirred for 4 h at r.t. under argon. $CuBr_2$ (2.70 g, 12.09 mmol), dried under high vacuum overnight at 70°C, was added and the resulting dark mixture was stirred at r.t. under argon. After completion of the reaction (17 h), the solution was filtered through a pad of celite and

the filtrate was washed with a solution of satd. aqueous NH_4Cl and aqueous NH_3 (9/1 (v/v), 2 x 200 mL) and brine (100 mL). The aqueous layers were extracted with CH_2Cl_2 (2 x 200 mL) and the combined organic layers were dried (Na_2SO_4) and concentrated. Column chromatography on silica (PE/EtOAc 98/2 to 97/3) gave the pseudodisaccharide **11** as clear oil (2.34 g, 3.54 mmol, 87%).

^{1}H NMR (500.1 MHz, $CDCl_3$): δ 7.47-7.27 (m, 15H, 3 C_6H_5), 5.16 (d, ^3J = 3.4 Hz, 1H, Fuc H-1), 5.03 (A of AB, ^2J = 11.6Hz, 1H, CH_2Ph), 4.89, 4.85, 4.78, 4.76 (4d, ^2J = 11.8Hz, 4H, CH_2Ph), 4.70 (B of AB, ^2J = 11.6Hz, 1H, CH_2Ph), 4.26 (q, ^3J = 6.4 Hz, 1H, Fuc H-5), 4.10 (dd, ^3J = 3.4, 10.2 Hz, 1H, Fuc H-2), 4.05 (dd, ^3J = 2.6, 10.2 Hz, 1H, Fuc H-3), 3.75 (m, 1H, H-1), 3.70 (m, 1H, Fuc H-4), 3.36 (t, ^3J = 6.4 Hz, H-2), 1.88-1.77 (m, 2H, H-3, H-6a), 1.76-1.68 (m, 2H, H-4a, H-5a), 1.43 (m, 1H, H-6b), 1.34-1.11 (m, 8H, Fuc-H6, -CH_3, H-4b, H-5b), 0.93 (s, 9H, SiC(CH_3)$_3$), 0.09 (s, 3H, SiCH_3), 0.06 (s, 3H, SiCH_3); ^{13}C NMR (125.8 MHz, $CDCl_3$): δ 139.1, 138.9, 138.8, 128.4, 128.3, 128.2, 127.6, 127.5 (18C, 3 C_6H_5), 96.8 (Fuc C-1), 81.5 (C-2), 79.3 (Fuc C-3), 78.2 (Fuc C-4), 76.7 (Fuc C-2), 74.9 (CH_2Ph), 73.6 (CH_2Ph), 73.3 (C-1), 73.0 (CH_2Ph), 66.4 (Fuc C-5), 35.6 (C-3), 33.2 (C-6), 31.1 (C-5), 26.1 (3C, SiC(CH$_3$)$_3$), 19.8 (C-4), 18.9 (Fuc C-6), 18.2 (SiC(CH$_3$)$_3$), 17.0 (CH$_3$), -3.9, -5.0 (2C, SiCH$_3$); $[\alpha]_D$ - 53.7 (*c* 2.1, $CHCl_3$); HR-MS (ESI) *m/z*: calcd for $C_{40}H_{56}NaO_6Si^+$ $[M+Na]^+$: 683.3738; found: 683.3740.

[(1*R*,2*R*,3*S*)-1-Hydroxy-3-methyl-cyclohex-2-yl] 2,3,4-tri-*O*-benzyl-α-L-fucopyranoside (**12**). Compound **11** (2.10 g, 3.18 mmol) was dissolved in a solution of TBAF in THF (20 mL, 1M) and stirred for 24 h at r.t.. The solution was diluted with CH_2Cl_2 (50 mL) and washed with H_2O (100 mL). The aqueous layer was extracted with CH_2Cl_2 (2 x 50 mL) and the combined organic layers were dried (Na_2SO_4) and concentrated. Column chromatography on silica (PE/EtOAc 80/20) gave **12** as white solid (1.74 g, 3.18 mmol, quant.); $[\alpha]_D$ - 42.0 (*c* 0.45, $CHCl_3$); NMR data were in accordance with literature.[6]

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2.3 Development of selectin antagonists based on differences and similarities between E- and P-selectin

2.3.1 E- and P-selectin: Differences and similarities guide the way to selectin antagonists

Author contributions: F.P.C. Binder: design and synthesis of selectin antagonists, manuscript; K. Lemme: biological characterization of selectin antagonists; M. Smieško: molecular modeling studies.

Manuscript

E- and P-selectin: Differences and Similarities Guide the Way to Selectin Antagonists

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Keywords

Carbohydrate mimetics, selectin antagonist, sialyl Lewis^x

Abstract

Selectins are key players in the inflammatory cascade as they initiate the migration of leukocytes to sites of inflammation. However, excessive invasion of leukocytes can cause acute and chronic inflammatory diseases. Consequently, blocking of selectins is regarded as a potential therapeutic approach. Based on the common binding epitope of all selectins, sialyl Lewis^x (sLe^x), we developed potent selectin antagonists, which exploit similarities and differences in the binding of sLe^x to P- and E-selectin. The nearly identical core conformation of sLe^x bound to P- and E-selectin allowed to introduce the D-Glc*N*Ac mimic (1*R*,2*R*,3*S*)-3-methylcyclohexane-1,2-diol to the design of P-selectin antagonists. Replacement of the carboxy group of sialic acid with a methylamide moiety was found to significantly reduce the affinity to E-selectin, while affinity to P-selectin was conserved. Finally, sialic acid was successfully replaced with small mimics resulting in structurally simplified E- and P-selectin antagonists with up to 20-fold improved binding affinity compared to the lead sLe^x.

Introduction

The selectin family consists of E, P-, and L-selectin. All members contain a Ca^{2+}-dependent carbohydrate recognition domain (CRD). Upon an inflammatory stimulus, two of these key components of our immune system, namely P- and E- selectin are up-regulated on endothelial cells and platelets. They mediate the rolling of leukocytes on the endothelial surface, which is followed by firm adhesion *via* integrins and extravasation to the site of the inflammatory stimulus. However, this important defense mechanism becomes harmful in acute and chronic inflammatory diseases like stroke, reperfusion injury, psoriasis, or rheumatoid arthritis, since in these states, the excessive extravasation of leukocytes leads to the destruction of tissues.[1] Inhibiting the interaction of selectins with their physiological ligands and thereby disabling the whole inflammatory cascade is considered a promising therapeutic approach.[2]

Most of the small molecule antagonists developed to date were derived from the tetrasaccharide sialyl Lewis^x (sLe^x, Neu5Ac(α2-3)Gal(β1-4)[Fuc(α1-3)]Glc*N*Ac, (**1**), Figure 1),[3,4] which is the minimum binding epitope of all three selectins. Since sLe^x (**1**) itself exhibits only weak binding (0.3-1.1 mM E-selectin, 7-9 mM P-selectin),[5] poor pharmacokinetics and complex synthesis, efforts were directed to identify drug-like glycomimetics, *i.e.* mimetics with appropriate pharmacodynamic and pharmacokinetic properties, especially with high affinity and oral availability. Although numerous high affinity antagonists have been reported,[3,6] none of them has been successful in therapeutic applications to date.[7]

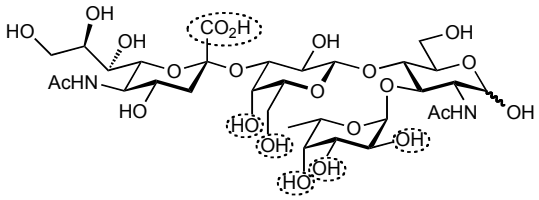

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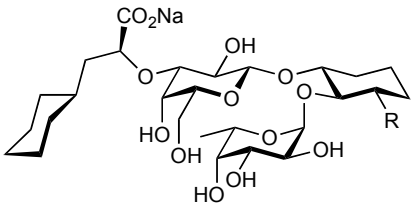
Figure 1. The tetrasaccharide sialyl Lewis^x (**1**) and its pharmacophoric groups: hydroxyl groups of L-fucose[8-10], hydroxyl groups in 4- and 6- position of D-galactose[11] and the carboxylic acid residue of sialic acid[10,12].

Results and discussion

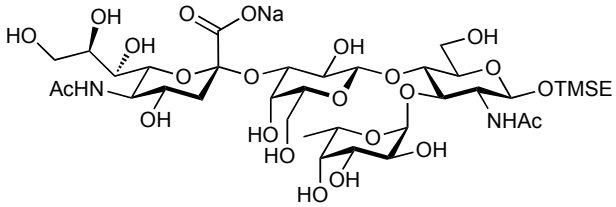
1. Similarities: Same core, same effect

In a rational approach towards selectin antagonists, carbohydrate residues were subsequently replaced with mimics conserving the pharmacophoric groups.[4,13] In the case of E-selectin antagonists, sialic acid (*N*-acetyl-D-neuraminic acid, D-Neu5Ac) was successfully replaced

with (*S*)-cyclohexyllactic acid for instance, which significantly improved binding affinity to E-selectin, but abrogated binding to P-selectin. *N*-Acetyl-D-glucoseamine (D-Glc*N*Ac) obviously does not contain pharmacophoric groups and was replaced by various mimics. It turned out, that it serves as a spacer to ensure the right core conformation, *i.e.* the right spatial orientation of L-fucose (L -Fuc) relative to D-galactose (D-Gal).[10,12,14-16] In fact, Ernst *et al.* showed, that mimics of D-Glc*N*Ac can increase the binding affinity by pre-organizing the core of E-selectin antagonists, forcing the molecule in a conformation closer to the bioactive conformation.[17,18] Following this concept, introduction of a methyl group vicinal to L-fucose afforded **3**, which is six-fold more potent than the lead **2** (Figure 2).[19]



2 R= H (CGP69669)
3 R= CH_3



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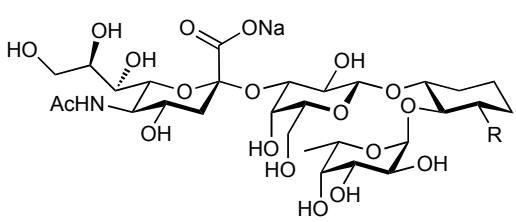
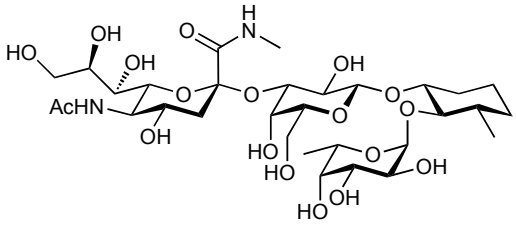


5 R= H ***E: 0.35*** ***P: 0.31***
6 R= CH_3 ***E: 0.03*** ***P: 0.05***



7 ***E: 0.29*** ***P: 0.07***

Figure 2. Selectin antagonists **2**, **3**, **5**, **6**, and **7** and the reference TMSE-sLe^x **4**. IC_{50} values are given as relative IC_{50} values (rIC_{50}) scaled on **4** (rIC_{50}= 1.0).

As a starting point for the development of novel selectin antagonists, we chose **5** (Figure 2), which is known to bind to both P- and E-selectin.[20] Since the core conformation of sLe^x bound to P-selectin is almost identical to the one of sLe^x bound to E-selectin,[5,9] we reasoned that introduction of a methyl group vicinal to L-fucose should be beneficial for P-selectin antagonists as well. The rIC_{50} values determined for **5** nicely reproduced the relative affinities reported by Toepfer *et al.*[20] The additional methyl group in **6** improved binding to E-selectin by factor ten, which is consistent with the improved binding of **3** compared to **2**. Towards P-selectin, binding affinity was improved by factor six, which demonstrates that the concept of

pre-organization works for P-selectin antagonists as well. Overall, **6** exhibited a 20-fold higher binding affinity to P-selectin than sLe^x (**1**), making it a solid starting point for further modifications.

2. Differences: The sialic acid binding domain

2.1. The carboxy group of sialic acid

Structure activity relationship (SAR) studies suggest that the carboxylic acid moiety is the most important functional group of D-Neu5Ac contributing to P- and E-selectin binding[10,12] and various models have been developed to rationalize the importance of the carboxylate. For E-selectin, a salt bridge with the guanidinium moiety of Arg97 was predicted.[5,21] For P-selectin, data were more conflicting[22] and either a salt bridge with the side chain of Lys113[14,23] or a hydrogen bond to Tyr48[5] were predicted. In 2000, Somers *et al.* published the crystal structures of E- and P-selectin in complex with sLe^x, which gave a precise picture of the interactions at molecular level.[9] For E-selectin, the crystal structure (pdb code 1g1t) confirmed the predicted salt bridge between the guanidinium moiety of Arg97 and the carboxylate of D-Neu5Ac and revealed an additional hydrogen bond to Tyr48 (Figure 3). However, in the case of P-selectin (pdb code 1g1r), the carboxylate of D-Neu5Ac was found not to be involved in a salt bridge but rather in a water mediated hydrogen bond to Ser97 and a hydrogen bond to Tyr48 (Figure 3).

We reasoned that this significant difference in interaction should allow the design of selective and less polar P-selectin antagonists, *e.g.* replacement of the carboxylate of D-Neu5Ac with a non-charged isosteric amide should result in loss of affinity to E-selectin, while affinity to P-selectin should be conserved. To test this hypothesis, we synthesized and tested the methylamide analogue of **6**, antagonist **7** (Figure 2). Indeed, binding affinity to P-selectin was conserved, while binding to E-selectin was reduced by factor ten. Interestingly, this relative change in affinity nicely correlates with the different binding affinities of sLe^x to P-selectin and sLe^x to E-selectin, indicating that the different type of interaction of the carboxylate is the major course for the different binding affinities for sLe^x to E- and P-selectin.

Our findings underline the importance of the charge-charge interaction for E-selectin antagonists and are in strong contrast to the computational modeling studies of Pichierri and Matsuo,[24] who reported that the carboxylic acid moiety of D-Neu5Ac was binding in the protonated state. Finally, we could demonstrate that negative charge is no prerequisite for P-selectin antagonists mimicking sLe^x (**1**), which is an important step towards selective and less polar P-selectin antagonists.

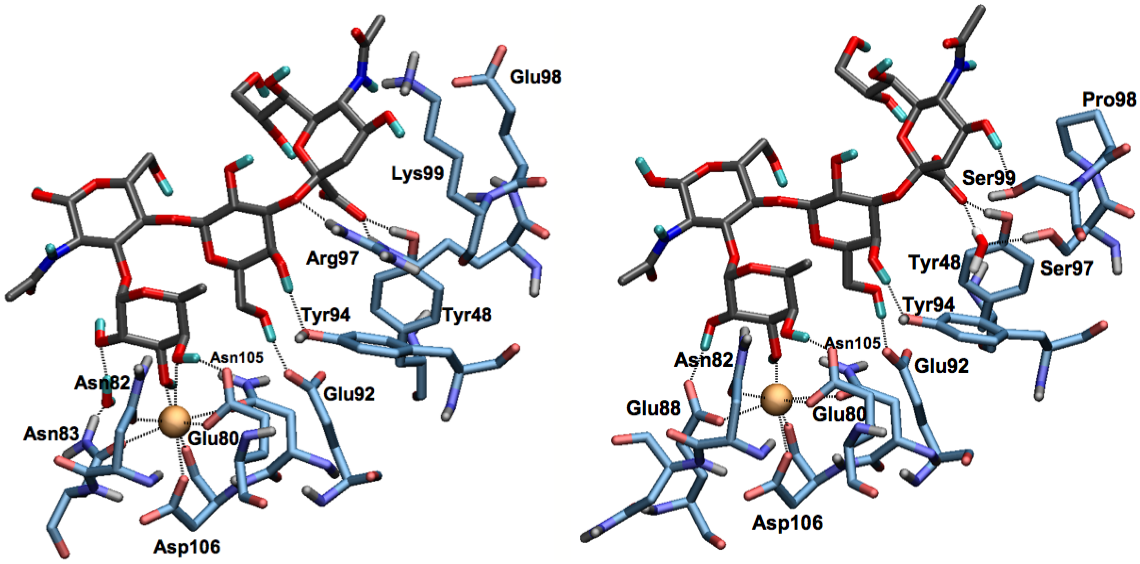


Figure 3. SLe^x bound to E-selectin (left) and sLe^x bound to P-selectin (right).[9]

2.2. Mimics of sialic acid

An obvious step for the design of sLe^x mimetics as selectin antagonists is the replacement of D-Neu5Ac, as it is expensive, polar, and apparently contributes to binding with the carboxylate only. Exclusively anionic groups like sulfates,[12,25] phosphates,[3,26] glycolic acid,[15,27] lactic acid,[17,28] and derivatives thereof were used for its replacement. Although effective -it is known that sulfates for example can fully replace sialic acid[12]- the replacement with sulfates and phosphates goes along with various drawbacks. First, the binding mode or even the binding site can change, which impedes the rational design of antagonists. It was demonstrated that 3-sulfo $Lewis^x$ binds to P-selectin Ca^{2+} independently,[12] clearly pointing to a binding mode different from the one observed in the crystal structure. Second, introduction of highly polar residues counteracts the optimization of the pharmacokinetic properties. Consequently, we decided to replace D-Neu5Ac with derivatives of lactic acid, as lactic acid itself already proved successful in the development of potent E-selectin antagonists.[17,28,29] With the results of section **2.1** in mind, we reasoned that amide analogues might pave the way to structurally simplified, non-charged P-selectin antagonists.

Besides the importance of the carboxylic acid residue of D-Neu5Ac, it is known that modifications of the glycerol side chain and the *N*-acetyl group of D-Neu5Ac have no influence on binding affinity.[12,30] While no SAR information is available for the 4-hydroxy group, the crystal structure of sLe^x (**1**) bound to P-selectin suggests a hydrogen bond to

Ser99, a hydrogen bond not available in the complex of E-selectin and sLe^x (**1**). Consequently, we developed antagonist **8**, which comprises a hydroxyl group to mimic the 4-hydroxyl group of D-Neu5Ac (Figure 4). Antagonist **8** had the same relative binding affinity to P- and E-selectin as **7**, providing evidence that the sialic acid mimic can fully replace sialic acid in E- and P-selectin antagonists. Replacement of the methylamide of **8** with a carboxylic acid residue once more enhanced binding affinity to E-selectin (**10**). Compound **9** served to explore the scope of further modifications that might increase binding affinity *via* additional lipophilic interactions. It turned out, that the bulky benzyl group is well tolerated by E-selectin, but does not enhance binding. Deletion of the hydroxyl group in the sialic acid mimic of **10** went along with a small drop in binding affinity for E-selectin (**11**).

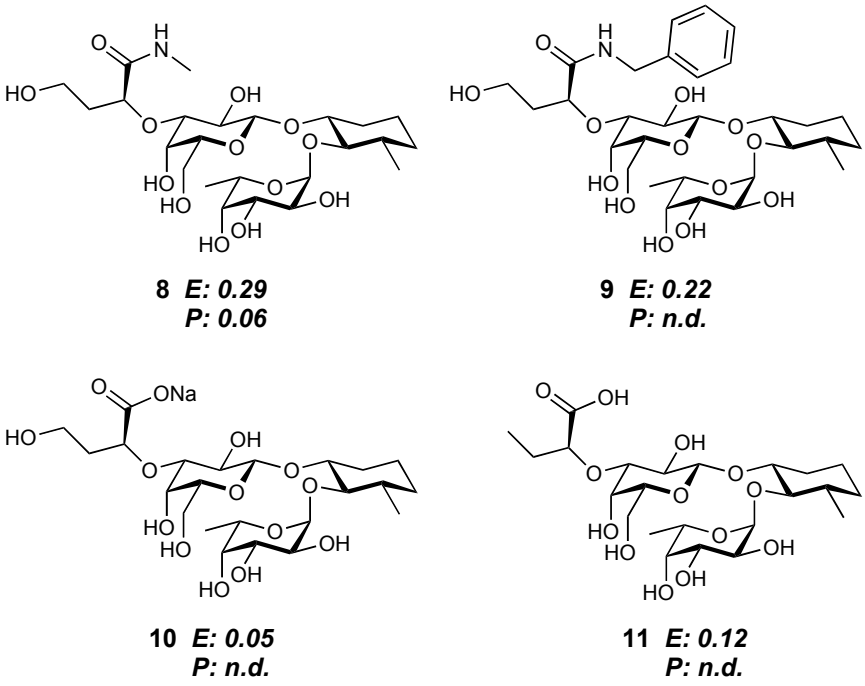


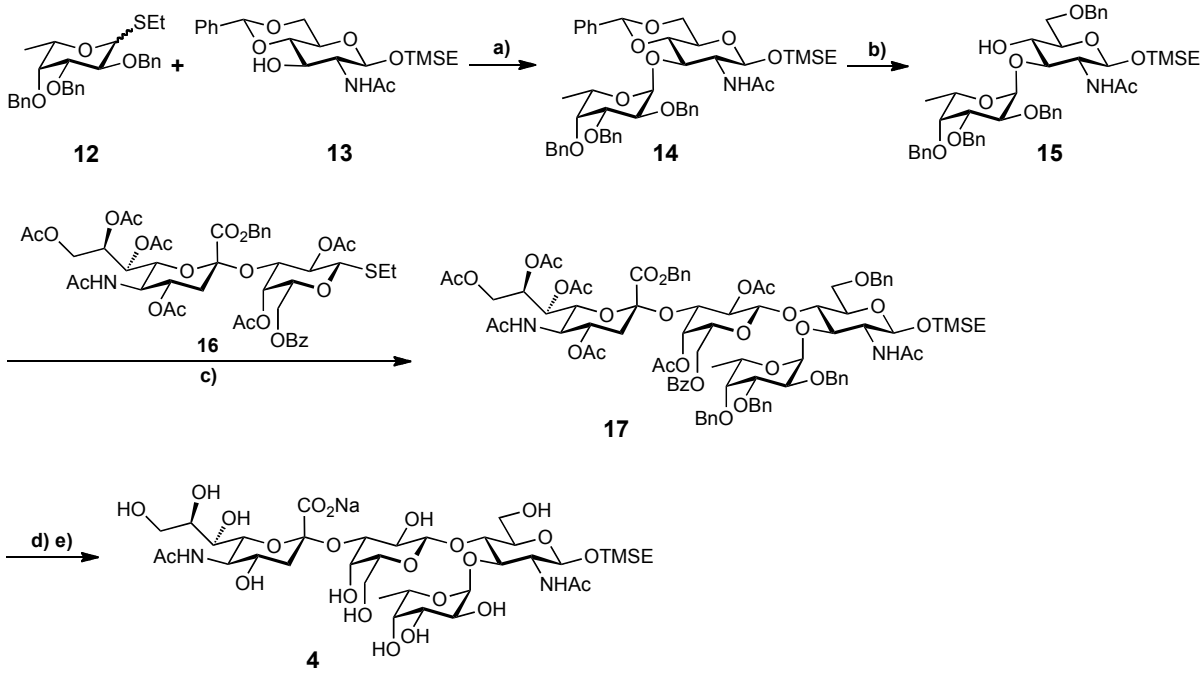
Figure 4. Relative IC_{50} values (rIC_{50}) of selectin antagonists **8** to **11**. IC_{50} values are scaled on sLe^x **4** (rIC_{50}=1); n.d. = binding affinity not determined.

3. Synthesis

As it is known that minor amounts of ion exchange resin can lead to false positive results in selectin assays,[31] no ion exchange resin was used throughout the synthesis.

3.1. Synthesis of TMSE-sLe^x 4

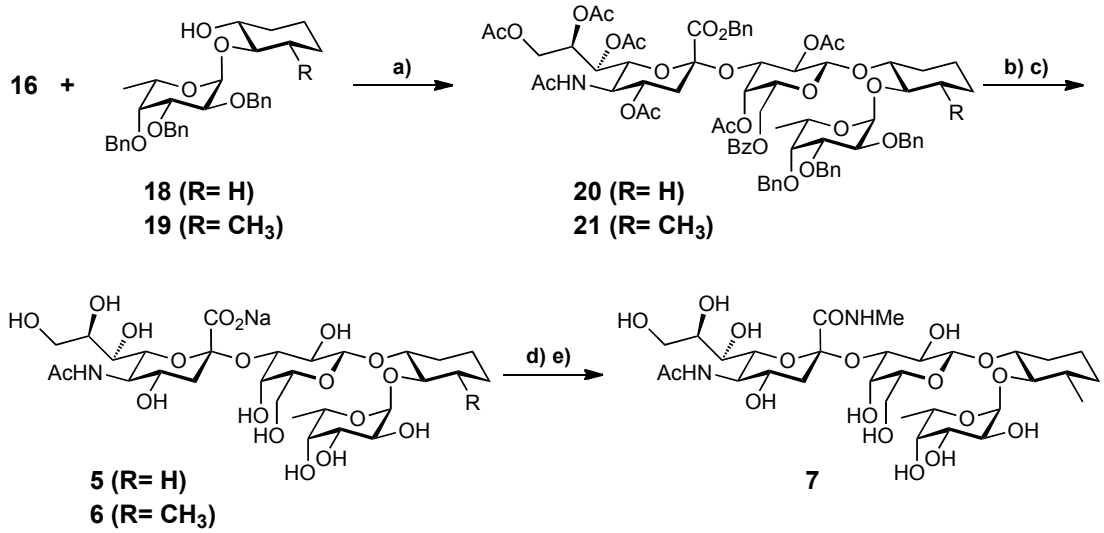
Thio fucoside **12**[18] and D-Glc*N*Ac acceptor **13**[32] were coupled under *in situ* anomerisation conditions[33] to give disaccharide **14**, which upon regioselective opening of the benzylidene acetal[34] yielded acceptor **15**. SLe^x precursor **17** was synthesized *via* DMTST[35] promoted coupling of **16**[36] and **15**. Hydrogenolytic debenzylation and saponification finally afforded TMSE protected sLe^x **4**.



Scheme 1. a) Br_2, Bu_4NBr, CH_2Cl_2, MS 4 Å, 0°C to r.t., 12 h, 55%; b) $Me_3N{\cdot}BH_3$, $AlCl_3$, H_2O, THF, r.t., 5 h, 78%; c) DMTST, CH_2Cl_2, MS 4 Å, r.t., 5 d, 20%; d) H_2, $Pd(OH)_2/C$, dioxane, H_2O, r.t., 24 h; e) aq. NaOH, MeOH, r.t., 20 h, 43% from **17**.

3.2. Synthesis of sLe^x mimetics 5 - 7

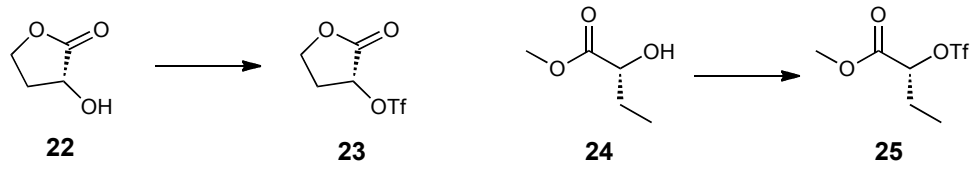
Pseudodisaccharides **18** and **19**, synthesized as reported recently,[15,37] were coupled to donor **16** using DMTST as promotor (Scheme 2). Hydrogenolytic debenzylation followed by saponification with sodium hydroxide afforded **5** and **6** in good to excellent yields. Aminolysis of the benzyl ester of **6** afforded methylamide **7** quantitatively.



Scheme 2. a) DMTST, CH_2Cl_2, MS 4 Å, r.t., 3 d (**20**: 85%, **21**: 55%); b) H_2, $Pd(OH)_2/C$, dioxane, H_2O, r.t., 12 h; c) aq. NaOH, MeOH, r.t. (**5**: 79% from **20**, **6**: 81% from **21**); d) BnBr, KF, DMF, r.t., 2 d; e) $MeNH_2$, THF, EtOH, r.t., 12 h, quant. from **6**.

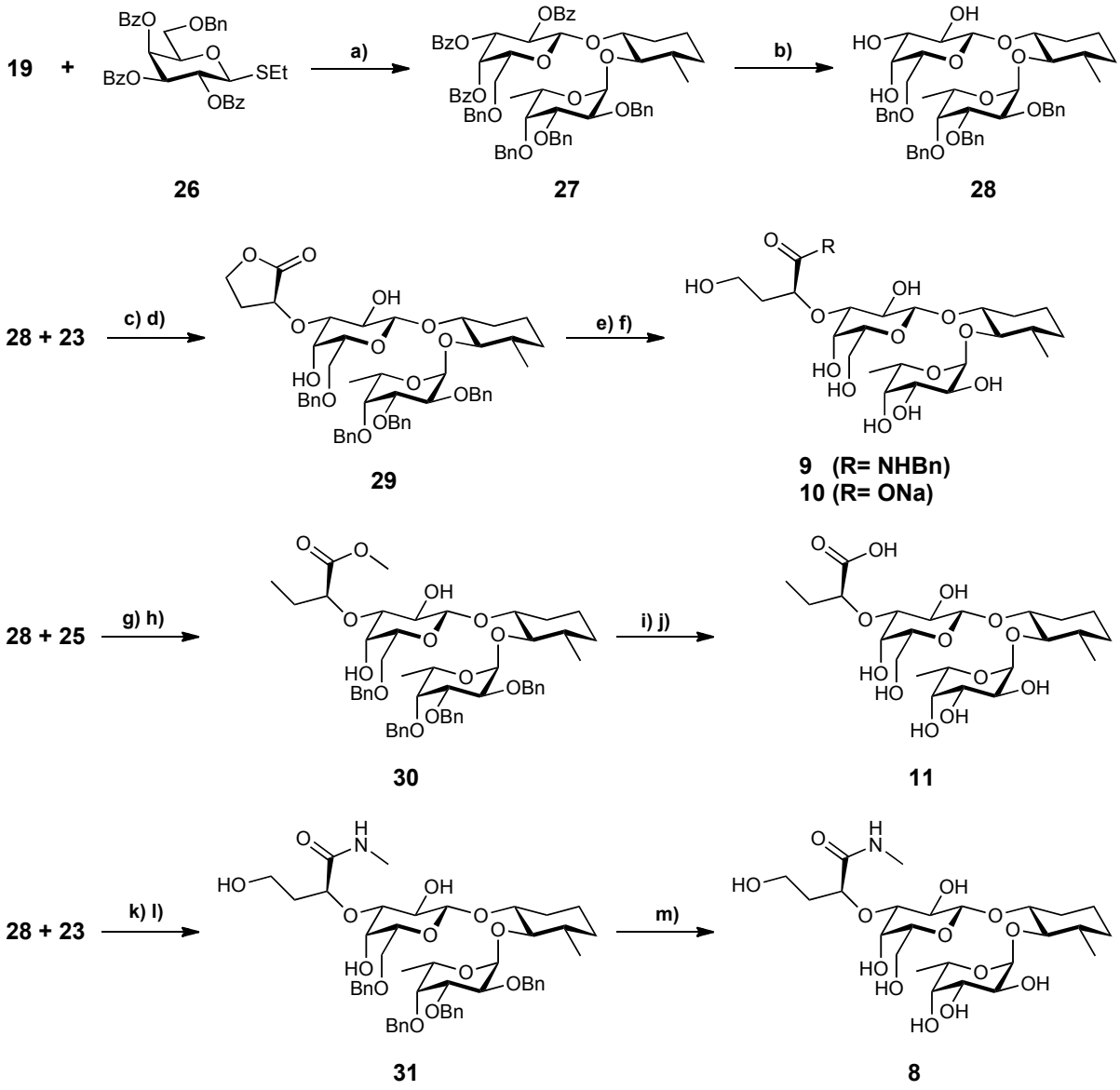
3.2. Synthesis of sLe^x mimetics 8 - 11

Mimetics **8**-**11** were synthesized by alkylation of the core building block **28** with triflates **23** and **25**[38] (Scheme 4), which were available from lactone **22** and ester **24**, respectively (Scheme 3).



Scheme 3. Tf_2O, DTBMP, CH_2Cl_2, -18°C to r.t., 3 h, (**23**: 74%, **25**: 66%).

Building block **28** was synthesized in two steps from **19** and galactoside **26**[39] *via* DMTST promoted coupling and debenzoylation under Zemplén conditions (Scheme 4). Regioselective alkylation of the 3-position of the D-Gal moiety in **28** with triflates **23** and **25** was achieved *via* tin acetal formation of **28** with Bu_2SnO. Alkylation with **23** afforded lactone **29**, which was deprotected and subsequently opened with benzylamine or sodium hydroxide to yield antagonists **9** and **10**, respectively. Compound **11** was synthesized analogously, using **25** instead of **23**. Finally, for **8**, alkylation with **23** and lactone opening with methylamine were combined to a one-pot reaction yielding **31** in 51% and allowing recovery of unreacted starting material **28** in 33%. Hydrogenolytic debenzylation finally afforded **8**.



Scheme 4. a) DMTST, CH_2Cl_2, MS 4 Å, r.t., 16 h, 67%; b) NaOMe, MeOH, r.t., 22 h, 87%; c) Bu_2SnO, toluene, MeOH, 60°C, 4 h; d) CsF, DME, r.t., 24 h, (**29**: 52%, **28**: 17%); e) H_2, $Pd(OH)_2/C$, dioxane, H_2O, r.t., 21 h; f) $BnNH_2$, THF, 20 h, (**9**: 76% from **29**) or aq. NaOH, dioxane, (**10**: 69% from **29**); g) Bu_2SnO, toluene, MeOH, 60°C, 4 h; h) CsF, DME, r.t., 24 h, 75%; i) H_2, $Pd(OH)_2/C$, dioxane, H_2O, r.t., 17 h; j) aqueous NaOH, dioxane, 14% from **30**; k) Bu_2SnO, toluene, MeOH, 50°C, 4 h; l) CsF, DME, $MeNH_2$, r.t., (**31**: 51%, **28**: 33%); m) H_2, $Pd(OH)_2/C$, dioxane, H_2O, r.t., 5 h, 37%.

4. Biological Evaluation

The affinity of selectin antagonists to E- and P-selectin was evaluated in a competitive binding assay, utilizing a polyacrylamide-type glycoconjugate as synthetic ligand for immobilized E-/P-selectin.[40] Briefly, microtiter plates were coated with either E-selectin/IgG, or P-selectin/IgG, blocked with BSA, and incubated with a fixed concentration of sLe^a-polyacrylamide (sLe^a-PAA) either in presence or absence of the antagonists. The

binding reaction was revealed by the addition of TMB substrate reagent and quantified spectrophotometrically at 450 nm. The IC_{50} defines the molar concentration of the test compound that reduces the maximal specific binding of sLe^a-PAA polymer to E-selectin/P-selectin by 50%. To ensure comparability of different antagonists, the reference compounds **2** (E-selectin)/**5** (P-selectin) were tested in parallel on each individual microtiter plate. The affinities are reported relative to **4** as rIC_{50}. The relative IC_{50} (rIC_{50}) is the ratio of the IC_{50} of the test compound to the IC_{50} of **4**.

Conclusion

Taking advantage of the similar binding mode of the sLe^x core to P- and E-selectin, the concept of pre-organization was applied successfully to the design of P-selectin antagonists. The D-Glc*N*Ac mimic (1*R*,2*R*,3*S*)-3-methylcyclohexane-1,2-diol improved the binding affinity 20-fold for P- and 30-fold for E-selectin. The isosteric exchange of the negatively charged carboxylate of D-Neu5Ac with the methylamide had no effect on P-selectin binding, while binding to E-selectin was weakened ten-fold. These results clearly demonstrate that negative charge is not essential for P-selectin antagonists mimicking sLe^x (**1**) and provide the opportunity to design selective and less polar P-selectin antagonists. Finally, D-Neu5Ac was successfully replaced with (*S*)-2,4-dihydroxybutyric acid and its methylamide analogue, resulting in the potent and structurally less complex selectin antagonists **8** and **10**.

Experimental part

NMR spectra were recorded on a Bruker Avance DMX-500 (500 MHz) spectrometer. Assignment of ^1H and ^{13}C NMR spectra was achieved using 2D methods (COSY, HSQC, HMQC, HMBC). Chemical shifts are given in ppm and were assigned in relation to the solvent signals on the δ-scale[41] or to tetramethylsilane (0 ppm) as internal standard. Coupling constants *J* are given in Hertz (Hz). Multiplicities were specified as follows: s (singlet), d (doublet), dd (doublet of a doublet), t (triplet), q (quartet), m (multiplet). For assignment of resonance signals to the appropriate nuclei the following abbreviations were used: Cy (cyclohexyl), Fuc (fucose), Gal (galactose), Glc*N*Ac (*N*-acetylglucosamine), Lac (lactone), MeCy (3-methylcyclohexane-1,2-diol), Sia (sialic acid). Reactions were monitored by TLC using glass plates coated with silica gel 60 F_{254} (Merck) and visualized by using UV light and/or by charring with a molybdate solution (a 0.02 M solution of ammonium cerium sulfate dihydrate and ammonium molybdate tetrahydrate in aqueous 10% H_2SO_4. Column chromatography was performed manually using silica gel 60 (40-63 μm) from Fluka or using

automated systems (RediSep Companion or RediSep Rf) from Teledyne Isco with normal phase RediSep columns from the same manufacturer or reversed-phase columns containing LiChroprep RP-18 (40-63 μm) from Merck KGaA, Darmstadt, Germany. LC-MS separations were carried out using Sunfire C_{18} columns (19 x 150 mm, 5.0 μm) on a Waters 2525 LC, equipped with Waters 2996 photodiode array and Waters micromass ZQ MS for detection. Size exclusion chromatography was performed with Bio-Gel® P-2 Gel (45-90 mm) from Bio-Rad.

Solvents were purchased from Sigma-Aldrich or Acros. Solvents were dried prior to use where indicated. Tetrahydrofurane (THF) was dried by refluxing with sodium/benzophenone and distilled immediately before use. Dichloromethane (CH_2Cl_2) and dimethoxyethane (DME) were dried by filtration over Al_2O_3 (Fluka, type 5016 A basic). Methanol was dried by distillation from sodium methoxide, DMF by distillation from calcium hydride. Optical rotations were measured using a Perkin-Elmer Polarimeter 341. Electron spray ionization mass spectra (ESI-MS) were obtained on a Waters micromass ZQ. HRMS analysis were carried out using a Agilent 1100 LC equipped with a photodiode array detector and a Micromass QTOF I equipped with a 4 GHz digital-time converter. Microanalysis was performed at the Institute of Organic Chemistry at the University of Basel, Switzerland. Purity of final compounds was determined on an Agilent 1100 HPLC; detector ELS, Waters 2420; column: Waters Atlantis dC18, 3 μm, 4.6 x 75 mm; eluents: A: water + 0.1% TFA; B: 90% acetonitrile + 10% water + 0.1% TFA; linear gradient: 0 - 1 min 5% B; 1 - 20 min 5 to 70% B; flow: 0.5 mL/min.

2-(Trimethylsilyl)ethyl (2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-(1→3)-2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranoside (14).

Bromine (0.180 mL, 3.50 mmol) was slowly added to a stirred solution of fucose donor **12** (1.52 g, 3.18 mmol) in anhydrous CH_2Cl_2 (90 mL) at 0°C under argon. After 10 min, excess bromine was quenched by addition of cyclohexene. Powdered activated molecular sieves 4 Å (9 g), TBAB (1.54 g, 4.78 mmol) and **13**[32] (0.65 g, 1.59 mmol) were added and the mixture was stirred at 0°C for 2 h before warming to r.t. and stirring at r.t. overnight. The reaction mixture was filtered (celite) and washed with water (100 mL) and brine (100 mL). The organic layer was dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Column chromatography on silica (PE/Et_2O) afforded **14** as white foam (0.72 g, 0.87 mmol, 55%).

^{1}H NMR (500.1 MHz, $CDCl_3$): δ 7.51-7.23 (m, 20H, Ar-H), 5.59 (d, ^3J = 6.7Hz, 1H, N*H*COCH$_3$), 5.49 (s, C*H*Ph), 5.04 (d, ^3J = 3.2Hz, Fuc-H1), 4.99 (d, ^3J = 8.3Hz, Glc*N*Ac-H1), 4.96-4.88 (m, 2H, C*H*$_2$Ph), 4.75 (s, 2H, C*H*$_2$Ph), 4.65 (B of AB, *J* = 11.7Hz, C*H*$_2$Ph), 4.58 (B' of A'B', *J* = 11.5Hz, C*H*$_2$Ph), 4.39-4.31 (m, 2H, Glc*N*Ac-H3, Glc*N*Ac-H6a), 4.12-4.04 (m, 2H, Fuc-H2, Fuc-H5), 3.98-3.86 (m, 2H, Fuc-H3, C*H*$_2$CH$_2$Si(CH$_3$)$_3$), 3.76 (dd, ^3J = ^2J = 10.0Hz, Glc*N*Ac-H6b), 3.63-3.45 (m, 4H, Fuc-H4, Glc*N*Ac-H4, Glc*N*Ac-H5, C*H*$_2$CH$_2$Si(CH$_3$)$_3$), 3.28 (m, 1H, Glc*N*Ac-H2), 1.62 (s, 3H, COC*H*$_3$), 1.01-0.82 (m, 2H, CH$_2$C*H*$_2$Si(CH$_3$)$_3$), 0.80 (d, ^3J = 6.4Hz, Fuc-H6), 0.01 (s, 9H, CH$_2$CH$_2$Si(C*H*$_3$)$_3$); ^{13}C NMR (125.8 MHz, $CDCl_3$): δ 170.6 (*C*OCH$_3$), 138.9, 138.8, 138.7, 129.2, 128.8, 128.5, 128.4, 128.3, 128.0, 127.9, 127.7, 127.5, 126.4 (24C, C_6H_5), 101.8 (*C*HPh), 100.1 (Glc*N*Ac-C1), 98.5 (Fuc-C1), 81.0 (Glc*N*Ac-C4), 80.0 (Fuc-C3), 77.7, 77.4 (Fuc-C2, Fuc-C4), 75.0 (*C*H$_2$Ph), 74.9 (Glc*N*Ac-C3), 74.3, 72.6 (*C*H$_2$Ph), 69.1 (Glc*N*Ac-C6), 67.5 (*C*H$_2$CH$_2$Si(CH$_3$)$_3$), 66.9 (Fuc-C5), 66.3 (Glc*N*Ac-C5), 59.1 (Glc*N*Ac-C2), 23.4 (CO*C*H$_3$), 18.2 (CH$_2$*C*H$_2$Si(CH$_3$)$_3$), 16.4 (Fuc-C6), -1.3 (3C, CH$_2$CH$_2$Si(*C*H$_3$)$_3$); $[\alpha]_D$ -73.3° (*c* 0.96, $CHCl_3$); MS (ESI) *m*/*z*: calcd for $C_{47}H_{59}NNaO_{10}Si$ $[M+Na]^+$: 848.4; found: 848.4; Elemental analysis calcd (%) for $C_{47}H_{59}NO_{10}Si$: C 68.34, H 7.20, N 1.70; found: C 68.46, H 7.27, N 1.66.

2-(Trimethylsilyl)ethyl (2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-(1→3)-2-acetamido-6-O-benzyl-2-deoxy-β-D-glucopyranoside (15).

To a solution of benzylidene acetal **14** (600 mg, 0.73 mmol) in anhydrous THF (14 mL) were added $Me_3N{\cdot}BH_3$ (212 mg, 2.91 mmol) and anhydrous $AlCl_3$ (580 mg, 4.35 mmol) with stirring under argon at r.t. Once all reagents were dissolved, H_2O (26 μL) was added and the mixture was stirred for 5 h. The reaction was quenched by addition of H_2O (12 mL) and 1 N aq HCl (12 mL) and extracted with CH_2Cl_2 (3 · 30 mL). The organic layers were washed with brine (30 mL), dried over Na_2SO_4, and concentrated. Column chromatography on silica gel (PE/EtOAc 7/3 to 1/1) afforded **15** as white foam (470 mg, 0.57 mmol, 78%).

^{1}H NMR (500.1 MHz, $CDCl_3$): δ 7.43-7.23 (m, 20H, Ar-H), 5.51 (d, ^3J = 7.1Hz, 1H, N*H*COCH$_3$), 4.96 (m, 2H, Fuc-H1, C*H*$_2$Ph), 4.91 (d, ^3J = 8.2Hz, Glc*N*Ac-H1), 4.86-4.57 (m, 7H, C*H*$_2$Ph), 4.13-4.04 (m, 2H, Fuc-H2, Fuc-H5), 4.02-3.89 (m, 3H, Fuc-H3, Glc*N*Ac-H3, C*H*$_2$CH$_2$Si(CH$_3$)$_3$), 3.83 (m, 1H, Glc*N*Ac-H6a), 3.73-3.66 (m, 2H, Fuc-H4, Glc*N*Ac-H6b), 3.59-3.47 (m, 2H, Glc*N*Ac-H5, C*H*$_2$CH$_2$Si(CH$_3$)$_3$), 3.43 (dd, ^3J = 8.8Hz, 1H, Glc*N*Ac-H4), 3.26 (m, Glc*N*Ac-H2), 1.60 (s, 3H, COC*H*$_3$), 1.15 (d, ^3J = 6.3Hz, Fuc-H6), 1.02-0.84 (m, 2H, CH$_2$C*H*$_2$Si(CH$_3$)$_3$), -0.01 (s, 9H, CH$_2$CH$_2$Si(C*H*$_3$)$_3$); ^{13}C NMR (125.8 MHz, $CDCl_3$): δ 170.9

(*C*OCH$_3$), 138.6, 138.4, 128.7, 128.6, 128.5, 128.4, 128.1, 128.0, 127.9, 127.8, 127.7, 127.6 (24C, C$_6$H$_5$), 99.4, 99.3 (Fuc-C1, Glc*N*Ac-C1), 84.1 (Glc*N*Ac-C3), 79.3 (Fuc-C3), 77.4 (Fuc-C4), 76.0 (Fuc-C2), 75.1 (*C*H$_2$Ph), 75.0 (Glc*N*Ac-C5), 74.1, 73.6, 73.0 (*C*H$_2$Ph), 70.7 (Glc*N*Ac-C4), 69.8 (Glc*N*Ac-C6), 68.1 (Fuc-C5), 67.0 (*C*H$_2$CH$_2$Si(CH$_3$)$_3$), 56.7 (Glc*N*Ac-C2), 23.4 (CO*C*H$_3$), 18.2 (CH$_2$*C*H$_2$Si(CH$_3$)$_3$), 16.8 (Fuc-C6), -1.3 (3C, CH$_2$CH$_2$Si(*C*H$_3$)$_3$); $[\alpha]_D$ -36.7° (*c* 0.55, CHCl$_3$); MS (ESI) *m*/*z*: calcd for C$_{47}$H$_{61}$NNaO$_{10}$Si $[M+Na]^+$: 850.4; found: 850.5.

2-(Trimethylsilyl)ethyl (benzyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosynate)-(2→3)-2,4-di-*O*-acetyl-6-*O*-benzoyl-β-D-galactopyranosyl-(1→4)-[2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl-(1→3)]-2-acetamido-6-*O*-benzyl-2-deoxy-β-D-glucopyranoside (17).

16 (300 mg, 0.31 mmol) and **15** (380 mg, 0.46 mmol) were dissolved in anhydrous CH$_2$Cl$_2$ (8 mL). Powdered activated molecular sieves 4 Å (0.8 g) were added and the mixture was stirred at r.t. under argon. After 3.5 h, a solution of DMTST (200 mg, 0.77 mmol) in anhydrous CH$_2$Cl$_2$ (2.0 mL) that had been stirred with molecular sieves 4 Å (0.2 g) for 3.5 h, was added. After stirring for 5 d, the solution was diluted with CH$_2$Cl$_2$ (10 mL), filtered and successively washed with satd aq NaHCO$_3$ (20 mL) and brine (20 mL). The aqueous layers were extracted with CH$_2$Cl$_2$ (3 · 20 mL) and the combined organic layers were dried over Na$_2$SO$_4$ and concentrated under reduced pressure. Column chromatography on silica (PE/EtOAc/MeOH 8/5/0.5 to 8/5/0.9) afforded **17** as white solid (110 mg, 0.06 mmol, 20%).
^{1}H NMR (500.1 MHz, CDCl$_3$): δ 8.09-721 (m, 30H, Ar-H), 6.22 (d, ^3J = 8.1Hz, 1H, Glc*N*Ac-N*H*), 5.62 (ddd, ^3J = 2.5, 5.9, 8.7Hz, 1H, Sia-H8), 5.43-5.32 (m, 2H, Sia-H7, PhC*H*$_2$), 5.22 (d, ^3J = 3.5Hz, 1H, Fuc-H1), 5.20 (d, ^3J = 3.5Hz, 1H, Gal-H4), 5.07-4.95 (m, 3H, Gal-H2, PhC*H*$_2$), 4.92 (m, 1H, Sia-H4), 4.88-4.72 (m, 7H, Gal-H1, Gal-H3, Glc*N*Ac-H1, PhC*H*$_2$), 4.68 (B of AB, *J* = 11.8Hz, 1H, PhC*H*$_2$), 4.61 (A of AB, *J* = 12.0Hz, 1H, PhC*H*$_2$), 4.61 (B of AB, *J* = 12.0Hz, 1H, PhC*H*$_2$), 4.41-4.29 (m, 2H, Gal-H6a, Sia-H9a), 4.23 (dd, ^3J = 7.0Hz, ^2J = 11.0Hz, 1H, Gal-H6b), 4.20-3.96 (m, 8H, Fuc-H2, Fuc-H5, Gal-H5, Glc*N*Ac-H3, Glc*N*Ac-H4, Glc*N*Ac-H6a, Sia-H5, Sia-H9b), 3.93-3.78 (m, 5H, Fuc-H3, Glc*N*Ac-H2, Glc*N*Ac-H5, Glc*N*Ac-H6b, C*H*$_2$CH$_2$Si(CH$_3$)$_3$), 3.56-3.47 (m, 3H, Fuc-H4, Sia-H6, C*H*$_2$CH$_2$Si(CH$_3$)$_3$), 2.64 (dd, ^3J = 4.5, ^2J = 12.6Hz, 1H, Sia-H3^{eq}), 2.27, 2.15, 2.11, 2.03, 2.00, 1.94, 1.88, (7s, 24H, 8 COC*H*$_3$), 1.75 (dd, ^3J =^2J = 12.4Hz, 1H, Sia-H3^{ax}) 1.13 (d, ^3J = 6.5Hz, 3H, Fuc-H6), 1.06-0.77 (m, 2H, CH$_2$C*H*$_2$Si(CH$_3$)$_3$), 0.00 (s, 9H, CH$_2$CH$_2$Si(C*H*$_3$)$_3$); ^{13}C NMR (125.8 MHz, CDCl$_3$): δ 170.7, 170.6, 170.5, 170.4, 170.1, 169.8 (8C, *C*OCH$_3$),

167.5 (Sia-C1), 165.8 (Ar*C*O), 139.2, 139.1, 138.8, 138.6, 134.8, 133.4, 129.8, 129.0, 128.8, 128.6, 128.5, 128.4, 128.3, 127.8, 127.7, 127.6, 127.5, 127.3 (36C, Ar-C), 99.2, 99.1 (Gal-C1, Glc*N*Ac-C1), 97.0 (Sia-C2), 96.5 (Fuc-C1), 79.5 (Fuc-C3), 77.7 (Fuc-C4), 76.6 (Fuc-C2), 74.6 (Ph*C*H_2), 74.2, 73.5, 73.4 (3C, Glc*N*Ac-C3, Glc*N*Ac-C4, Glc*N*Ac-C5), 73.0, 72.9 (3C, C*H*$_2$Ph), 72.2 (Sia-C6), 71.2, 70.7, 70.4, (3C, Gal-C2, Gal-C3, Gal-C5), 69.5 (Glc*N*Ac-C6), 69.4 (Sia-C4), 68.5 (Ph*C*H_2), 67.8, 67.7, 67.2 66.9 (4C, Fuc-C5, Gal-C4, Sia-C7, Sia C8), 66.6 (*C*$H_2CH_2Si(CH_3)_3$), 62.6 (Sia-C9), 61.7 (Gal-C6), 53.4 (Glc*N*Ac-C2), 49.0 (Sia-C5), 37.6 (Sia-C3), 23.3, 23.2, 21.5, 21.0, 20.9, 20.8, 20.7 (8C, CO*C*H_3), 18.0 (CH_2*C*$H_2Si(CH_3)_3$), 16.8 (Fuc-C6), -1.3 (3C, CH_2CH_2Si(*C*H_3)$_3$); $[\alpha]_D$ -24.3° (*c* 0.58, $CHCl_3$); MS (ESI) *m*/*z*: calcd for $C_{90}H_{110}N_2NaO_{30}Si$ $[M+Na]^+$: 1749.7; found: 1749.8.

2-(Trimethylsilyl)ethyl (sodium 5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosynate)-(2→3)-β-D-galactopyranosyl-(1→4)-[α-L-fucopyranosyl-(1→3)]-2-acetamido-2-deoxy-β-D-glucopyranoside (4).

Compound **17** (90 mg, 0.052 mmol) was dissolved in dioxane/water (4/1, 10 mL) under argon. $Pd(OH)_2$/C (20 mg, 10% $Pd(OH)_2$) was added and the resulting mixture was hydrogenated (4 bar H_2) at r.t. After 24 h, the mixture was filtered and the solvent was removed under reduced pressure. The residue was redissolved in 1 N aq NaOH (10 mL) and MeOH (2 mL) and stirred at r.t. After 20 h the mixture was neutralized with 1 N HCl, volatiles were removed under reduced pressure, and crude product was purified *via* SEC and RP chromatography (H_2O/MeOH). Lyophilization from water afforded **4** as white fluffy solid (21 mg, 0.022 mmol, 43%).

^{1}H NMR (500.1 MHz, D_2O, CD_3OD): δ 5.09 (d, ^3J = 4.0Hz, 1H, FucH-1), 4.86-4.74 (m, Fuc-H5), 4.56 (d, ^3J = 8.1Hz, 1H, Glc*N*Ac-H1), 4.52 (d, ^3J = 7.8Hz, 1H, Gal-H1), 4.08 (dd, ^3J = 3.1, 9.8Hz, 1H, Gal-H3), 4.06-3.55 (m, 22H, (C*H*$_2$CH$_2$Si(CH$_3$)$_3$), Fuc-H2, Fuc-H3, Fuc-H4, Gal-H4, Gal-H5, Gal-H6a, Gal-H6b, Glc*N*Ac-H2, Glc*N*Ac-H3, Glc*N*Ac-H4, Glc*N*Ac-H5, Glc*N*Ac-H6a, Glc*N*Ac-H6b, Sia-H4, Sia-H5, Sia-H6, Sia-H7, Sia-H8, Sia-H9a, Sia-H9b), 3.52 (dd, ^3J = 7.8, 9.8Hz, Gal-H2), 2.76 (dd, ^3J = 4.6Hz, ^2J = 12.1Hz, 1H, Sia-H3^{eq}), 2.03, 2.01 (2s, 6H, COC*H*$_3$), 1.79 (t, ^2J =^3J = 12.1Hz, 1H, Sia-H3^{ax}), 1.17 (d, ^3J = 6.6Hz, 3H, Fuc-H6), 1.05-0.80 (m, 2H, (CH$_2$C*H*$_2$Si(CH$_3$)$_3$), 0.00 (s, 9H, (CH$_2$CH$_2$Si(C*H*$_3$)$_3$); ^{13}C NMR (125.8 MHz, D_2O, CD_3OD): δ 176.0, 175.1, 174.8 (3C, NH*C*OCH$_3$, Sia-C1) 102.6 (Gal-C1), 101.2, 100.6 (2C, Glc*N*Ac-C1, Sia-C2), 100.6 (Fuc-C1), 99.6, 76.6, 76.3, 76.0, 75.9, 74.4, 73.9, 72.9, 72.8, 70.2, 69.3, 69.1, 68.7, 68.3, 67.7, 63.6, 62.4, 60.6, 56.7, 52.7, 40.8 (Sia-C3), 23.3, 23.0 (2C, NHCO*C*H$_3$) 18.1 (CH$_2$*C*H$_2$Si(CH$_3$)$_3$), 16.2 (Fuc-C6), -1.5 (3C,

$CH_2CH_2Si(CH_3)_3$); $[\alpha]_D$ -43.9° (*c* 0.70, MeOH); HR-MS (ESI) *m*/*z*: calcd for $C_{36}H_{64}N_2NaO_{23}Si^+$ $[M+H]^+$: 943.3561; found: 943.3553; HPLC purity: > 99.5 %.

(Benzyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosynate)-(2→3)-2,4-di-*O*-acetyl-6-*O*-benzoyl-β-D-galactopyranosyl-(1→1)-[2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl-(1→2)]-(*R*,*R*)-cyclohexane-1,2-diol (20).

Compound **16** (720 mg, 0.75 mmol) and **18** (460 mg, 0.86 mmol) were dissolved in anhydrous CH_2Cl_2 (12 mL). Powdered activated molecular sieves 4 Å (1.2 g) were added and the mixture was stirred at r.t. under argon. After 3.5 h a solution of DMTST (510 mg, 1.97 mmol) in anhydrous CH_2Cl_2 (8 mL) that had been stirred with molecular sieves 4 Å (0.8 g) for 3.5 h was added. After stirring for 60 h, the solution was diluted with CH_2Cl_2 (40 mL), filtered and successively washed with satd aq $NaHCO_3$ (60 mL) and brine (60 mL). The aqueous layers were extracted with CH_2Cl_2 (2 · 60 mL) and the combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure. Column chromatography on silica (PE/EtOAc/MeOH 8/5/0.3 to 8/5/0.7) afforded **20** as white foam (912 mg, 0.64 mmol, 85%).

^{1}H NMR (500.1 MHz, $CDCl_3$): δ 8.05-7.11 (m, 25H, Ar-H), 5.47 (ddd, ^3J = 2.6, 6.1, 9.0Hz, 1H, Sia-H8), 5.25-5.18 (m, 2H, Sia-H7, PhCH_2), 5.02 (d, ^3J = 3.2Hz, 1H, Gal-H4), 4.90 (A' of AB, *J* = 11.9Hz, 1H, PhCH_2), 4.87-4.78 (m, 5H, Fuc-H1, Gal-H2, SiaN*H*, PhCH_2), 4.75 (m, 1H, Sia-H4), 4.71-4.58 (m, 5H, Gal-C1, PhCH_2), 4.50 (dd, ^3J = 3.4, 10.2Hz, 1H, Gal-H3), 4.46 (m, 1H, Fuc-H5), 4.26 (dd, ^3J = 2.6, ^2J = 12.4Hz, 1H, Sia-H9a), 4.22 (dd, ^3J = 6.3Hz, ^2J = 10.8Hz, 1H, Gal-H6a), 4.08-3.83 (m, 6H, Fuc-H2, Fuc-H3, Gal-H6b, Sia-H5, Sia-H9b), 3.60 (m, 1H, Cy), 3.57 (m, 1H, Fuc-H4), 3.52 (m, 1H, Cy), 3.39 (dd, 1H, ^3J = 2.7, 10.7Hz, Sia-H6), 2.52 (dd, ^3J = 4.6, ^2J = 12.6Hz, 1H, Sia-H3^{eq}), 2.10, 2.04, 1.99, 1.94, 1.90, 1.82, 1.75, (7s, 21H, COCH_3), 2.13-1.11 (8H, Cy), 1.62 (m, 1H, Sia-H3^{ax}) 1.07 (d, ^3J = 6.5Hz, 3H, Fuc-H6); ^{13}C NMR (125.8 MHz, $CDCl_3$): δ 170.7, 170.6, 170.4, 170.0, 169.9, 169.6 (7C, *C*OCH_3), 167.5 (Sia-C1), 165.8 (Ar*C*O), 139.5, 139.0, 134.9, 133.3, 130.0, 129.9, 128.9, 128.8, 128.7, 128.5, 128.4, 128.3, 128.1, 127.6, 127.4, (30C, Ar-CH), 99.2 (Gal-C1), 96.8 (Sia-C2), 94.8 (Fuc-C1), 80.1 (Fuc-C3), 78.9 (Cy), 78.0 (Fuc-C4), 76.8 (Fuc-C2), 75.6 (Cy), 74.5, 73.2, (3C, Ph*C*H_2), 72.1, 71.8 (Gal-C3, Sia-C6), 70.1 (2C, Gal-C2, Gal-C5), 69.4 (Sia-C4), 68.4 (Ph*C*H_2), 67.8, 67.7, 67.2 (Gal-C4, Sia-C7, Sia-C8), 66.2 (Fuc-C5), 62.8 (Sia-C9), 61.6 (Gal-C6), 49.0 (Sia-C5), 37.5 (Sia-C3), 29.5, 29.0 23.2, 23.1 (4C, Cy), 23.3, 21.5, 21.0, 20.9, 20.8 (7C, CO*C*H_3), 16.8 (Fuc-C6); $[\alpha]_D$ -24.4° (*c* 0.89, $CHCl_3$); MS (ESI) *m*/*z*:

calcd for $C_{76}H_{89}NNaO_{26}$ $[M+Na]^+$: 1454.6; found: 1454.7; elemental analysis calcd (%) for $C_{76}H_{89}NO_{26} + H_2O$ (1450.53): C 62.93, H 6.32, N 0.97; found: C 63.09, H 6.20, N 0.81.

(Benzyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosynate)-(2→3)-2,4-di-*O*-acetyl-6-*O*-benzoyl-β-D-galactopyranosyl-(1→1)-[2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl-(1→2)]-(1*R*,2*R*,3*S*)-3-methyl-cyclohexane-1,2-diol (21).

Compound **16** (300 mg, 0.31 mmol) and **19** (256 mg, 0.47 mmol) were dissolved in anhydrous CH_2Cl_2 (6.0 mL). Powdered activated molecular sieves 4 Å (0.6 g) were added and the mixture was stirred at r.t. under argon. After 3.5 h a solution of DMTST (246 mg, 0.95 mmol) in anhydrous CH_2Cl_2 (1.9 mL) that had been stirred with molecular sieves 4 Å (0.19 g) for 3.5 h, was added. After stirring for 3 d, the solution was diluted with CH_2Cl_2 (40 mL), filtered, and successively washed with satd aq $NaHCO_3$ (50 mL) and brine (50 mL). The aqueous layers were extracted with CH_2Cl_2 (2 · 50 mL) and the combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure. Column chromatography on silica (PE/EtOAc/MeOH 8/5/0.5 to 8/5/0.7) afforded **21** as white foam (248 mg, 0.17 mmol, 55%).

^{1}H NMR (500.1 MHz, $CDCl_3$): δ 8.04-7.14 (m, 25H, Ar-H), 5.48 (ddd, ^3J = 2.6, 6.2, 9.1Hz, 1H, Sia-H8), 5.21 (A of AB, ^2J = 12.0Hz, 1H, PhCH_2), 5.20 (dd, ^3J = 2.8, 9.4Hz, 1H, Sia-H7), 5.01 (d, ^3J = 3.7Hz, 2H, Fuc-H1, Gal-H4), 4.90 (A' of A'B', J = 12.0Hz, 1H, PhCH_2), 4.88-4.64 (m, 10H, Fuc-H5, Gal-H2, SiaN*H*, Sia-H4, 3 PhCH_2), 4.56 (d, ^3J = 8.0Hz, 1H, Gal-H1), 4.48 (dd, ^3J = 3.3, 10.2Hz, 1H, Gal-H3), 4.25 (dd, ^3J = 2.6Hz, ^2J = 12.4Hz, 1H, Sia-H9a), 4.17 (dd, ^3J = 6.4Hz, ^2J = 10.8Hz, 1H, Gal-H6a), 4.05 (dd, ^3J = 3.7, 10.3Hz, 2H, Fuc-H2), 4.02-3.93 (m, 3H, Fuc-H3, Gal-H6b, Sia-H5), 3.90-3.83 (m, 2H, Gal-H5, Sia-H9b), 3.59 (m, 1H, Fuc-H4), 3.50 (m, 1H, MeCy-H1), 3.37 (dd, 1H, ^3J = 2.7, 10.8Hz, Sia-H6), 3.18 (t, ^3J = 9.2Hz, MeCy-H2), 2.50 (dd, ^3J = 4.6Hz, ^2J =12.7Hz, 1H, Sia-H3^{eq}), 2.09, 2.05 (2s, 6H, 2 COCH_3), 1.99 (m,1H, MeCy), 1.99, 1.94, 1.90, 1.74, 1.72 (5s, 15H, 5 COCH_3), 1.65-1.47 (m, 4H, Sia-H3^{ax}, MeCy) 1.21-1.11 (m, 5H, Fuc-H6, MeCy), 1.03 (d, ^3J = 6.4Hz, 3H, MeCy-CH_3), 0.98 (m, 1H, MeCy); ^{13}C NMR (125.8 MHz, $CDCl_3$): δ 170.8, 170.7, 170.6, 170.4, 169.9, 169.5 (7C, *C*OCH$_3$), 167.5 (Sia-C1), 165.8 (Ar*C*O), 139.3, 139.1, 138.7, 134.9, 133.3, 128.9, 128.7, 128.6, 128.5, 128.4, 128.3, 127.6, 127.4, 127.3 (30C, Ar-C), 99.4 (Gal-C1), 98.3 (Fuc-C1), 96.9 (Sia-C2), 82.2 (MeCy-C2), 80.6, 80.5 (Fuc-C3, MeCy-C1), 77.8 (Fuc-C4), 76.7 (Fuc-C2), 74.6, 74.3, 72.7 (3C, Ph*C*H$_2$), 72.0, 71.9 (Gal-C3, Sia-C6), 70.0, 69.8 (Gal-C2, Gal-C5), 69.4 (Sia-C4), 68.4 (Ph*C*H$_2$), 67.8 (2C, Gal-C4, Sia-C8), 67.1 (Sia C7),

66.3 (Fuc-C5), 62.8 (Sia-C9), 61.5 (Gal-C6), 49.0 (Sia-C5), 39.2 (MeCy-C3) 37.5 (Sia-C3), 33.6 (MeCy-C4), 30.9 (MeCy-C6), 23.3 (*C*H_3CO), 23.1 (MeCy-C5), 21.5, 21.0, 20.9, 20.8 (6C, CO*C*H_3), 18.9 (MeCy-*C*H_3), 17.1 (Fuc-C6); $[\alpha]_D$ -18.9° (*c* 1.01, $CHCl_3$); MS (ESI) *m*/*z*: calcd for $C_{77}H_{91}NNaO_{26}$ $[M+Na]^+$: 1468.6; found: 1468.6; elemental analysis calcd (%) for $C_{77}H_{91}NO_{26}$ + 0.5 H_2O (1455.55): C 63.54, H 6.37, N 0.96; found: C 63.58, H 6.35, N 0.80.

(Sodium 5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosynate)-(2→3)-β-D-galactopyranosyl-(1→1)-[α-L-fucopyranosyl-(1→2)]-(*R*,*R*)-cyclohexane-1,2-diol (5).

Compound **20** (912 mg, 0.64 mmol) was dissolved in dioxane/water (4/1, 50 mL) under argon. $Pd(OH)_2$/C (100 mg, 10% $Pd(OH)_2$) was added and the resulting mixture was hydrogenated (4 bar H_2) at r.t. After 24 h, the mixture was filtered and the solvent was removed under reduced pressure. The residue was redissolved in 1 N aq NaOH (9 mL) and 9 mL MeOH and stirred at r.t. After 3 h the mixture was neutralized with aqueous 1 N HCl, volatiles were removed under reduced pressure, and the crude product was purified *via* SEC and RP chromatography (H_2O/MeOH). Lyophilization from water afforded **5** as a white fluffy solid (371 mg, 0.50 mmol, 79%). Analytical data were in accordance with literature[20]; HR-MS (ESI) *m*/*z*: calcd for $C_{29}H_{48}NNa_2O_{19}$ $[M+Na]^+$: 760.2610; found: 760.2608; HPLC-purity: > 99.5 %.

(Sodium 5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosynate)-(2→3)-β-D-galactopyranosyl-(1→1)-[α-L-fucopyranosyl-(1→2)]-(1*R*,2*R*,3*S*)-3-methyl-cyclohexane-1,2-diol (6).

Compound **21** (310 mg, 0.21 mmol) was dissolved in dioxane/water (4/1, 10 mL) under argon. $Pd(OH)_2$/C (40 mg, 10% $Pd(OH)_2$) was added and the resulting mixture was hydrogenated (4 bar H_2) at r.t. After 24 h, the mixture was filtered and the solvent removed under reduced pressure yielding 220 mg of a white solid, which was directly used for saponification. The crude product (75 mg) was stirred in aqueous NaOH (1 N, 1.5 mL) for 24 h at r.t., lyophilized and purified *via* SEC and RP chromatography (H_2O/MeOH). Lyophilization from water afforded **6** as white fluffy solid (42 mg, 0.056 mmol, 81%).

^{1}H NMR (500.1 MHz, D_2O): δ 5.07 (d, ^3J = 3.6Hz, 1H, Fuc-H1), 5.05-4.71 (m, Fuc-H5), 4.53 (d, ^3J = 7.8Hz, 1H, Gal-H1), 4.05 (dd, ^3J = 2.4, 9.6Hz, 1H, Gal-H3), 3.92 (m, Gal-H4), 3.90-3.50 (m, 15H, Fuc-H2, Fuc-H3, Fuc-H4, Gal-H2, Gal-H5, Gal-H6a, Gal-H6b, MeCy-H1, Sia-H4, Sia-H5, Sia-H6, Sia-H7, Sia-H8, Sia-H9a, Sia-H9b), 3.20 (t, ^3J = 9.6Hz, 1H,

MeCy-H2), 2.73 (dd, ^3J = 4.4Hz, ^2J = 12.1Hz, 1H, Sia-H3^{eq}), 2.13 (m, 1H, MeCy-H6a), 2.00 (s, 3H, COCH_3), 1.78 (t, ^3J =^2J = 12.1Hz, 1H, Sia-H3^{ax}) 1.69-1.52 (m, 3H, MeCy-H3, MeCy-H4a, MeCy-H5a), 1.33-1.17 (m, 2H, MeCy-H5b, MeCy-H6b), 1.15 (d, ^3J = 6.4Hz, 1H, Fuc-H6), 1.11-0.99 (m, 4H, MeCy-CH_3, MeCy-H4b); ^{13}C NMR (125.8 MHz, D_2O, CD_3OD): δ 176.0 (*C*OCH$_3$), 175.0 (Sia-C1), 100.8 (Sia-C2), 100.5 (Gal-C1), 99.8 (Fuc-C1), 85.0 (MeCy-C2), 79.4 (MeCy-C1), 76.9 (Gal-C3), 75.5 (Gal-C5), 73.8 (Sia-C6), 73.0 (Fuc-C4), 72.7 (Sia-C8), 70.2 (Fuc-C3), 69.9 (Gal-C2), 69.4, 69.2, 69.1 (3C, Fuc-C2, Sia-C4, Sia-C7), 68.5 (Gal-C4), 67.5 (Fuc-C5), 63.6 (Sia-C9), 62.6 (Gal-C6), 52.7 (Sia-C5), 40.6 (Sia-C3), 39.8 (MeCy-C3), 34.2 (MeCy-C4), 31.1 (MeCy-C6), 23.6 (MeCy-C5), 23.0 (CO*C*H$_3$), 19.2 (MeCy-*C*H$_3$), 16.4 (Fuc-C6); $[\alpha]_D$ -47.4° (*c* 0.89, MeOH); HR-MS (ESI) *m*/*z*: calcd for $C_{30}H_{50}NNa_2O_{19}$ $[M+Na]^+$: 774.2767; found: 774.2768; HPLC-purity: > 99.5 %.

(5-Acetamido-3,5-dideoxy-*N*-methyl-D-*glycero*-α-D-*galacto*-2-nonulopyranosynylamide)-(2→3)-β-D-galactopyranosyl-(1→1)-[α-L-fucopyranosyl-(1→2)]-(1*R*,2*R*,3*S*)-3-methyl-cyclohexane-1,2-diol (7).

A solution of **6** (40 mg, 0.053 mmol), BnBr (0.019 mL, 0.160 mmol) and KF (8 mg, 0.138 mmol) in anhydrous DMF (3.0 mL) was stirred at r.t. under argon for 2 d. Water (3 mL) was added and the mixture was lyophilized to yield the crude benzyl ester as white solid (80 mg), which was directly used in the next step. The crude ester (20 mg) was dissolved in $MeNH_2$ in THF (2 M, 4 mL) and $MeNH_2$ in EtOH (8 M, 3 mL) and stirred at r.t. under argon for 12 h. Volatiles were removed under reduced pressure and purification *via* RP chromatography (H_2O/MeOH) and lyophilization from water afforded **7** as white fluffy solid (10 mg, 0.013 mmol, quant.).

^{1}H NMR (500.1 MHz, D_2O): δ 5.04 (d, ^3J = 3.7Hz, 1H, Fuc-H1), 4.97-4.70 (m, Fuc-H5), 4.50 (d, ^3J = 7.9Hz, 1H, Gal-H1), 3.97 (dd, ^3J = 2.8, 9.7Hz, 1H, Gal-H3), 3.87-3.53 (m, 15H, Fuc-H2, Fuc-H3, Fuc-H4, Gal-H4, Gal-H5, Gal-H6a, Gal-H6b, MeCy-H1, Sia-H4, Sia-H5, Sia-H6, Sia-H7, Sia-H8, Sia-H9a, Sia-H9b), 3.49 (m, 1H, Gal-H2), 3.17 (t, ^3J = 9.6Hz, 1H, MeCy-H2), 2.74 (s, 3H, CONHCH_3), 2.70 (dd, ^3J = 4.4Hz, ^2J = 12.3Hz, 1H, Sia-H3^{eq}), 2.08 (m, 1H, MeCy-H6a), 1.98 (s, 3H, COCH_3), 1.87 (t, ^3J = ^2J = 12.3Hz, 1H, Sia-H3^{ax}) 1.66-1.47 (m, 3H, MeCy-H3, MeCy-H4a, MeCy-H5a), 1.27-1.14 (m, 2H, MeCy-H5b, MeCy-H6b), 1.11 (d, ^3J = 6.5Hz, 1H, Fuc-H6), 1.07-0.96 (m, 4H, MeCy-CH_3, MeCy-H4b); ^{13}C NMR (125.8 MHz, D_2O, CD_3OD): δ 176.1 (*C*OCH$_3$), 170.8 (Sia-C1), 100.8 (Sia-C2), 100.4 (Gal-C1), 99.8 (Fuc-C1), 84.9 (MeCy-C2), 79.6 (MeCy-C1), 76.7 (Gal-C3), 75.3 (Gal-C5), 74.5 (Sia-C6), 73.0 (Fuc-C4), 72.0 (Sia-C8), 70.2 (Fuc-C3), 69.8 (Gal-C2), 69.2 (2C, Fuc-C2,

Gal-C4), 68.5 (Sia-C7), 68.2 (Sia-C4), 67.4 (Fuc-C5), 64.0 (Sia-C9), 62.3 (Gal-C6), 52.5 (Sia-C5), 39.8 (MeCy-C3), 38.4 (Sia-C3), 34.1 (MeCy-C4), 31.2 (MeCy-C6), 26.7 (CONH*C*H_3), 23.6 (MeCy-C5), 23.0 (CO*C*H_3), 19.1 (MeCy-*C*H_3), 16.4 (Fuc-C6); $[\alpha]_D$ -52.0° (*c* 0.66, MeOH); HR-MS (ESI) *m*/*z*: calcd for $C_{31}H_{54}N_2NaO_{18}$ $[M+Na]^+$: 765.3264 ; found: 765.3260; HPLC-purity: > 99.5 %.

(*R*)-2-Oxotetrahydrofuran-3-yl trifluoromethanesulfonate (23).

(*R*)-3-Hydroxydihydrofuran-2(3*H*)-one (150 mg, 1.47 mmol) and 2,6-di-*tert*-butyl-4-methylpyridine (512 mg, 2.49 mmol) were dissolved in anhydrous CH_2Cl_2 (7.0 mL) under argon. The solution was cooled to -18°C and triflic anhydride (0.37 mL, 0.62 mmol) was added slowly. The solution was stirred at -18°C for 3 h, warmed to r.t., diluted with CH_2Cl_2 (13 mL), and washed with aqueous KH_2PO_4 (2 M, 20 mL). The aqueous layer was extracted with CH_2Cl_2 (2 · 20 mL) and the combined organic layers were dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Column chromatography on silica (PE/EtOAc) afforded the triflate as slightly orange solid (255 mg, 1.09 mmol, 74%), which was directly used in the next step.

^{1}H NMR (500.1 MHz, $CDCl_3$): δ 5.42 (t, ^3J = 8.8Hz, H-3), 4.56 (ddd, ^2J = ^3J = 9.5Hz, ^3J = 2.8 Hz, H-5a), 4.36 (ddd, ^2J = ^3J = 9.5Hz, ^3J = 6.4Hz, H-5b), 2.85 (m, 1H, H-4a), 2.62 (m, 1H, H-4b); ^{13}C NMR (125.8 MHz, $CDCl_3$): δ 168.6 (CO), 118.5 (q, J = 320Hz, *C*F_3), 77.7 (C3), 64.8 (C5), 29.4 (C4); MS (ESI) *m*/*z*: calcd for $C_5H_5F_3NaO_5S^+$ $[M+Na]^+$: 257.0; found: 256.8.

(*R*)-Methyl 2-(((trifluoromethyl)sulfonyl)oxy)butanoate (25).

In analogy to **23,** (*R*)-methyl 2-hydroxybutanoate (200 mg, 1.69 mmol) was reacted with triflic anhydride (0.430 mL, 2.56 mmol) and DTBMP (560 mg, 2.72 mmol) in anhydrous CH_2Cl_2 (8.0 mL) under argon. Workup and column chromatography on silica (PE/EtOAc) afforded the triflate as clear oil (280 mg, 1.12 mmol, 66%), which was directly used in the next step. Analytical data were in accordance with literature.[38]

2,3,4-Tri-*O*-benzoyl-6-*O*-benzyl-β-D-galactopyranosyl-(1→1)-[2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl-(1→2)]-(1*R*,2*R*,3*S*)-3-methyl-cyclohexane-1,2-diol (27)

Powdered activated molecular sieves 4 Å (3.0 g) were added to a solution of **19** (1.50 g, 2.74 mmol) and galactoside **26**[39] (2.60 g, 4.15 mmol) in anhydrous CH_2Cl_2 (20 ml) and the mixture was stirred at r.t. under argon for 4 h. DMTST (2.12 g, 8.21 mmol) was dissolved in

anhydrous CH_2Cl_2 (6.0 ml), powdered activated molecular sieves 4 Å (0.6 g) were added and the suspension was stirred at r.t. under argon for 4 h as well. Subsequently, the two suspensions were combined and stirred at r.t. under argon for 16 h. The mixture was diluted with CH_2Cl_2 (30 mL), filtered (celite), and washed with satd. aqueous $NaHCO_3$ (100 mL) and brine (100 mL). The aqueous layers were extracted with CH_2Cl_2 (3 · 50 mL) and the combined organic layers were dried over Na_2SO_4 and concentrated. Column chromatography on silica (PE/EtOAc 5/1) afforded **27** as white foam (2.03 g, 1.83 mmol, 67%).

^{1}H NMR (500.1 MHz, $CDCl_3$): δ 7.98, 7.94, 7.80 (3m, 6H, Ar-H), 7.56-7.07 (m, 29H, Ar-H), 5.97 (d, ^3J = 3.4Hz, Gal-H-4), 5.71 (dd, ^3J = 8.2, 10.3Hz, Gal-H2), 5.52 (dd, ^3J = 3.5, 10.3Hz, Gal-H3), 5.12 (d, ^3J = 3.3Hz, 1H, Fuc-H1), 4.94 (A of AB, ^2J = 11.5Hz, 1H, CH_2Ph), 4.90 (q, ^3J = 6.5, 6.4Hz, 1H, Fuc-H5), 4.84-4.79 (m, 2H, CH_2Ph, Gal-H1), 4.74 (B' of A'B', ^2J = 11.5Hz, 1H, CH_2Ph), 4.70 (A of AB, ^2J = 11.5Hz, 1H, CH_2Ph), 4.94 (A of AB, ^2J = 11.5Hz, 1H, CH_2Ph), 4.58-4.53 (m, 2H, CH_2Ph), 4.48 (A of AB, ^2J = 11.9Hz, 1H, CH_2Ph), 4.35 (B of AB, ^2J = 11.9Hz, 1H, CH_2Ph), 4.12-4.03 (m, 3H, Fuc-H2, Fuc-H3, Gal-H5), 3.75-3.64 (m, 3H, Fuc-H4, Gal-H6a, MeCy-H1), 3.60 (m, 1H, Gal-H6b), 3.29 (t, ^3J = 9.2Hz, 1H, MeCy), 2.03 (m, 1H, MeCy), 1.70-1.49 (m, 3H, MeCy), 1.43 (d, ^3J = 6.5Hz, 3H, Fuc-H6), 1.29-1.10 (m, 2H, MeCy), 1.07 (d, ^3J = 6.6Hz, 3H, MeCy-CH_3), 0.92 (m, 1H, MeCy); ^{13}C NMR (125.8 MHz, $CDCl_3$): δ 165.8, 165.7, 165.1 (3C, *C*OC_6H_5), 139.3, 139.1, 138.6, 137.6133.6, 133.3, 129.9, 129.8, 129.7, 129.1, 128.7, 128.5, 128.4, 128.3, 127.9, 127.8, 127.7, 127.5, 127.3 (42 C, Ar-C), 100.0 (Gal-C1), 97.9 (Fuc-C1), 81.3 (MeCy-C2), 81.0, (MeCy-C1), 80.4 (Fuc-C3), 79.6 (Fuc-C4), 76.4 (Fuc-C2), 75.2, 74.4, 73.8 (3C, *C*H_2Ph), 72.8 (2C, *C*H_2Ph, Gal-C5), 72.4 (Gal-C3), 69.8 (Gal-C2), 68.8 (Gal-C4), 67.8 (Gal-C6), 66.6 (Fuc-C5), 39.0 (MeCy-C3), 33.0 (MeCy-C4), 30.9 (MeCy-C6), 22.8 (MeCy-C5), 18.9 (MeCy-*C*H_3), 17.1 (Fuc-C6); $[\alpha]_D$ +1.22° (*c* 1.28, $CHCl_3$); MS (ESI) *m*/*z*: calcd for $C_{68}H_{70}NaO_{14}^+$ $[M+Na]^+$: 1133.5; found: 1133.5; elemental analysis calcd (%) for $C_{68}H_{70}O_{14}$ + 0.5 H_2O: C 72.90, H 6.39; found: C 73.03, H 6.59.

6-*O*-Benzyl-β-D-galactopyranosyl-(1→1)-[2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl-(1→2)]-(1*R*,2*R*,3*S*)-3-methyl-cyclohexane-1,2-diol (28).

A freshly prepared solution of NaOMe in MeOH (3 N, 0.18 mL) was slowly added to a solution of **27** (500 mg, 0.45 mmol) in anhydrous MeOH (7.0 mL) under argon at r.t. After 22 h, the mixture was neutralized with aqueous 1 N HCl and concentrated under reduced pressure. Column chromatography on silica (CH_2Cl_2/MeOH 35/1) afforded **28** as white foam (311 mg, 0.39 mmol, 87%).

^{1}H NMR (500.1 MHz, $CDCl_3$): δ 7.31-7.14 (m, 20H, Ar-H), 5.03 (d, ^3J = 3.6Hz, 1H, Fuc-H1), 4.86 (A of AB, ^2J = 11.5Hz, 1H, CH_2Ph), 4.74 (A of AB, ^2J = 11.5Hz, 1H, CH_2Ph), 4.71-4.64 (m, 2H, CH_2Ph), 4.60 (B of AB, ^2J = 11.5Hz, 1H, CH_2Ph), 4.54-4.43 (m, 4H, Fuc-H5, CH_2Ph), 4.25 (1H, ^3J = 6.8Hz, Gal-H1), 4.00 (dd, ^3J = 3.6, 10.3Hz, 1H, Fuc-H2), 3.96-3.90 (m, 2H, Fuc-H3, Gal-H4), 3.75-3.57 (m, 4H, Fuc-H4, Gal-H6a,b, MeCy-H1), 3.55-3.45 (m, 3H, Gal-H2, Gal-H3, Gal-H5), 3.17 (t, ^3J = 9.4Hz, 1H, MeCy-H2), 2.06 (m, 1H, MeCy), 1.64-1.47 (m, 3H, MeCy), 1.29-1.08 (m, 2H, MeCy), 1.06 (d, ^3J = 6.5Hz, 3H, Fuc-H6), 1.02 (d, ^3J = 6.3Hz, 3H, MeCy-CH_3), 0.94 (m, 1H, MeCy); ^{13}C NMR (125.8 MHz, $CDCl_3$): δ 139.0, 138.5, 137.9, 128.7, 128.5, 128.3, 128.0, 127.7, 127.6, 127.5, 127.4 (24C, Ar-C), 99.7 (Gal-C1), 98.0 (Fuc-C1), 84.1 (MeCy-C2), 79.9 (Fuc-C3), 78.7 (MeCy-C1), 78.3 (Fuc-C4), 76.5 (Fuc-C2), 75.0, 74.4, 73.6 (3C, *C*H_2Ph), 73.6 73.1, 71.5 (Gal-C2, Gal-C3, Gal-C5), 72.7 (*C*H_2Ph), 69.5 (Gal-C6), 68.7 (Gal-C4), 66.7 (Fuc-C5), 38.8 (MeCy-C3), 33.7 (MeCy-C4), 31.1 (MeCy-C6), 23.2 (MeCy-C5), 19.1 (MeCy-*C*H_3), 17.0 (Fuc-C6); $[\alpha]_D$ - 56.1° (*c* 0.94, $CHCl_3$); MS (ESI) *m*/*z*: calcd for $C_{47}H_{58}NaO_{11}^+$ $[M+Na]^+$: 821.4; found: 821.5; elemental analysis calcd (%) for $C_{47}H_{58}O_{11}$: C 70.66, H 7.32; found: C 70.50, H 7.56.

6-*O*-Benzyl-3-*O*-((*S*)-2-oxotetrahydrofuran-3-yl)-β-D-galactopyranosyl-(1→1)-[2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl-(1→2)]-(1*R*,2*R*,3*S*)-3-methyl-cyclohexane-1,2-diol (29).

Compound **28** (600 mg, 0.75 mmol) and Bu_2SnO (300 mg, 1.21 mmol) were suspended in anhydrous toluene (5.3 mL) and anhydrous MeOH (2.7 mL) and stirred at 60°C under argon for 4 h. Volatiles were evaporated under reduced pressure and the resulting white solid was dried in high vacuum overnight. The residue was dissolved in anhydrous DME (10 mL) under argon, and extensively dried CsF (345 mg, 2.27 mmol) and **23** (530 mg, 2.26 mmol) were added. After 24 h, the solution was diluted with CH_2Cl_2 (100 mL) and washed with a 20% aqueous solution of KF (2 · 100 mL) and brine (100 mL). The aqueous layers were extracted with CH_2Cl_2 (2 · 100 mL) and the combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure. Column chromatography on silica (CH_2Cl_2/*i*-propanol) afforded **29** (360 mg, 0.39 mmol, 52%) as white fluffy solid. **28** was recovered in 17% (100 mg, 0.13 mmol).

^{1}H NMR (500.1 MHz, $CDCl_3$): δ 7.30-7.13 (m, 20H, Ar-H), 5.00 (d, ^3J = 3.4Hz, 1H, Fuc-H1), 4.86 (A of AB, ^2J = 11.4Hz, 1H, CH_2Ph), 4.74 (A' of A'B', ^2J = 11.7Hz, 1H, CH_2Ph), 4.70-4.59 (m, 3H, CH_2Ph), 4.55 (dd, ^3J = 6.5Hz, 1H, Fuc-H5), 4.51 (B of AB, ^2J = 11.4Hz, CH_2Ph), 4.48-4.43 (m, 3H, CH_2Ph, Lac-H-2), 4.34 (m, 1H, Lac-H-4a), 4.24 (d, ^3J = 7.7Hz, 1H, Gal-H1), 4.12 (m, 1H, lac-H-4b), 4.07 (d, ^3J = 2.6Hz, 1H, Gal-H4), 3.99 (dd, ^3J = 3.4,

10.3Hz, 1H, Fuc-H2), 3.95 (dd, ^3J = 2.6, 10.3Hz, 1H, Fuc-H3), 3.74 (dd, ^3J = 6.4Hz, ^2J = 9.5Hz, 1H, Gal-H6a), 3.71-3.60 (m, 4H, Gal-H2, Gal-H6b, MeCy-H1, Fuc-H4), 3.58 (dd, ^3J = 2.6, 9.3Hz, 1H, Gal-H3), 3.51 (q, ^3J = 6.4Hz, 1H, Gal-H5), 3.16 (t, ^3J = 9.4Hz, 1H, MeCy-H2), 2.53 (m, 1H, lac-H-3a), 2.32 (m, 1H, lac-H-3b), 2.04 (m, 1H, MeCy), 1.61-1.49 (m, 3H, MeCy), 1.29-1.08 (m, 2H, MeCy), 1.06 (d, ^3J = 6.4Hz, 3H, Fuc-H6), 1.03 (d, ^3J = 6.3Hz, 3H, MeCy-CH_3), 0.93 (m, 1H, MeCy); ^{13}C NMR (125.8 MHz, $CDCl_3$): δ 176.8 (*C*O), 139.1, 138.6, 138.0, 128.6, 128.5, 128.4, 128.3, 127.9, 127.8, 127.6, 127.5, 127.4 (24C, Ar-C), 100.1 (Gal-C1), 98.3 (Fuc-C1), 83.8 (MeCy-C2), 82.9 (Gal-C3), 80.0 (Fuc-C3), 79.0 (MeCy-C1), 78.3 (Fuc-C4), 76.3 (Fuc-C2), 75.1, 74.5, 74.3 (3C, *C*H_2Ph), 73.8 (lac-C2), 73.1 (Gal-C5), 72.6 (*C*H_2Ph), 70.8 (Gal-C2), 69.2 (Gal-C6), 67.3 (Gal-C4), 66.6 (Fuc-C5), 65.4 (lac-C-4), 39.0 (MeCy-C3), 33.7 (MeCy-C4), 31.2 (MeCy-C6), 30.1 (lac-C3), 23.2 (MeCy-C5), 19.1 (MeCy-*C*H_3), 16.9 (Fuc-C6); $[\alpha]_D$ -56.5 (*c* 0.90, $CHCl_3$); MS (ESI) *m*/*z*: calcd for $C_{51}H_{62}NaO_{13}^+$ $[M+Na]^+$: 905.41; found: 605.64; elemental analysis calcd (%) for $C_{51}H_{62}O_{13}$ (883.03): C 69.37, H 7.08; found: C 69.14, H 6.93.

3-*O*-(Sodium (*S*)-1-carboxy-3-hydroxypropyl)-β-D-galactopyranosyl-(1→1)-[α-L-fucopyranosyl-(1→2)]-(1*R*,2*R*,3*S*)-3-methyl-cyclohexane-1,2-diol (10)

and

3-*O*-((*S*)-4-Hydroxy-1-(benzylamino)-1-oxobutan-2-yl)-β-D-galactopyranosyl-(1→1)-[α-L-fucopyranosyl-(1→2)]-(1*R*,2*R*,3*S*)-3-methyl-cyclohexane-1,2-diol (9).

Compound **29** (270 mg, 0.31 mmol) was dissolved in dioxane/water (4/1, 3.5 mL) under argon. $Pd(OH)_2$/C (30 mg, 10% $Pd(OH)_2$) was added and the resulting mixture was hydrogenated (1 bar H_2) at r.t. After 21 h, the mixture was filtered and the solvent removed under reduced pressure to give the debenzylated intermediate as white foam, which was directly used in the next step (160 mg, 0.31 mmol).

10: The intermediate (23 mg, 0.044 mmol) was stirred in dioxane (0.5 mL) and aqueous NaOH (1 N, 0.5 mL) for 24 h. Dioxane was removed under reduced pressure and the residue was purified *via* RP chromatography (H_2O/MeOH) and lyophilized from water to afford **10** as white fluffy solid (17 mg, 0.03 mmol, 69%).

^{1}H NMR (500.1 MHz, CD_3OD): δ 5.00 (d, ^3J = 4.0Hz, 1H, Fuc-H1), 4.93-4.85 (m, 1H, Fuc-H5), 4.30 (d, ^3J = 7.9Hz, 1H, Gal-H1), 3.93 (dd, ^3J = 3.3, 9.9Hz, 1H, COC*H*CH_2CH_2OH), 3.89-3.79 (m, 3H, $COCHCH_2CH_2OH$, Fuc-H3, Gal-H4), 3.78-3.61 (m, 7H, $COCHCH_2CH_2OH$, Fuc-H2, Fuc-H4, Gal-H2, Gal-H6a,b, MeCy-H1), 3.44 (m, 1H, Gal-H5), 3.27 (t, ^3J = 3.1, 9.5Hz, 1H, Gal-H3), 3.21 (t, ^3J = 9.3Hz, 1H, MeCy), 2.14-2.02 (m, 2H,

COCHC*H*$_2$CH$_2$OH, MeCy), 1.88 (m, 1H, COCHC*H*$_2$CH$_2$OH), 1.71-1.58 (m, 3H, MeCy), 1.39-1.22 (m, 2H, MeCy), 1.19 (d, ^3J = 6.6Hz, 3H, Fuc-H6), 1.13 (d, ^3J = 6.3Hz, 3H, MeCy-C*H*$_3$), 1.06 (m, 1H, MeCy); ^{13}C NMR (125.8 MHz, CD$_3$OD): δ 182.0 (*C*OCHCH$_2$CH$_2$OH), 102.2 (Gal-C1), 100.3 (Fuc-C1), 85.6 (Gal-C3), 84.5 (MeCy-C2), 81.4 (CO*C*HCH$_2$CH$_2$OH), 79.8 (MeCy-C1), 75.9 (Gal-C5), 73.8 (Fuc-C4), 71.3, 71.1 (Fuc-C3, Gal-C2), 70.3 (Fuc-C2), 67.6 (Fuc-C5, Gal-C4), 63.2 (Gal-C6), 61.0 (COCHCH$_2$*C*H$_2$OH), 40.4 (MeCy-C3), 37.7 (COCH*C*H$_2$CH$_2$OH), 35.0 (MeCy-C4), 31.9 (MeCy-C6), 24.2 (MeCy-C5), 19.6 (MeCy-*C*H$_3$), 16.7 (Fuc-C6); [α]$_D$ -89.0 (*c* 0.67, MeOH); HR-MS (ESI) *m*/*z*: calcd for $C_{23}H_{39}Na_2O_{14}^+$ [M+Na]$^+$: 585.2130 ; found: 585.2127; HPLC-purity: > 99.5 %.

9: Benzyl amine (0.063 mL, 0.577 mmol) was added to a stirred solution of the intermediate (25 mg, 0.048 mmol) in anhydrous THF (1.0 mL) at r.t. under argon. After 20 h, volatiles were evaporated under reduced pressure, the residue was purified *via* RP chromatography (H$_2$O/MeOH) and lyophilized from water to afford **9** as white fluffy solid (23 mg, 0.037 mmol, 76%).

^{1}H NMR (500.1 MHz, CD$_3$OD): δ 7.35-7.23 (m, 5H, C$_6$H$_5$), 5.00 (d, ^3J = 4.0Hz, 1H, Fuc-H1), 4.93-4.83 (m, 1H, Fuc-H5), 4.45 (A of AB, ^2J = 14.8Hz, 1H, C*H*$_2$Ph), 4.38 (B of AB, ^2J = 14.8Hz, 1H, C*H*$_2$Ph), 4.28 (d, ^3J = 7.7Hz, 1H, Gal-H1), 4.25 (dd, ^3J = 3.8, 8.9Hz, 1H, COC*H*CH$_2$CH$_2$OH), 3.88-3.79 (m, 3H, COCHCH$_2$C*H*$_2$OH, Fuc-H3, Gal-H4), 3.78-3.61 (m, 6H, COCHCH$_2$C*H*$_2$OH, Fuc-H2, Fuc-H4, Gal-H2, Gal-H6a, MeCy-H1), 3.58 (dd, ^3J = 4.8, 11.4Hz, Gal-H6b), 3.38-3.32 (m, 2H, Gal-H3, Gal-H5), 3.20 (t, ^3J = 9.3Hz, 1H, MeCy), 2.09 (m, 1H, MeCy), 2.03 (m, 1H, COCHC*H*$_2$CH$_2$OH), 1.88 (m, 1H, COCHC*H*$_2$CH$_2$OH), 1.72-1.55 (m, 3H, MeCy), 1.40-1.21 (m, 2H, MeCy), 1.17 (d, ^3J = 6.6Hz, 3H, Fuc-H6), 1.13 (d, ^3J = 6.3Hz, 3H, MeCy-C*H*$_3$), 1.07 (m, 1H, MeCy); ^{13}C NMR (125.8 MHz, CD$_3$OD): δ 176.0 (*C*OCHCH$_2$CH$_2$OH), 139.8, 129.6, 128.6, 128.4 (6C, *C*$_6$H$_5$), 102.2 (Gal-C1), 100.4 (Fuc-C1), 84.8, 84.6 (Gal-C3, MeCy-C2), 80.3, 79.9 (CO*C*HCH$_2$CH$_2$OH, MeCy-C1), 75.9 (Gal-C5), 73.8 (Fuc-C4), 71.5, 71.4 (Fuc-C3, Gal-C2), 70.3 (Fuc-C2), 68.3 (Gal-C4), 67.5 (Fuc-C5), 62.9 (Gal-C6), 59.4 (COCHCH$_2$*C*H$_2$OH), 43.8 (*C*H$_2$Ph), 40.4 (MeCy-C3), 37.3 (COCH*C*H$_2$CH$_2$OH), 34.9 (MeCy-C4), 31.8 (MeCy-C6), 24.2 (MeCy-C5), 19.6 (MeCy-*C*H$_3$), 16.7 (Fuc-C6); [α]$_D$ -77.6 (*c* 0.86, MeOH); HR-MS (ESI) *m*/*z*: calcd for $C_{30}H_{47}NNaO_{13}^+$ [M+Na]$^+$: 652.2945 ; found: 652.2940; HPLC-purity: > 99.5 %.

6-*O*-Benzyl-3-*O*-((*S*)-1-methoxy-1-oxobutan-2-yl)-β-D-galactopyranosyl-(1→1)-[2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl-(1→2)]-(1*R*,2*R*,3*S*)-3-methyl-cyclohexane-1,2-diol (30).

Compound **28** (150 mg, 0.19 mmol) and Bu_2SnO (70 mg, 0.28 mmol) were suspended in anhydrous toluene (1.4 mL) and anhydrous MeOH (0.6 mL) and stirred at 60°C under argon for 4 h. Volatiles were evaporated under reduced pressure and the resulting white solid was dried in high vacuum overnight. The residue was dissolved in anhydrous DME (3.0 mL) under argon, and extensively dried CsF (90 mg, 0.59 mmol) and **25** (130 mg, 0.52 mmol) were added. The reaction was monitored by TLC (CH_2Cl_2/*i*-propanol 95/5) and after 18 h, the solution was diluted with CH_2Cl_2 (30 mL) and washed with a 20% aqueous solution of KF (2 · 30 mL) and brine (30 mL). The aqueous layers were extracted with CH_2Cl_2 (2 · 30 mL) and the combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure. Column chromatography on silica (CH_2Cl_2/*i*-propanol) afforded **30** (126 mg, 0.14 mmol, 75%) as white fluffy solid.

^{1}H NMR (500.1 MHz, $CDCl_3$): δ 7.31-7.13 (m, 20H, Ar-H), 5.01 (d, ^3J = 3.3Hz, 1H, Fuc-H1), 4.87 (A of AB, ^2J = 11.5Hz, 1H, C*H*$_2$Ph), 4.74 (A' of A'B', ^2J = 11.7Hz, 1H, C*H*$_2$Ph), 4.70-4.64 (m, 2H, C*H*$_2$Ph), 4.64-4.56 (m, 2H, C*H*$_2$Ph, Fuc-H5), 4.53 (B of AB, ^2J = 11.5Hz, CH_2Ph), 4.49-4.41 (m, 2H, C*H*$_2$Ph), 4.22 (d, ^3J = 7.8Hz, 1H, Gal-H1), 4.06 (dd, ^3J = 4.1, 8.2Hz, COC*H*CH_2CH_3), 3.99 (dd, ^3J = 3.3, 10.3Hz, 1H, Fuc-H2), 3.95 (dd, ^3J = 2.4, 10.3Hz, 1H, Fuc-H3), 3.86 (d, ^3J = 2.9Hz, 1H, Gal-H4), 3.76-3.58 (m, 8H, Fuc-H4, Gal-H2, Gal-H6a,b, MeCy-H1, COC*H*$_3$), 3.47 (t, ^3J = 6.1Hz, 1H, Gal-H5), 3.23 (dd, ^3J = 3.2, 9.3Hz, 1H, Gal-H3), 3.17 (t, ^3J = 9.3Hz, 1H, MeCy-H2), 2.03 (m, 1H, MeCy), 1.86-1.64 (m, 2H, COCHC*H*$_2$CH$_3$), 1.60-1.48 (m, 3H, MeCy), 1.29-1.10 (m, 2H, MeCy), 1.07 (d, ^3J = 6.5Hz, 3H, Fuc-H6), 1.02 (d, ^3J = 6.3Hz, 3H, MeCy-C*H*$_3$), 0.96 (t, ^3J = 7.4Hz, 3H, COCHCH$_2$C*H*$_3$), 0.91 (m, 1H, MeCy); ^{13}C NMR (125.8 MHz, $CDCl_3$): δ 174.7 (*C*O_2CH_3), 139.2, 138.6, 138.2, 128.6, 128.5, 128.4, 128.3, 128.2, 127.8, 127.7, 127.6, 127.5, 127.4 (24C, Ar-C), 100.2 (Gal-C1), 98.1 (Fuc-C1), 83.5 (MeCy-C2), 82.9 (Gal-C3), 80.2 (CO*C*HCH_2CH_3), 80.0 (Fuc-C3), 78.8 (MeCy-C1), 78.5 (Fuc-C4), 76.2 (Fuc-C2), 75.1, 74.3, 73.7 (3C, *C*H_2Ph), 73.2 (Gal-C5), 72.7 (*C*H_2Ph), 70.9 (Gal-C2), 69.2 (Gal-C6), 66.8, 66.7 (Fuc-C5, Gal-C4), 52.3 (CO_2*C*H_3), 38.9 (MeCy-C3), 33.7 (MeCy-C4), 31.2 (MeCy-C6), 26.9 (COCH*C*H_2CH_3) 23.2 (MeCy-C5), 19.1 (MeCy-*C*H_3), 16.9 (Fuc-C6), 10.0 (COCHCH$_2$*C*H_3); $[\alpha]_D$ -59.1 (*c* 1.44, $CHCl_3$); MS (ESI) *m/z*: calcd for $C_{52}H_{66}NaO_{13}^+$ $[M+Na]^+$: 921.44; found: 921.50; elemental analysis calcd (%) for $C_{52}H_{66}O_{13}$ (899.07): C 69.47, H 7.40; found: C 69.38 , H 7.38.

3-*O*-(Sodium (*S*)-1-carboxypropyl)-β-D-galactopyranosyl-(1→1)-[α-L-fucopyranosyl-(1→2)]-(1*R*,2*R*,3*S*)-3-methyl-cyclohexane-1,2-diol (11).

Compound **30** (95 mg, 0.11 mmol) was dissolved in dioxane/water (4/1, 5 mL) under argon. $Pd(OH)_2/C$ (30 mg, 10% $Pd(OH)_2$) was added and the resulting mixture was hydrogenated (1 bar H_2) at r.t. After 17 h, the mixture was filtered and the solvent removed under reduced pressure yielding 57 mg of a white solid, which was directly used for saponification. The crude product was stirred in a solution of aqueous NaOH (1 N, 0.5 mL) and dioxane (1.5 mL) at r.t. for 24 h, lyophilized from water and purified *via* HPLC. Lyophilization from water afforded **11** as white fluffy solid (8 mg, 0.015 mmol, 14%).

^{1}H NMR (500.1 MHz, CD_3OD): δ 5.00 (d, ^3J = 3.9Hz, 1H, Fuc-H1), 4.93-4.77 (m, 1H, Fuc-H5), 4.27 (d, ^3J = 7.7Hz, 1H, Gal-H1), 4.16 (dd, ^3J = 4.3, 7.7Hz, 1H, COC*H*CH_2CH_3), 3.91 (d, ^3J = 2.2Hz, 1H, Gal-H4), 3.84 (dd, ^3J = 3.2, 10.2Hz, 1H, Fuc-H3), 3.78-3.60 (m, 6H, Fuc-H2, Fuc-H4, Gal-H2, Gal-H6a,b, MeCy-H1), 3.41 (t, ^3J = 5.8Hz, 1H, Gal-H5), 3.34-3.26 (m, 1H, Gal-H3), 3.20 (t, ^3J = 9.3Hz, 1H, MeCy-H2), 2.10 (m, 1H, MeCy), 1.90, 1.77 (2m, 2H, COCHC$H_2$$CH_3$), 1.71-1.55 (m, 3H, MeCy), 1.38-1.22 (m, 2H, MeCy), 1.19 (d, ^3J = 6.5Hz, 3H, Fuc-H6), 1.13 (d, ^3J = 6.3Hz, 3H, MeCy-CH_3), 1.11-1.01 (m, 4H, $COCHCH_2CH_3$, MeCy); ^{13}C NMR (125.8 MHz, CD_3OD): δ 102.4 (Gal-C1), 100.4 (Fuc-C1), 84.6, 84.4 (Gal-C3, MeCy-C2), 80.0 (MeCy-C1), 75.9 (Gal-C5), 73.8 (Fuc-C4), 71.8, 71.4 (Fuc-C3, Gal-C2), 70.3 (Fuc-C2), 68.1 (Gal-C4), 67.5 (Fuc-C5), 62.9 (Gal-C6), 40.4 (MeCy-C3), 34.9 (MeCy-C4), 31.9 (MeCy-C6), 27.6 (COCH*C*H_2CH_3), 24.2 (MeCy-C5), 19.6 (MeCy-*C*H_3), 16.7 (Fuc-C6), 10.1 ($COCHCH_2CH_3$); $[\alpha]_D$ -79.1 (*c* 0.62, MeOH); HR-MS (ESI) *m*/*z*: calcd for $C_{23}H_{40}NaO_{13}^+$ $[M+Na]^+$: 547.2361 ; found: 547.2363; HPLC-purity: > 99.5 %.

6-*O*-Benzyl-3-*O*-((*S*)-4-hydroxy-1-(methylamino)-1-oxobutan-2-yl)-β-D-galactopyranosyl-(1→1)-[2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl-(1→2)]-(1*R*,2*R*,3*S*)-3-methyl-cyclohexane-1,2-diol (31).

Compound **28** (60 mg, 0.075 mmol) and Bu_2SnO (28 mg, 0.112 mmol) were suspended in anhydrous toluene (1.4 mL) and anhydrous MeOH (0.6 mL) and stirred at 50°C under argon for 4 h. Volatiles were evaporated under reduced pressure and the resulting white solid was dried in high vacuum overnight. The residue was dissolved in anhydrous DME (1.0 mL) under argon, and extensively dried CsF (35 mg, 0.23 mmol) and **23** (53 mg, 0.226 mmol) were added. The reaction was monitored by TLC (CH_2Cl_2/*i*-propanol 95/5) and upon completion NH_2Me in THF (2 M, 4 mL) was added. The solution was stirred for 30 min, diluted with CH_2Cl_2 (30 mL) and washed with a 20% aqueous solution of KF (2 ·30 mL) and

brine (30 mL). The aqueous layers were extracted with CH_2Cl_2 (3 · 30 mL) and the combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure. Column chromatography on silica (CH_2Cl_2/*i*-propanol) afforded **31** (35 mg, 0.038 mmol, 51%) as white fluffy solid. Starting material **28** was recovered in 33% (20 mg, 0.025 mmol).

^{1}H NMR (500.1 MHz, $CDCl_3$): δ 7.49 (d, ^3J = 4.1Hz, CON*H*CH$_3$), 7.29-7.14 (m, 20H, Ar-H), 5.01 (d, ^3J = 3.6Hz, 1H, Fuc-H1), 4.56 (A of AB, ^2J = 11.5Hz, 1H, C*H*$_2$Ph), 4.76-4.63 (m, 3H, C*H*$_2$Ph), 4.56 (B of AB, ^2J = 11.5Hz, 1H, C*H*$_2$Ph), 4.52-4.45 (m, 3H, Fuc-H5, C*H*$_2$Ph), 4.40 (B of AB, ^2J = 11.9Hz, 1H, C*H*$_2$Ph), 4.26 (1H, ^3J = 7.7Hz, Gal-H1), 4.04 (m, 1H, COC*H*CH$_2$CH$_2$OH), 3.99 (dd, ^3J =3.6, 10.3Hz, 1H, Fuc-H2), 3.95-3.89 (m, 2H, Fuc-H3, Gal-H4), 3.84-3.61 (m, 6H, Gal-H2, Gal-H6a,b, MeCy-H1, COCHCH$_2$C*H*$_2$OH), 3.57 (m, 1H, Fuc-H4), 3.38 (m, 1H, Gal-H5), 3.16 (t, ^3J = 9.3Hz, 1H, MeCy-H2), 2.75 (d, ^3J = 4.1Hz, 3H, CONHC*H*$_3$), 2.11-2.01 (m, 2H, MeCy, COCHC*H*$_2$CH$_2$OH), 1.84 (m, 1H, COCHC*H*$_2$CH$_2$OH), 1.61-1.49 (m, 3H, MeCy), 1.30-1.10 (m, 2H, MeCy), 1.08 (d, ^3J = 6.4Hz, 3H, Fuc-H6), 1.01 (d, ^3J = 6.2Hz, 3H, MeCy-C*H*$_3$), 0.92 (m, 1H, MeCy); ^{13}C NMR (125.8 MHz, $CDCl_3$): δ 174.0 (*C*ONHCH$_3$), 139.0, 138.4, 137.4, 128.7, 128.5, 128.4, 128.3, 128.2, 127.7, 127.6, 127.5, 127.3 (24C, Ar-C), 100.1 (Gal-C1), 97.9 (Fuc-C1), 84.1, 83.9 (Gal-C3, MeCy-C2), 81.1 (CO*C*HCH$_2$CH$_2$OH), 79.9 (Fuc-C3), 78.8 (MeCy-C1), 78.4 (Fuc-C4), 76.4 (Fuc-C2), 75.0, 74.3, 73.9 (3C, *C*H$_2$Ph), 72.8 (*C*H$_2$Ph), 72.5 (Gal-C5), 70.3 (Gal-C6), 69.7 (Gal-C2), 67.8 (Gal-C4), 66.8 (Fuc-C5), 59.9 (COCHCH$_2$*C*H$_2$OH), 38.6 (MeCy-C3), 35.7 (COCH*C*H$_2$CH$_2$OH), 33.7 (MeCy-C4), 31.0 (MeCy-C6), 26.0 (CONH*C*H$_3$), 23.2 (MeCy-C5), 19.0 (MeCy-*C*H$_3$), 17.0 (Fuc-C6); $[\alpha]_D$ -50.9 (*c* 0.66, $CHCl_3$); MS (ESI) *m*/*z*: calcd for $C_{52}H_{67}NaNO_{13}^+$ $[M+Na]^+$: 936.45; found: 936.63; elemental analysis calcd (%) for $C_{52}H_{67}NO_{13}$ + 0.5 H_2O (923.10): C 67.66, H 7.43, N 1.52; found: C 67.65, H 7.43, N 1.68.

3-*O*-((*S*)-4-hydroxy-1-(methylamino)-1-oxobutan-2-yl)-β-D-galactopyranosyl-(1→1)-[α-L-fucopyranosyl-(1→2)]-(1*R*,2*R*,3*S*)-3-methyl-cyclohexane-1,2-diol (8).

Compound **31** (90 mg, 0.098 mmol) was dissolved in dioxane/water (4/1, 3.5 mL) under argon. $Pd(OH)_2$/C (5 mg, $Pd(OH)_2$) was added and the resulting mixture was hydrogenated (1 bar H_2) at r.t. After 5 h the mixture was filtered and the solvent removed under reduced pressure. Purification *via* HPLC and lyophilization from water afforded **8** as white fluffy solid (20 mg, 0.036 mmol, 37%).

^{1}H NMR (500.1 MHz, CD_3OD): δ 5.00 (d, ^3J = 4.0Hz, 1H, Fuc-H1), 4.93-4.84 (m, 1H, Fuc-H5), 4.30 (d, ^3J = 7.7Hz, 1H, Gal-H1), 4.19 (dd, ^3J = 3.8, 8.7Hz, 1H, COC*H*CH$_2$CH$_2$OH), 3.88 (d, ^3J = 2.8Hz, Gal-H4), 3.86-3.80 (m, 2H, COCHCH$_2$C*H*$_2$OH, Fuc-H3), 3.77-3.63 (m,

7H, $COCHCH_2CH_2OH$, Fuc-H2, Fuc-H4, Gal-H2, Gal-H6a,b, MeCy-H1), 3.40 (m, 1H, Gal-H5), 3.37-3.27 (m, 1H, Gal-H3), 3.20 (t, ^3J = 9.3Hz, 1H, MeCy), 2.78 (s, 3H, $NHCH_3$), 2.11 (m, 1H, MeCy), 2.02 (m, 1H, $COCHCH_2CH_2OH$), 1.87 (m, 1H, $COCHCH_2CH_2OH$), 1.73-1.55 (m, 3H, MeCy), 1.40-1.23 (m, 2H, MeCy), 1.19 (d, ^3J = 6.6Hz, 3H, Fuc-H6), 1.13 (d, ^3J = 6.3Hz, 3H, MeCy-CH_3), 1.06 (m, 1H, MeCy); ^{13}C NMR (125.8 MHz, CD_3OD): δ 176.7 ($COCHCH_2CH_2OH$), 102.3 (Gal-C1), 100.4 (Fuc-C1), 84.8, 84.6 (Gal-C3, MeCy-C2), 80.0 ($COCHCH_2CH_2OH$, MeCy-C1), 76.0 (Gal-C5), 73.9 (Fuc-C4), 71.5 (Fuc-C3, Gal-C2), 70.3 (Fuc-C2), 68.0 (Gal-C4), 67.5 (Fuc-C5), 62.8 (Gal-C6), 59.5 ($COCHCH_2CH_2OH$), 40.4 (MeCy-C3), 37.2 ($COCHCH_2CH_2OH$), 34.9 (MeCy-C4), 31.9 (MeCy-C6), 26.1 ($NHCH_3$), 24.2 (MeCy-C5), 19.6 (MeCy-CH_3), 16.8 (Fuc-C6); $[\alpha]_D$ -73.0 (*c* 0.70, MeOH); HR-MS (ESI) *m*/*z*: calcd for $C_{24}H_{45}NNaO_{13}$ $[M+Na]^+$: 576.2627; found: 576.2627; HPLC-purity: > 99.5 %.

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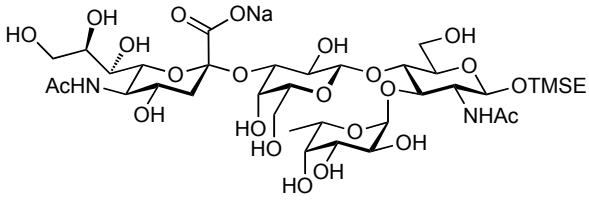
2.3.2 Further selectin antagonists and synthesis of building blocks

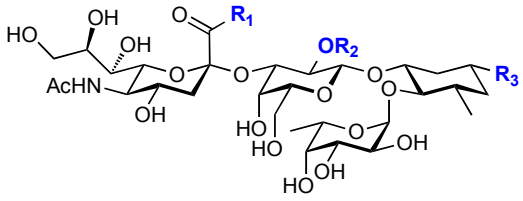
In the course of our studies on selectin antagonists presented in the previous section, further antagonists were developed that have not been described to date. In this section, the rational for their development, biological data, and synthesis are discussed. Furthermore, the synthesis of building blocks used in, but not covered by section 2.3.1 is outlined.

2.3.2.1 Rational and biological data

Table 2.3.1 Relative IC_{50} values (rIC_{50}) of selectin antagonists. IC_{50} values were measured by Katrin Lemme and GMI using **1.1a** (P-sel) and **CGP69669** (E-sel) as reference compounds on each microtiter plate; n.d. = binding affinity not determined.



1.12 (TMSE-sLe^x)



Entry	Compound	R^1	R^2	R^3	rIC_{50} (E)	rIC_{50} (P)
1	**1.12**	-	-	-	1.0	1.0
2	**1.1b**	ONa	H	H	0.08	0.05
3	**3.7***	$NHCH_3$	H	H	0.25	0.07
4	**3.1**	NH_2	H	H	0.27	0.22
5	**3.2**	ONa	$COCH_3$	H	0.03	0.03
6	**3.3**	ONa	H	CO_2Na	0.06	0.06
7	**3.4**	ONa	$COCH_3$	CO_2CH_3	n.d.	n.d.
8	**3.5**	ONa	H	$CO_2NH(CH_2)_2NHCOCH_2(O(CH_2)_2)_2NH_2$	n.d.	n.d.

All IC_{50} values of selectin antagonists are referenced to TMSE protected sLe^x (**1.12**, entry 1 in Table 2.3.1). To evaluate the impact of the different modifications, affinity data is also provided for **1.1b**.

Compound 3.7*: Design and synthesis of **3.7*** are described in section 2.3.1.

Compound 3.1: To study the influence of the methyl group in methylamide **3.7***, amide **3.1** was synthesized. While binding affinity to E-selectin was identical, three-fold weaker binding was observed for P-selectin. This difference might result from the higher quality of the hydrogen bond formed by the methylamide compared to the amide.

Compound 3.2: Various esters and amides in 2-position of D-Gal were found to exert a positive effect on binding affinity to E-selectin (see section: 1.4.2.). However, this effect was only observed for cyclohexyllactic acid derivatives of sLe^x. We wondered if it can also be observed for antagonists containing sialic acid and if it can be found for P-selectin antagonists as well. Consequently, mimetic **3.2** was synthesized and tested. Affinity to both E- and P-selectin was increased approximately two fold, which is in the same range as observed for E-selectin antagonists containing cyclohexyllactic acid. These results suggest, that modifications of the 2-position of D-Gal which are beneficial for E-selectin antagonists, can be transferred to P-selectin antagonists as well.

Compound 3.3: Structure activity relationship studies revealed that a methyl ester at the position of the former ring oxygen of D-Glc*N*Ac could significantly improve the binding affinity of E-selectin antagonists (see section 1.4.2.). According to the crystal structure of sLe^x bound to E-selectin, this part of the mimic has no contact to the protein. Therefore, it was reasoned that the gain in affinity is due to stabilization of the ring conformation. To explore the effect of an additional substituent on the binding affinity to E- and P-selectin, **3.3** was synthesized. However, **3.3** did not exhibit a significant improvement in affinity for both selectins. Possible explanations might be the compensation of favorable conformational stabilization by unfavorable changes in the solvation properties, or a change in the binding mode.

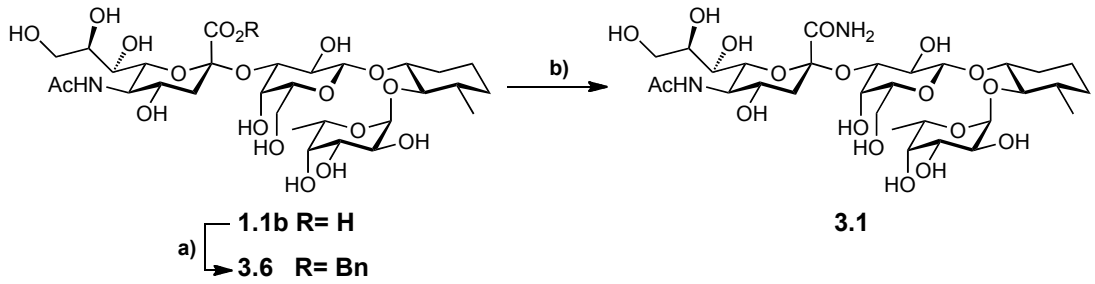
Compound 3.4: No data was available for **3.4**, which combines the acetyl ester in position 2 of D-Gal and the methyl ester at the former ring oxygen position of D-Glc*N*Ac.

Compound 3.5: Linker modified **3.5** was synthesized for the preparation of an affinity column, as well as for immobilization on a Biacore chip (inverse experiment, see thesis of Céline Weckerle[1]). The linker was attached to the D-Glc*N*Ac mimic, since SAR studies of similarly modified selectin ligands had revealed no negative influence on the binding affinity compared to unmodified ligands.[1]

2.3.2.2 Synthesis

2.3.2.2.1 Synthesis of (5-Acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosynylamide)-(2→3)-β-D-galactopyranosyl-[α-L-fucopyranosyl-(1→2)]-(1*R*,2*R*,3*S*)-3-methyl-cyclohexane-1,2-diol (3.1).

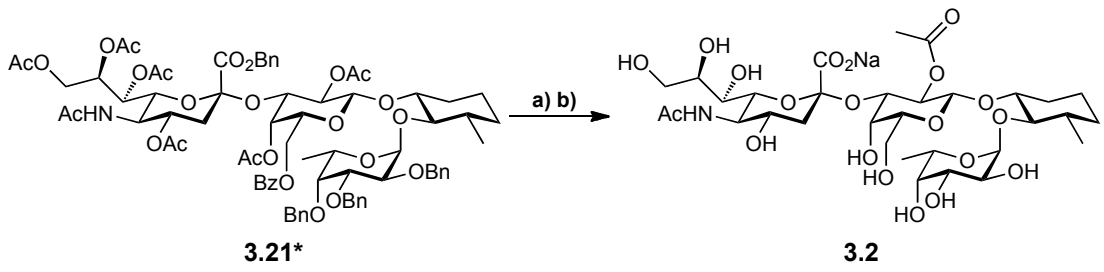
Amide **3.1** was obtained from the benzyl ester of **1.1b** (Scheme 2.3.1). Ester formation proceeded chemoselectively with benzyl bromide in presence of KF and aminolysis with NH_3 afforded **3.1** in 95% over two steps.



Scheme 2.3.1 a) BnBr, KF, DMF, r.t., 2 d; b) NH_3, dioxane, MeOH, r.t., 12 h, 95% from **1.1b.**

2.3.2.2.2 Synthesis of (Sodium 5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosynate)-(2→3)-2-*O*-acetyl-β-D-galactopyranosyl-(1→1)-[α-L-fucopyranosyl-(1→2)]-(1*R*,2*R*,3*S*)-3-methyl-cyclohexane-1,2-diol (3.2).

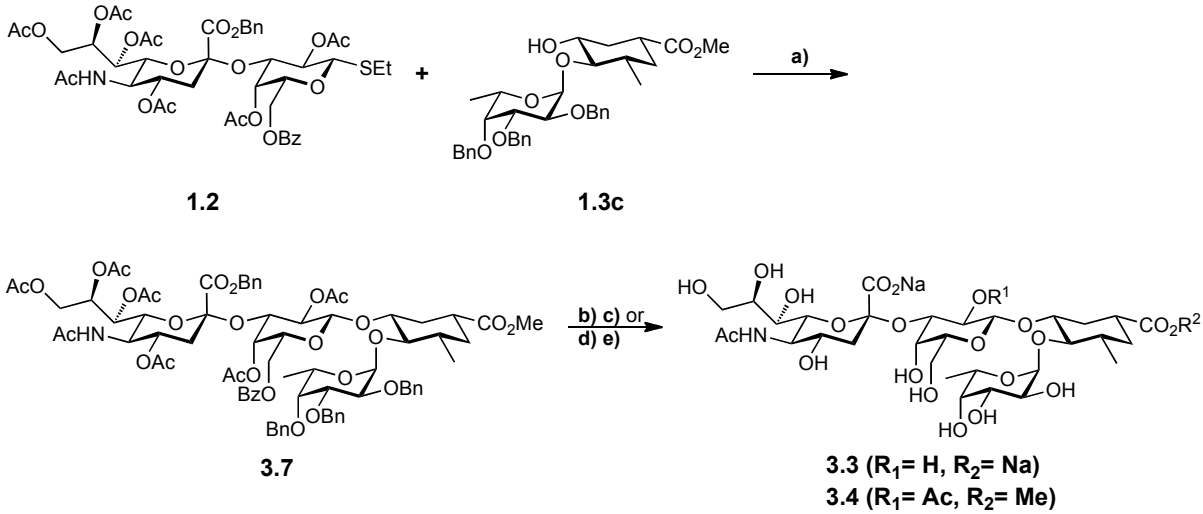
Debenzylation by hydrogenolysis of **3.21*** followed by mild transesterification with NaOMe in methanol afforded monoacetylated **3.2** in 44% yield over two steps (Scheme 2.3.2).



Scheme 2.3.2 a) H_2, $Pd(OH)_2$/C, dioxane, H_2O, r.t., 24 h; b) NaOMe, MeOH, r.t., 4.5 h, 44%.

2.3.2.2.3 Synthesis of (Sodium 5-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosynate)-(2→3)-β-D-galactopyranosyl-(1→3)-[a-L-fucopyranosyl-(1→4)]-(1R,3R,4R,5S)-1-sodium carboxylate-5-methyl-cyclohexane-3,4-diol (3.3) and (Sodium 5-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosynate)-(2→3)-2-O-acetyl-β-D-galactopyranosyl-(1→3)-[α-L-fucopyranosyl-(1→4)]-(1R,3R,4R,5S)-1-methoxycarbonyl-5-methyl-cyclohexane-3,4-diol (3.4).

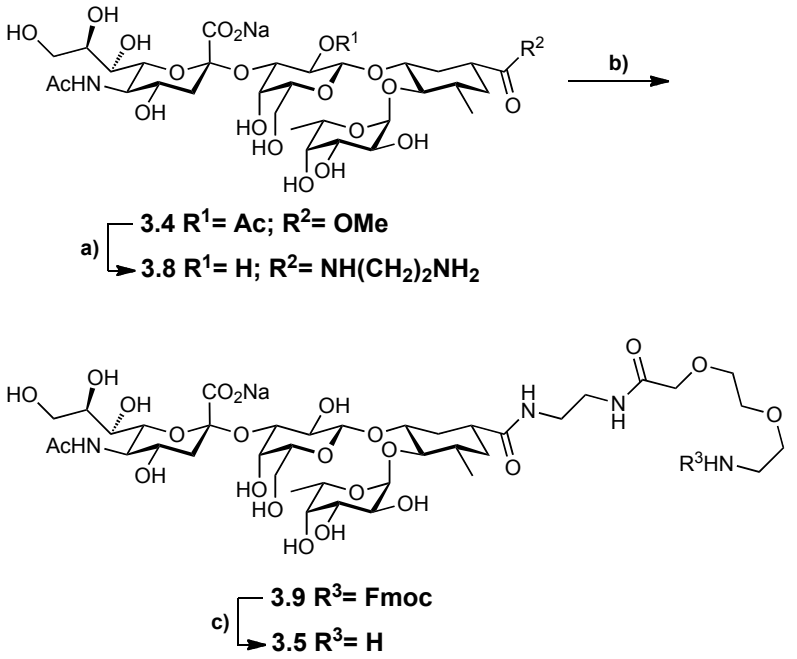
DMTST promoted coupling of **1.2** with **1.3c** afforded **3.7** in 65% yield (Scheme 2.3.3). Debenzylation by hydrogenolysis of **3.7** was either followed by saponification with aqueous sodium hydroxide to yield **3.3**, or by mild transesterification to give **3.4**.



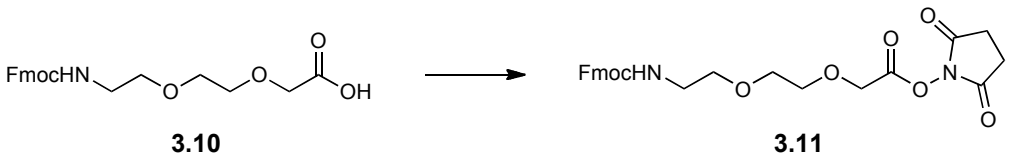
Scheme 2.3.3 a) DMTST, CH_2Cl_2, MS 4 Å, r.t., 42 h, 65%; b) H_2, $Pd(OH)_2$/C, dioxane, water, 24 h; c) NaOH, H_2O, r.t., 8 h, 85% **3.3** from **3.7**; d) H_2, $Pd(OH)_2$/C, dioxane, water, 2 d; e) NaOMe, MeOH, r.t., 2 h, 84% **3.4** from **3.7.**

2.3.2.2.4 Synthesis of (5-Acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosidonic acid)-(2→3)-β-D-galactopyranosyl-(1→3)-[a-L-fucopyranosyl-(1→4)]-(1R,3R,4R,5S)-1((2-(2-(2-(2-aminoethoxy)ethoxy)acetamido)ethyl)carbamoyl)-5-methyl-cyclohexane-3,4-diol (3.5).

In analogy to the synthesis of **GMI1070**,[2] **3.4** was first reacted with 1,2-diaminoethane at 80°C to cleave the acetyl ester and to introduce an amino side chain at the D-Glc*N*Ac mimic (Scheme 2.3.4). To remove side products that might hamper coupling of **3.8** to the linker, **3.8** was purified *via* size exclusion chromatography before coupling to Fmoc protected succinimidyl ester **3.11** (Scheme 2.3.5) in 26% over two steps. Finally, Fmoc was cleaved with piperidine to give **3.5**, which was directly used for coupling.



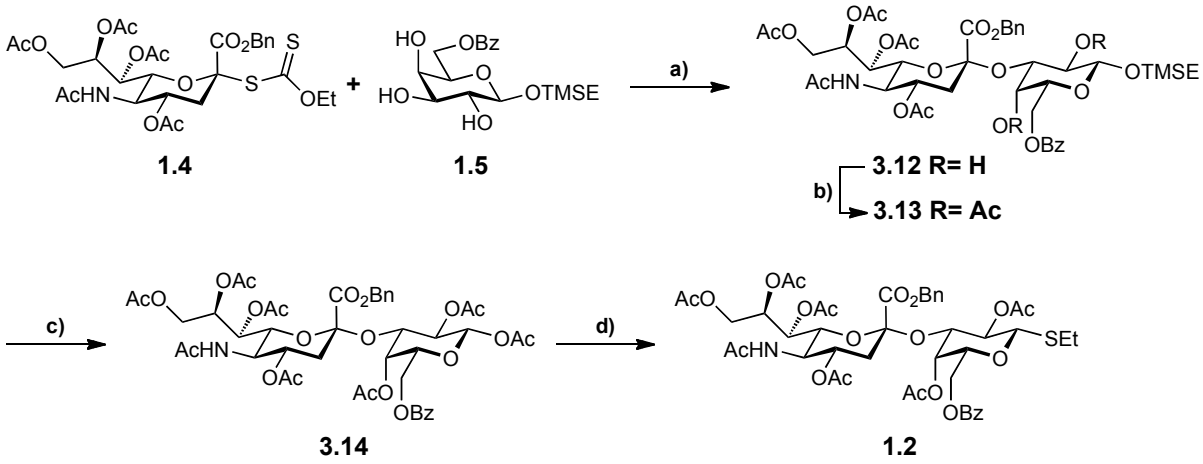
Scheme 2.3.4 a) 1,2-diaminoethane, 80°C, 5 h; b) **3.11**, aq. $NaHCO_3$, MeCN, r.t., 5 h, 26% from **3.4**; c) piperidine, MeCN, r.t., 2 h.



Scheme 2.3.5 NHS, DIC, THF, r.t., 14 h, 97%.

2.3.2.2.5 Synthesis of ethyl (benzyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosynate)-(2→3)-2,4-di-*O*-acetyl-6-*O*-benzoyl-1-thio-β-D-galactopyranoside (1.2).

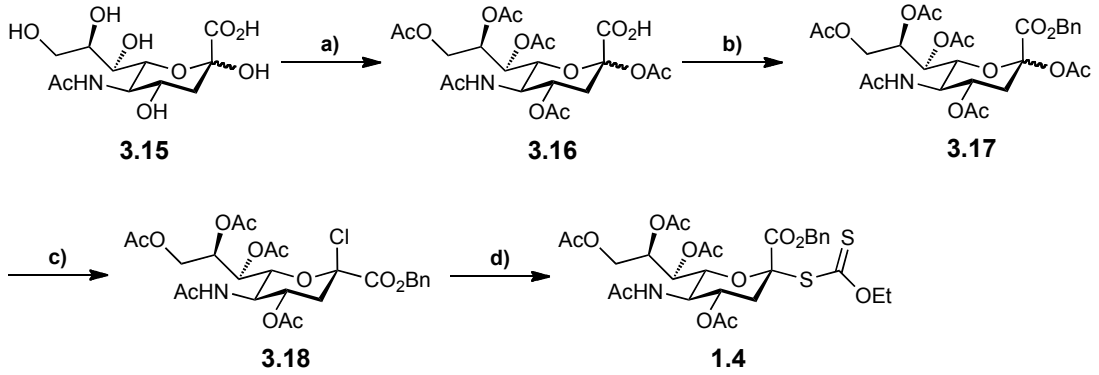
In analogy to the synthesis of methyl (methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→3)-2,4,6-tri-*O*-benzoyl-1-thio-β-D-galactopyranoside,[3,4] building block **1.2**[5] was synthesized from sialic acid donor **1.4** and galactoside **1.5** (Scheme 2.3.6). Using NIS-TfOH as promoter, the glycosyl donor **1.4** was successfully introduced in 3-position of **1.5**. Cumbersome purification was avoided by direct acetylation of roughly purified **3.12**, giving **3.13** in 28% yield. Cleavage of the TMSE group with $BF_3{\cdot}Et_2O$ in presence of acetic anhydride afforded **3.14**, which was further transformed to the title compound **1.2** using $BF_3{\cdot}Et_2O$ as promoter.



Scheme 2.3.6 a) NIS, TfOH, MeCN, CH_2Cl_2, MS 3 Å, -70 to -30°C, 12 h; b) Ac_2O, DMAP, pyridine, r.t., 24 h, 28% from **1.4**; c) Ac_2O, toluene, MS 3 Å, $BF_3{\cdot}Et_2O$, r.t., 16 h, quant.; d) EtSH, $BF_3{\cdot}Et_2O$, CH_2Cl_2, r.t., 2 h, 88%.

2.3.2.2.6 Synthesis of *O*-Ethyl *S*-(benzyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosynate)dithiocarbonate (1.4).

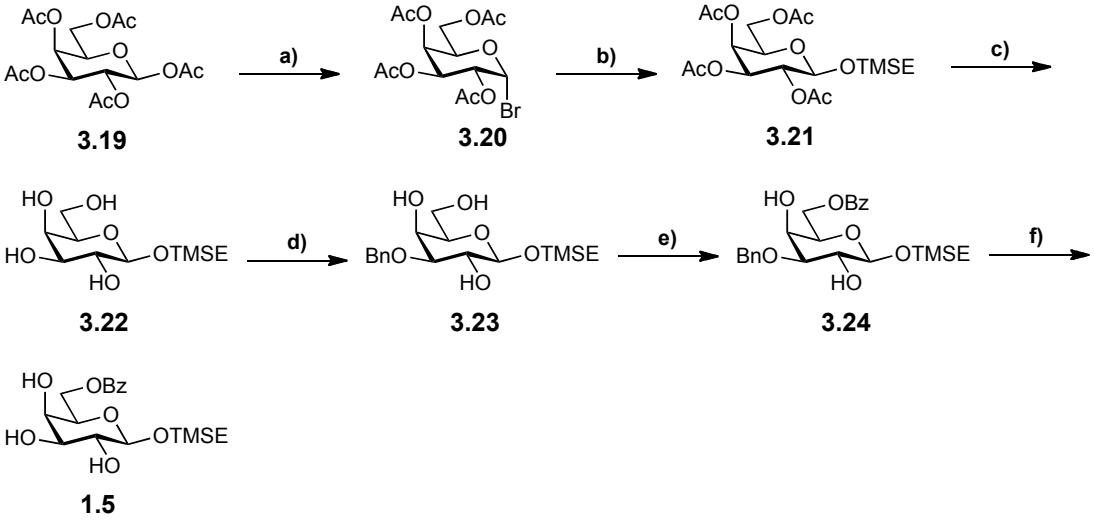
Following a procedure developed by Gan-Pang Gao,[6] sialyl 2-xanthate **1.4** was synthesized in four steps starting from sialic acid **3.15** (Scheme 2.3.7). DMAP catalyzed acetylation with acetic anhydride in pyridine followed by esterification with benzyl bromide in presence of KF afforded fully protected **3.17** in good yield. Treatment with acetyl chloride and conc. hydrochloric acid afforded the 2-*β*-chloro derivative **3.18**, which was promoted to **1.4** using tetrabutylammonium hydrogensulfate (TBAHS) in aqueous $NaHCO_3$ and ethyl acetate.



Scheme 2.3.7 a) Ac_2O, DMAP, pyridine, 0°C to r.t., 16 h; b) BnBr, KF, DMF, r.t., 21 h, 72% from **3.15**; c) AcCl, HCl (conc.), CH_2Cl_2, -20°C to r.t., 27 h; d) K-xanthogenate, TBAHS, $NaHCO_3$ (aq., satd.), EtOAc, r.t., 3 h, 60% from **3.17.**

2.3.2.2.7 Synthesis of 2-(trimethylsilyl)ethyl 6-*O*-benzoyl-β-D-galactopyranoside (1.5).

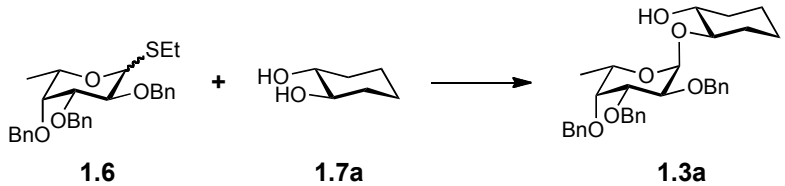
Starting from per acetylated D-galactose **3.19**, the TMSE group was introduced *via* galactosyl bromide **3.20** using Helferich conditions (Scheme 2.3.8). Deprotection of **3.21** under Zemplén conditions afforded **3.22**. Following a procedure published by Murase et al,[3] selective benzoylation in 6-position of **3.22** was done in a three step sequence. Regioselective benzylation in 3-position *via* the tin-acetal (→ **3.23**) was followed by low temperature benzoylation (→ **3.24**) and subsequent hydrogenolytic debenzylation to afford **1.5**.



Scheme 2.3.8 a) HBr, AcOH, CH_2Cl_2, 0°C to r.t., 2.5 h, 92%; b) 2-(Trimethylsilyl)ethanol, HgO, $HgBr_2$, $CaSO_4$, CH_2Cl_2, r.t., 12 h, 85%; c) NaOMe, MeOH, r.t., 1 h, quant.; d) BnBr, Bu_2SnO, Bu_4NBr, toluene, 80°C to 60°C, 8 h, 82%; e) BzCl, pyridine, CH_2Cl_2, -50°C to -20°C, 1.5 h, 60%; f) H_2, $Pd(OH)_2/C$, dioxane, H_2O, 12 h, quant.

2.3.2.2.8 Synthesis of 2,3,4-Tri-*O*-benzyl-α-L-fucopyranosyl-(1→2)-(*R*,*R*)-cyclohexane-1,2-diol (1.3a).

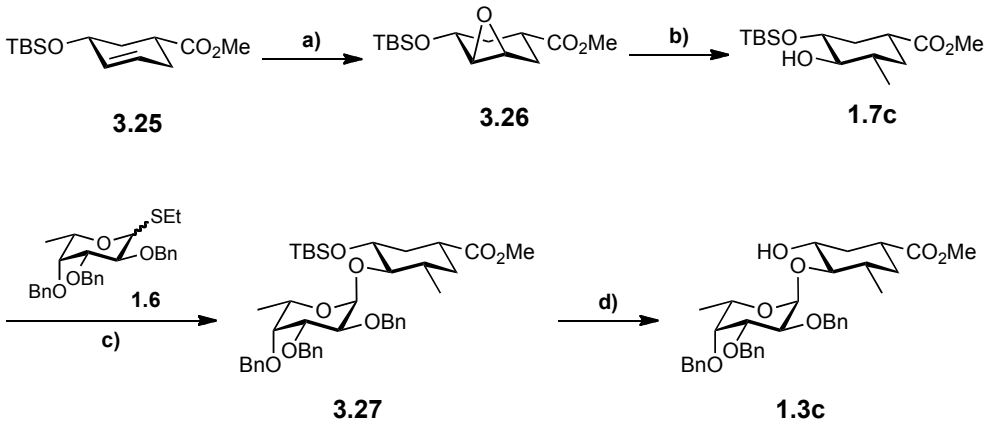
Thio fucoside **1.6**[7] was reacted with commercially available (*R*,*R*)-cyclohexane-1,2-diol **1.7a** under Lemieux *in situ* anomerisation conditions affording **1.3a**[8] α-selectively in 67% yield (Scheme 2.3.9).



Scheme 2.3.9 Br_2, CH_2Cl_2, DMF, Bu_4NBr, 0°C to r.t., 12 h, 67%.

2.3.2.2.9 Synthesis of [(1*R*,3*R*,4*R*,5*S*)-1-methoxycarbonyl-5-methyl-cyclohex-4-yl] 2,3,4-tri-*O*-benzyl-α-L-fucopyranosid (1.3c).

Pseudodisaccharide **1.3c** was synthesized from in-house building block **3.25** following literature procedures (Scheme 2.3.10).[9] Epoxidation of **3.25**, followed by Lewis acid catalyzed epoxide opening with the higher-order cyanocuprate $Me_2Cu(CN)Li_2$ afforded compound **1.7c**, which was fucosylated with **1.6**[7] under in situ anomerisation conditions. Cleavage of the TBS protecting group with tetrabutylammonium fluoride finally afforded **1.3c**.



Scheme 2.3.10 a) *m*-CPBA, CH_2Cl_2, 10°C to r.t., 2 h (81%); b) MeLi, CuCN, $BF_3{\cdot}Et_2O$, THF, -78°C, 5 h (52%); c) Bu_4NBr, DTBMP, MS 4 Å, $CuBr_2$, DMF, CH_2Cl_2, r.t., 20 h (56%); d) TBAF, THF, r.t., 24 h (88%).

2.3.2.3 Experimental

General experimental conditions are described in section 2.3.1.

(5-Acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosynylamide)-(2→3)-β-D-galactopyranosyl-[α-L-fucopyranosyl-(1→2)]-(1*R*,2*R*,3*S*)-3-methyl-cyclohexane-1,2-diol (3.1).

A solution of **1.1b** (40 mg, 0.053 mmol), BnBr (0.019 mL, 0.160 mmol) and KF (8 mg, 0.138 mmol) in anhydrous DMF (3.0 mL) was stirred at r.t. under argon for 2 d. Water (3 mL) was added and the mixture was lyophilized to yield the crude benzyl ester as white solid (80 mg), which was directly used in the next step. The crude ester **3.6** (20 mg) was dissolved in NH_3 in dioxane (0.5 M, 2 mL) and NH_3 in MeOH (7 M, 2 mL) and stirred at r.t. under argon. Volatiles were evaporated under reduced pressure and purification *via* SEC and lyophilization from water afforded **3.1** as white fluffy solid (9.2 mg, 0.013 mmol, 95%).

^{1}H NMR (500.1 MHz, D_2O): δ 5.05 (d, ^{3}J = 3.6Hz, 1H, Fuc-H1), 5.01-4.72 (m, Fuc-H5), 4.51 (d, ^{3}J = 7.9Hz, 1H, Gal-H1), 4.02 (dd, ^{3}J = 2.8, 9.7Hz, 1H, Gal-H3), 3.93 (m, Gal-H4), 3.89-3.54 (m, 14H, Fuc-H2, Fuc-H3, Fuc-H4, Gal-H5, Gal-H6a, Gal-H6b, MeCy-H1, Sia-H4, Sia-H5, Sia-H6, Sia-H7, Sia-H8, Sia-H9a, Sia-H9b), 3.51 (m, 1H, Gal-H2), 3.18 (t, ^{3}J = 9.6Hz, 1H, MeCy-H2), 2.68 (dd, ^{3}J = 4.4Hz, ^{2}J = 12.4Hz, 1H, Sia-H3^{eq}), 2.09 (m, 1H, MeCy), 1.99 (s, 3H, COCH_3), 1.87 (t, ^{3}J = ^{2}J = 12.4Hz, 1H, Sia-H3^{ax}) 1.70-1.46 (m, 3H, MeCy), 1.31-1.13 (m, 2H, MeCy), 1.11 (d, ^{3}J = 6.5Hz, 1H, Fuc-H6), 1.04 (m, 4H, MeCy-CH_3, MeCy); ^{13}C NMR (125.8 MHz, D_2O, CD_3OD): δ 176.0 (*C*OCH_3), 173.1 (Sia-C1), 100.7 (Sia-C2), 100.4 (Gal-C1), 99.8 (Fuc-C1), 84.9 (MeCy-C2), 79.6 (MeCy-C1), 76.8 (Gal-C3), 75.3 (Gal-C5), 74.6 (Sia-C6), 73.0 (Fuc-C4), 72.0 (Sia-C8), 70.2 (Fuc-C3), 69.9 (Gal-C2), 69.2 (2C, Fuc-C2, Gal-C4), 68.6 (Sia-C7), 68.3 (Sia-C4), 67.4 (Fuc-C5), 64.0 (Sia-C9), 62.4 (Gal-C6), 52.5 (Sia-C5), 39.8 (MeCy-C3), 38.4 (Sia-C3), 34.1 (MeCy-C4), 31.2 (MeCy-C6), 23.6 (MeCy-C5), 23.0 (CH_3CO), 19.1 (MeCy-*C*H_3), 16.4 (Fuc-C6); $[\alpha]_D$ -49.6° (*c* 0.61, MeOH); HR-MS (ESI) *m*/*z*: calcd for $C_{30}H_{52}N_2NaO_{18}$ $[M+Na]^+$: 751.3107 ; found: 751.3105; HPLC-purity: > 99.5 % (B).

(Sodium 5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosynate)-(2→3)-2-*O*-acetyl-β-D-galactopyranosyl-(1→1)-[α-L-fucopyranosyl-(1→2)]-(1*R*,2*R*,3*S*)-3-methyl-cyclohexane-3,4-diol (3.2).

3.21* (310 mg, 0.21 mmol) was dissolved in dioxane/water (4/1, 10 mL) under argon. $Pd(OH)_2$/C (40 mg, 10% $Pd(OH)_2$) was added and the resulting mixture was hydrogenated (4 bar H_2) at r.t. After 24 h, the mixture was filtered and the solvent removed under reduced pressure yielding 220 mg of a white solid. 50 mg of this residue were dissolved in anhydrous MeOH (5 mL) under argon. A solution of NaOMe in anhydrous MeOH (1 N, 125 mL) was added under stirring. After 4.5 h, the solution was neutralized with 1 N HAc in MeOH. Volatiles were evaporated under reduced and the crude product was purified *via* SEC. Lyophilization from water afforded **3.2** as white fluffy solid (17 mg, 0.021 mmol, 44%).

^{1}H NMR (500.1 MHz, D_2O): δ 5.02 (d, ^{3}J = 3.9Hz, 1H, Fuc-H1), 4.95-4.76 (m, 2H, Fuc-H5, Gal-H2), 4.71 (d, ^{3}J = 8.1Hz, 1H, Gal-H1), 4.25 (dd, ^{3}J = 2.9, 10.1Hz, 1H, Gal-H3), 3.94-3.81 (m, 4H, Fuc-H3, Gal-H4, Sia-H8, Sia-H9a), 3.79-3.55 (m, 10H, Fuc-H2, Fuc-H4, Gal-H5, Gal-H6a, Gal-H6b, MeCy-H1, Sia-H4, Sia-H5, Sia-H7, Sia-H9b), 3.40 (dd, ^{3}J = 1.8, 10.3Hz, 1H, Sia-H6), 3.13 (t, ^{3}J = 9.5Hz, 1H, MeCy-H2), 2.65 (t, ^{3}J = 4.6Hz, ^{2}J = 12.4Hz, 1H, Sia-H3^{eq}), 2.19 (s, 3H, COCH_3), 2.09 (m, 1H), 2.00 (s, 3H, COCH_3), 1.66-1.61 (m, 4H, MeCy, Sia-H3^{ax}), 1.25-1.10 (m, 3H, Fuc-H6, MeCy), 1.08-0.95 (m, 4H, MeCy-CH_3, MeCy);

^{13}C NMR (125.8 MHz, D_2O, CD_3OD): δ 176.3, 175.0 (2C, *C*OCH_3), 174.9 (Sia-C1), 100.4 (Sia-C2), 99.8 (Fuc-C1), 99.5 (Gal-C1), 84.5 (MeCy-C2), 80.7 (MeCy-C1), 75.6 (Gal-C5), 74.5 (Gal-C3), 73.9 (Sia-C6), 73.1 (Fuc-C4), 72.8 (Sia-C8), 71.8 (Gal-C2), 70.3 (Fuc-C3), 69.2 (3C, Fuc-C2, Sia-C4, Sia-C7), 68.7 (Gal-C4), 67.5 (Fuc-C5), 63.6 (Sia-C9), 62.6 (Gal-C6), 52.9 (Sia-C5), 40.7 (Sia-C3), 39.7 (MeCy-C3), 34.1 (MeCy-C4), 31.7 (MeCy-C6), 23.5 (MeCy-C5), 22.9, 21.8 (*C*H_3CO), 19.1 (MeCy-*C*H_3), 16.4 (Fuc-C6); $[\alpha]_D$ -58.82 (*c* 0.91, MeOH); HR-MS (ESI) *m*/*z*: calcd for $C_{32}H_{52}NNa_2O_{20}$ $[M+Na]^+$: 816.2873 ; found: 816.2874; HPLC-purity: > 99.5 % (B).

(Benzyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosynate)-(2→3)-2,4-di-*O*-acetyl-6-*O*-benzoyl-β-D-galactopyranosyl-(1→3)-[2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl-(1→4)]-(1*R*,3*R*,4*R*,5*S*)-1-methoxycarbonyl-5-methyl-cyclohexane-3,4-diol (3.7).

1.2 (160 mg, 0.17 mmol) and **1.3c** (200 mg, 0.33 mmol) were dissolved in anhydrous CH_2Cl_2 (14 mL). Powdered activated molecular sieves 4 Å (1.4 g) were added and the mixture was stirred at r.t. under argon. After 3.5 h, a solution of DMTST (130 mg, 0.50 mmol) in anhydrous CH_2Cl_2 (1.0 mL) that had been stirred over powdered activated molecular sieves 4 Å (0.1 g) for 3.5 h was added. After stirring for 42 h, the solution was diluted with CH_2Cl_2 (30 mL), filtered and successively washed with satd aq $NaHCO_3$ (50 mL) and brine (50 mL). The aqueous layers were extracted with CH_2Cl_2 (2 · 50 mL) and the combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure. Column chromatography on silica (MTBE) afforded **3.7** as white foam (167 mg, 0.11 mmol, 65%).

^{1}H NMR (500.1 MHz, $CDCl_3$): δ 8.10-7.20 (m, 25H, Ar-H), 5.52 (ddd, ^3J = 2.9, 5.6, 8.8Hz, 1H, Sia-H8), 5.32-5.25 (m, 2H, PhCH_2, SiaH-7), 5.10 (d, ^3J = 3.1Hz, 1H, Gal-H4), 5.06 (d, ^3J = 3.8Hz, 1H, Fuc-H1), 5.00-4.70 (m, 11H, Fuc-H5, Gal-H2, SiaN*H*, Sia-H4, 4 PhCH_2), 4.64 (d, ^3J = 8.0Hz, 1H, Gal-H1), 4.58 (dd, J = 3.3, 10.1Hz, 1H, Gal-H3), 4.31 (dd, ^3J = 2.8Hz, ^3J = 12.4Hz, 1H, Sia-H9a), 4.24 (dd, ^3J = 6.3Hz, ^3J =10.8Hz, 1H, Gal-H6a), 4.13 (dd, ^3J = 3.7, 10.3Hz, 1H, Fuc-H2), 4.08-4.00 (m, 3H, Fuc-H3, Gal-H6b, Sia-H5), 4.00-3.92 (m, 2H, Gal-H5, Sia-H9b), 3.69-3.59 (m, 5H, CO_2CH_3, Fuc-H4, MeCy-H3), 3.45 (dd, 1H, ^3J = 2.7, 10.7Hz, Sia-H6), 3.27 (t, ^3J = 9.6Hz, MeCy-H4), 2.57 (dd, ^3J = 4.6Hz, ^3J = 12.7Hz, 1H, Sia-H3^{eq}), 2.39-2.28 (m, 2H, MeCy), 2.18, 2.12, 2.07, 2.01, 1.97, 1.85, 1.81 (7s, 21H, COCH_3), 1.85 (m, 1H, MeCy) 1.72-1.63 (m, 2H, Sia-H3^{ax}, MeCy), 1.45 (m, 1H, MeCy) 1.23-1.19 (m, 4H, Fuc-H6, MeCy), 1.12 (d, ^3J = 6.5Hz, 3H, MeCy-CH_3); ^{13}C NMR (125.8 MHz, $CDCl_3$): δ 174.8, 170.8, 170.7, 170.5, 170.4, 169.9, (8C, 7 *C*OCH_3, *C*O_2Me), 167.5 (Sia-C1),

165.8 (Ar*C*O), 139.3, 139.1, 138.6, 134.9, 133.4, 130.0, 129.9, 128.9, 128.8, 128.7, 128.6, 128.5, 128.4, 128.3, 127.7, 127.6, 127.4, 127.3 (30C, Ar-C), 99.5 (Gal-C1), 98.5 (Fuc-C1), 96.9 (Sia-C2), 81.6 (MeCy-C4), 80.6 (Fuc-C3), 79.4 (MeCy-C3), 77.7 (Fuc-C4), 76.5 (Fuc-C2), 74.8, 74.3, 72.7 (3C, Ph*C*H_2), 72.1 (Sia-C6), 71.8 (Gal-C3), 70.1, 69.8, 69.4 (Gal-C2, Gal-C5, Sia-C4), 68.5 (Ph*C*H_2), 67.8, 67.7 (Gal-C4, Sia-C8), 67.1 (Sia C7), 66.4 (Fuc-C5), 62.4 (Sia-C9), 61.5 (Gal-C6), 51.9 (CO_2*C*H_3), 49.0 (Sia-C5), 40.5 (MeCy), 38.4, 37.5, (2C, MeCy, Sia-C3), 36.4, 33.2 (2C, MeCy), 23.3, 21.5, 21.0, 20.9, 20.8 (7C, CO*C*H_3), 18.7 (MeCy-*C*H_3), 17.0 (Fuc-C6); $[\alpha]_D$ -7.7 (*c* 0.75, $CHCl_3$); MS (ESI) *m*/*z*: calcd for $C_{79}H_{93}NNaO_{28}$ $[M+Na]^+$: 1526.6 ; found: 1526.6; elemental analysis calcd (%) for $C_{79}H_{93}NO_{28}$ + H_2O (1522.59): C 62.32, H 6.29, N 0.92; found: C 62.12, H 6.31, N 0.83.

(Sodium 5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosynate)-(2→3)-β-D-galactopyranosyl-(1→3)-[α-L-fucopyranosyl-(1→4)]-(1*R*,3*R*,4*R*,5*S*)-1-sodium carboxylate-5-methyl-cyclohexane-3,4-diol (3.3).

3.7 (80 mg, 0.053 mmol) was dissolved in dioxane/water (4/1, 5 mL) under argon. $Pd(OH)_2$/C (30 mg, 10% $Pd(OH)_2$) was added and the resulting mixture was hydrogenated (4 bar H_2) at r.t. After 24 h, the mixture was filtered and the solvent was removed under reduced pressure. The residue was redissolved in 1 N aq NaOH (2 mL) and stirred at r.t. for 8 h. The mixture was lyophilized and the resulting solid was dissolved in 1.0 mL water. The pH was adjusted to 9.0 with 1 N aq HAc and the crude product purified *via* SEC. Lyophilization from water afforded **3.3** as white fluffy solid (37 mg, 0.045 mmol, 85%).

^{1}H NMR (500.1 MHz, D_2O): δ 4.97 (d, ^3J = 4.0Hz, 1H, Fuc-H1), 4.79-4.64 (m, Fuc-H5), 4.45 (d, ^3J = 7.9Hz, 1H, Gal-H1), 3.96 (dd, ^3J = 3.0, 9.8Hz, 1H, Gal-H3), 3.82 (d, ^3J = 3.0Hz, 1H, Gal-H4), 3.80-3.62 (m, 7H, Cy-H3, Fuc-H3, Fuc-H4, Sia-H4, Sia-H5, Sia-H8, Sia-H9a), 3.61-3.40 (m, 8H, Fuc-H2, Gal-H2, Gal-H5, Gal-H6a,b, Sia-H-6, Sia-H7, Sia-H9b), 3.14 (t, ^3J = 9.7Hz, 1H, Cy-H4), 2.62 (dd, ^3J = 4.6Hz, ^2J = 12.4Hz, 1H, Sia-H3^{eq}), 2.16 (m, 2H, Cy-H2a, Cy-H6a), 1.90 (s, 3H, CO*C*H_3), 1.69 (m, 2H, Cy-H1, Sia-H3^{ax}), 1.56 (m, 1H, Cy-H5), 1.30 (q, *J* = 12.4Hz, 1H, Cy-H2b), 1.11 (q, *J* = 12.8Hz, 1H, Cy-H6b), 1.05 (d, ^3J = 6.6Hz, 1H, Fuc-C6), 0.99 (d, ^3J = 6.5Hz, 1H, MeCy-C*H*$_3$); ^{13}C NMR (125.8 MHz, D_2O): δ 184.7 (Cy-*C*O_2Na), 175.9 (*C*OCH_3), 175.1 (Sia-C1), 100.8 (Sia-C2), 100.4 (Gal-C1), 99.8 (Fuc-C1), 84.4 (Cy-C4), 78.4 (Cy-C3), 76.7 (Gal-C3), 75.4 (Gal-C5), 73.7 (Sia-C6), 73.0 (Fuc-C4), 72.7 (Sia-C8), 70.2 (Fuc-C3), 69.9 (Gal-C2), 69.4, 69.2, 69.1 (3C, Fuc-C2, Sia-C4, Sia-C7), 68.5 (Gal-C4), 67.5 (Fuc-C5), 63.5 (Sia-C9), 62.5 (Gal-C6), 52.6 (Sia-C5), 40.5 (Sia-C3), 38.7 (Cy-C5), 37.7 (Cy-C6), 34.4 (Cy-C2), 23.0 (CO*C*H_3), 19.0 (Cy-*C*H_3),

16.4 (Fuc-C6); $[\alpha]_D$ -58.3 (*c* 0.94, MeOH); HR-MS (ESI) *m/z*: calcd for $C_{31}H_{50}NNa_2O_{21}$ $[M+H]^+$: 818.2665; found: 818.2664; HPLC-purity: > 99.5 % (E).

(Sodium 5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosynate)-(2→3)-2-*O*-acetyl-β-D-galactopyranosyl-(1→3)-[α-L-fucopyranosyl-(1→4)]-(1*R*,3*R*,4*R*,5*S*)-1-methoxycarbonyl-5-methyl-cyclohexane-3,4-diol (3.4).

$Pd(OH)_2$/C (5 mg, 10% $Pd(OH)_2$/C) was added to a solution of **3.7** (40 mg, 0.027 mmol) in dioxane/H_2O (4/1, 10 mL) and the resulting mixture was hydrogenated (4 bar H_2) at r.t. After 2 d, the reaction mixture was filtered, concentrated, and dried at high vacuum 12 h The residue was redissolved in absolute MeOH (4.0 mL) and a freshly prepared solution of NaOMe in MeOH (1M, 0.160 mL) was added. The solution was stirred under argon for 2 h, neutralized with HCl in MeOH (1 M, 0.160 mL), and concentrated under reduced pressure. Purification *via* RP chromatography (H_2O/MeOH) afforded **3.4** (19 mg, 0.022 mmol, 84%) as white fluffy solid.

^{1}H NMR (500.1 MHz, CD_3OD): δ 5.03 (dd, ^3J = 8.0, 9.8Hz, Gal-H2), 4.97 (d, ^3J = 4.0Hz, 1H, Fuc-H1), 4.95-4.85 (m, Fuc-H5), 4.51 (d, ^3J = 8.0Hz, 1H, Gal-H1), 4.19 (dd, ^3J = 3.0, 9.8Hz, 1H, Gal-H3), 3.98 (m, 1H, Sia-H8), 3.90 (d, ^3J = 3.0Hz, 1H, Gal-H4), 3.89-3.83 (m, 2H, Fuc-H3, Sia-H9a), 3.78-3.58 (m, 11H, Fuc-H2, Fuc-H4, Gal-H6a,b, Cy-H3, Cy-CO_2CH_3, Sia-H4, Sia-H5, Sia-H9b), 3.51-3.43 (2H, Gal-H5, Sia-H7), 3.39 (dd, ^3J = 2.0, 10.3Hz, Sia-H6), 3.17 (t, ^3J = 9.6Hz, 1H, Cy-H4), 2.78 (dd, ^3J = 4.8Hz, ^2J = 12.3Hz, 1H, Sia-H3^{eq}), 2.46 (m, 1H, Cy-H1), 2.29 (m, 1H, Cy-H2a), 2.17 (s, 3H, $COCH_3$), 2.01 (s, 3H, $COCH_3$), 1.84 (m, 1H, Cy-H6a), 1.69 (m, 1H, Cy-H5), 1.55 (t, ^3J = 12.3Hz, 1H, Sia-H3^{ax}), 1.33 (m, 1H, Cy-H2b), 1.26-1.21 (m, 2H, Cy-H6b, Fuc-H6), 1.14 (d, ^3J = 6.5Hz, 1H, Cy-CH_3); ^{13}C NMR (125.8 MHz, CD_3OD): δ 176.5 (Cy-CO_2CH_3) 175.7, 175.4 (2C, CH_3CO, Sia-C1), 172.7 ($COCH_3$), 100.6, 100.5, 100.4 (3C, Fuc-C1, Gal-C1, Sia-C2), 83.0 (Cy-C4), 79.4 (Cy-C3), 76.3 (Gal-C5), 75.5 (Gal-C3), 74.8 (Sia-C6), 73.9 (Fuc-C4), 72.9 (Sia-C8), 71.9 (Gal-C2), 71.3 (Fuc-C3), 70.3, 69.4 (3C, Fuc-C2, Sia-C4, Sia-C7), 68.8 (Gal-C4), 67.7 (Fuc-C5), 64.7 (Sia-C9), 63.3 (Gal-C6), 54.1 (Sia-C5), 52.3 ($CyCO_2CH_3$), 42.3 (Sia-C3), 41.4 (Cy-C1), 39.3 (Cy-C5), 37.3 (Cy-C6), 34.5 (Cy-C2), 22.5, 21.6 (2C, $COCH_3$), 19.3 (Cy-CH_3), 16.7 (Fuc-C6); $[\alpha]_D$ -13.9 (*c* 0.47, MeOH); HR-MS (ESI) *m/z*: calcd for $C_{34}H_{54}NNa_2O_{22}^+$ $[M+Na^+]^+$: 874.2927; found: 874.2929; HPLC purity: 99% (B).

(5-Acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosidonic acid)-(2→3)-β-D-galactopyranosyl-(1→3)-[α-L-fucopyranosyl-(1→4)]-(1*R*,3*R*,4*R*,5*S*)-1-((1-(9*H*-fluoren-9-yl)-3,12-dioxo-2,7,10-trioxa-4,13-diazapentadecan-15-yl)carbamoyl)-5-methyl-cyclohexane-3,4-diol (3.9).

A solution of **3.4** (13 mg, 15.7 μmol) in 1,2-diaminoethane (2.0 mL, 30 mmol) was stirred in a sealed vessel under argon at 80°C for 5 h. The solution was concentrated under reduced pressure, roughly purified *via* SEC, and lyophilized from water to give still impure amine **3.8** (7.0 mg), which was directly used in the next step. The residue was dissolved in aqueous $NaHCO_3$ (50 mM, 2.0 mL) and MeCN (1.0 mL) and a solution of **3.11** (4.0 mg, 8.3 μmol) in MeCN (1.0 mL) was added. The reaction mixture was stirred at r.t. for 5 h, lyophilized and purified *via* RP chromatography (H_2O/MeOH) to give **3.9** (4.9 mg, 4.1 μmol, 26%) as white fluffy solid.

^{1}H NMR (500.1 MHz, CD_3OD): δ 7.80, 7.65, 7.40, 7.31 (4m, 8H, Ar-H), 4.98 (d, ^3J = 3.9Hz, 1H, Fuc-H1), 4.96-4.85 (m, 1H, Fuc-H5), 4.40-4.32 (m, 3H, Gal-H1, 2H linker), 4.21 (t, ^3J = 6.7Hz, 1H, Fmoc-H2), 4.02-3.96 (m, 3H, Gal-H3, 2H linker), 3.92-3.26 (12H linker), 3.92-3.52 (Fuc-H2, Fuc-H3, Fuc-H4, Gal-H2, Gal-H4, Gal-H6a, Gal-H6b, Cy-H3, Sia-H4, Sia-H5, Sia-H6, Sia-H8, Sia-H9a, Sia-H9b), 3.48 (m, 1H, Sia-H7), 3.41 (m, 1H, Gal-H5), 3.23 (t, ^3J = 9.5Hz, 1H, Cy-H4), 2.85 (m, 1H, Sia-H3^{eq}), 2.24 (m, 2H, Cy-H2a, Cy-H6a), 2.00 (s, 3H, COCH_3), 1.79-1.63 (m, 3H, Cy, Sia-H3^{ax}), 1.53 (q, J = 12.3Hz, 1H, Cy), 1.31 (m, 1H, Cy), 1.17 (d, ^3J = 6.5Hz, 1H, Fuc-C6), 1.13 (d, ^3J = 6.2Hz, 1H, MeCy-CH_3); ^{13}C NMR (125.8 MHz, CD_3OD): δ 177.6, 127.5, 175.2, 173.2 (4C, Cy-*C*ONH, 2 *C*ONH, Sia-C1), 159.0 (NH*C*O_2CH_2), 145.3, 142.6 (4C, Ar-C_i), 128.8, 128.2, 126.2, 121.0 (8C, Ar-*C*H), 102.2, 101.0, 100.4 (Fuc-C1, Gal-C1, Sia-C2), 83.6 (Cy-C4), 78.7 (Cy-C3), 77.9 (Gal-C3), 76.3 (Gal-C5), 74.8, 73.8, 73.0, 72.0, 71.2, 71.0, 70.4, 70.3, 70.1, 69.5, 68.9 (13C, Fuc-C2, Fuc-C3, Fuc-C4, Gal-C2, Gal-C4, Sia-C4, Sia-C6, Sia-C7, Sia-C8, 4C linker), 67.7 (Fmoc-C1), 67.5 (Fuc-C5), 64.6 (Sia-C9), 63.3 (Gal-C6), 53.9 (Sia-C5), 48.4 (Fmoc-C2), 43.2 (Cy-C1), 42.1 (Sia-C3), 41.7, 39.8, (3C, linker), 39.2 (Cy-C5), 37.6 (Cy-C6), 34.9 (Cy-C2), 22.6 (CO*C*H_3), 19.4 (Cy-*C*H_3), 16.7 (Fuc-C6); MS (ESI) *m*/*z*: calcd for $C_{54}H_{78}N_4NaO_{25}$ $[M+Na]^+$: 1205.48; found: 1205.46.

(5-Acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosidonic acid)-(2→3)-β-D-galactopyranosyl-(1→3)-[α-L-fucopyranosyl-(1→4)]-(1*R*,3*R*,4*R*,5*S*)-1((2-(2-(2-(2-aminoethoxy)ethoxy)acetamido)ethyl)carbamoyl)-5-methyl-cyclohexane-3,4-diol (3.5).

A solution of **3.9** (1.0 mg, 0.85 μmol) in MeCN/piperidine (20% v/v piperidine, 0.5 mL) was stirred at r.t. for 2 h. Volatiles were evaporated under reduced pressure and the residue was redissolved in H_2O (4 mL) and washed with CH_2Cl_2 (2 · 4 mL). Lyophilization of the aqueous layer afforded **3.5**, which was directly used.

MS (ESI) *m*/*z*: calcd for $C_{39}H_{69}N_4O_{23}$ $[M+H]^+$: 961.43; found: 961.51.

2,5-Dioxopyrrolidin-1-yl 1-(9*H*-fluoren-9-yl)-3-oxo-2,7,10-trioxa-4-azadodecan-12-oate (3.11).

A solution of 8-(Fmoc-amino)-3,6-dioxaoctanoic acid **3.10** (60 mg, 0.156 mmol), NHS (24 mg, 0.208 mmol) and DIC (40 μL, 0.258 mmol) in anhydrous THF (3.0 mL) was stirred under argon at r.t. for 16 h. The solution was diluted with THF (5 mL) and washed with satd. aq $NaHCO_3$ (10 mL) and brine (10 mL). The aqueous layers were extracted with Et_2O (3 · 10 mL), dried over Na_2SO_4 and the organic layers were concentrated under reduced pressure. The residue was dissolved in MeCN (4 mL) to precipitate diisopropyl urea, filtered and the filtrate was concentrated under reduced pressure to give **3.11** (73 mg, 0.151 mmol, 97%), which was directly used in the next step.

MS (ESI) *m*/*z*: calcd. for $C_{25}H_{26}N_2NaO_8$ $[M+Na]^+$: 505.16; found: 505.11

2-(Trimethylsilyl)ethyl (benzyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosynate)-(2→3)-2,4-di-*O*-acetyl-6-*O*-benzoyl-β-D-galactopyranoside (3.13).

1.4 (5.00 g, 7.44 mmol) and **1.5** (3.00 g, 7.80 mmol) were dissolved in anhydrous MeCN/CH_2Cl_2 (3/2, 150 mL). Powdered activated molecular sieves 3 Å (15 g) were added and the mixture was stirred under argon at r.t. for 3 h. The mixture was then cooled to -70°C and NIS (3.20 g, 14.22 mmol) in MeCN was added. Within 20 min, 5.7 mL of a 0.4 N solution of trifluormethanesulfonic acid in MeCN was added dropwise and the solution was stirred at -70°C for 20 min. After stirring overnight at -30°C the mixture was diluted with CH_2Cl_2, filtered and successively washed with 20% aq $Na_2S_2O_3$, satd aq $NaHCO_3$, and brine. The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. Column chromatography on silica (CH_2Cl_2/i-propanol 30/1) afforded still impure **3.12** (2.92 g), which

was directly used in the next step. The residue (2.50 g) and Ac_2O (3.0 mL, 31.7 mmol) were dissolved in anhydrous pyridine (6.0 mL) and catalytic amounts of DMAP were added. After stirring for 24 h at r.t., volatiles were evaporated under reduced pressure. The residue was dissolved in EtOAc and washed with water, aqueous HCl (1M), satd. aqueous $NaHCO_3$ and brine. The aqueous layers were extracted with CH_2Cl_2 (2 ·50 mL), the combined organic layers were dried with Na_2SO_4 and concentrated under reduced pressure. Column chromatography on silica (MTBE) afforded **3.13** (1.80 g, 2.47 mmol, 28% over 2 steps) as white foam.

^{1}H NMR (500.1 MHz, $CDCl_3$): δ 8.08-7.28 (m, 10H, Ar-H), 5.51 (ddd, ^3J = 2.6, 5.4, 8.4Hz, 1H, Sia-H8), 5.32-5.26 (m, 2H, PhCH_2, Sia-H7), 5.15 (d, ^3J = 10.3Hz, 1H, Sia-N*H*), 5.13 (d, ^3J = 3.0Hz, 1H, Gal-H4), 5.03 (dd, ^3J = 8.1, 10.0Hz, 1H, Gal-H2), 4.94 (B of AB, J = 12.1Hz, 1H, PhCH_2), 4.81 (td, ^3J = 4.6, 11.9Hz, 1H, Sia-H4), 4.61 (d, ^3J = 8.1Hz, 1H, Gal-H1), 4.60 (dd, ^3J = 3.0, 10.0Hz, 1H, Gal-H3), 4.42 (dd, ^3J = 6.8Hz, ^2J = 11.1Hz, 1H, Gal-H6a), 4.30 (dd, ^3J = 2.4Hz, ^2J = 12.4Hz, 1H, Sia-H9a), 4.21 (dd, ^3J = 6.8Hz, ^2J = 11.1Hz, 1H, Gal-H6b), 4.05-3.94 (m, 4H, Gal-H5, Sia-H5, Sia-H9b, OCH_2CH$_2$), 3.58 (td, J = 4.6, 12.6Hz, 1H, OCH_2CH$_2$), 3.47 (dd, 1H, ^3J = 2.6, 10.8Hz, Sia-H6), 2.59 (dd, ^3J = 4.6Hz, ^2J = 12.4Hz, 1H, Sia-H3^{eq}), 2.18, 2.09, 2.06, 2.03, 2.00, 1.94, 1.79 (7s, 21H, 7 COCH_3), 1.66 (dd, ^2J =^3J = 12.4Hz, 1H, Sia-H3^{ax}), 1.06-0.86 (m, 2H, OCH$_2$CH_2), -0.03 (s, 9H, Me_3Si); ^{13}C NMR (125.8 MHz, $CDCl_3$): δ 170.6, 170.6, 170.4, 170.3, 169.7, 169.7 (7C, CH_3CO), 167.4 (Sia-C1), 165.8 (Ar*C*O), 134.8 (Bn, Ar-C^i), 133.2 (Ar-CH), 129.8 (Ar-C^i), 129.7, 128.8, 128.6, 128.4 (9C, Ar-CH), 100.6 (Gal-C1), 96.8 (Sia-C2), 72.0 (Sia-C6), 71.7 (Gal-C3), 70.3 (Gal-C5), 70.0 (Gal-C2), 69.3 (Sia-C4), 68.4 (Ph*C*H$_2$), 67.8, 67.6 (Gal-C4, Sia-C8), 67.5 (O*C*H$_2$CH$_2$Si), 67.0 (Sia C7), 62.4 (Sia-C9), 61.9 (Gal-C6), 48.9 (Sia-C5), 37.5 (Sia-C3), 23.1, 21.4, 21.1, 20.8, 20.8 (7C, *C*H$_3$CO), 18.0 (OCH$_2$*C*H$_2$), -1.4 (3C, Si(CH_3)$_3$); $[\alpha]_D$ -0.5° (*c* 1.08, $CHCl_3$); MS (ESI) *m*/*z*: calcd for $C_{48}H_{63}NNaO_{21}Si$ $[M+Na]^+$: 1040.4; found: 1040.5; elemental analysis calcd (%) for $C_{48}H_{63}NO_{21}Si$ (1018.10): C 56.63, H 6.24, N 1.38; found: C 56.58, H 6.32, N 1.26.

(Benzyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosynate)-(2→3)-1,2,4-tri-*O*-acetyl-6-*O*-benzoyl-β-D-galactopyranoside (3.14).

3.13 (1.00 g, 0.98 mmol) and Ac_2O (1.39 mL, 14.7 mmol) were dissolved in anhydrous toluene (16 mL). Powdered activated molecular sieves 4 Å (2.0 g) were added and the mixture was stirred at r.t. under argon for 30 min. Freshly distilled $BF_3{\cdot}Et_2O$ (0.50 mL, 3.98

mmol) was added dropwise and stirring was continued for 16 h. After dilution with CH_2Cl_2 (30 mL), the reaction mixture was filtered and the filtrate was washed with satd. aqueous $NaHCO_3$ (30 mL) and brine (30 mL). The aqueous layers were extracted with CH_2Cl_2 (2 ·30 mL) and the combined organic layers were dried over Na_2SO_4 and concentrated. Column chromatography on silica (MTBE/acetone 4/1) afforded **3.14** as white foam (0.94 g, quant.).
^{1}H NMR (500.1 MHz, $CDCl_3$): δ 8.09-7.32 (m, 10H, Ar-H), 5.87 (d, ^3J = 8.3Hz, 1H, Gal-H1), 5.52 (ddd, ^3J = 2.4, 6.4, 8.8Hz, 1H, Sia-H8), 5.34 (A of AB, J = 12.0Hz, 1H, PhCH_2), 5.30 (dd, ^3J = 2.4, 8.7Hz, 1H, Sia-H7), 5.23-5.17 (m, 2H, Gal-H2, Gal-H4), 4.98 (m, 2H, Sia-NH, PhCH_2), 4.88-4.79 (m, 2H, Gal-H3, Sia-H4), 4.45-4.36 (m, 2H, Gal-H6a, Sia-H9a), 4.27-4.19 (m, 2H, Gal-H5, Gal-H6b), 4.05 (dd, ^3J =^3J = 10.5Hz, 1H, Sia-H5), 3.94 (dd, ^3J = 6.4Hz, ^2J =12.4Hz, 1H, Sia-H9b), 3.49 (dd, 1H, ^3J = 2.4, 10.5Hz, Sia-H6), 2.63 (dd, ^3J = 4.6Hz, ^2J =12.6Hz, 1H, Sia-H3^{eq}), 2.22, 2.14, 2.12, 2.11, 2.06, 2.05, 1.98, 1.82 (8s, 24H, 8 COCH_3), 1.69 (m, 1H, Sia-H3^{ax}); ^{13}C NMR (125.8 MHz, $CDCl_3$): δ 170.9, 170.8, 170.6, 170.5, 170.2, 169.9, 169.8, 169.1 (8C, COCH$_3$), 167.4 (Sia-C1), 165.9 (ArCO), 134.8, 129.9, 129.8, 129.1, 128.8, 128.5 (12C, Ar-C), 97.0 (Sia-C2), 92.3 (Gal-C1), 72.4 (Sia-C6), 71.5, 71.4 (Gal-C3, Gal-C5), 69.3 (Sia-C4), 68.9 (Gal-C2), 68.6 (PhCH$_2$), 68.2 (Sia-C8), 67.5, 67.3 (Gal-C4, Sia-C7), 62.7 (Sia-C9), 61.8 (Gal-C6), 49.0 (Sia-C5), 37.6 (Sia-C3), 23.3, 21.6, 21.1, 21.0, 20.9 (8C, COCH$_3$); $[\alpha]_D$ 13.4° (*c* 1.27, $CHCl_3$); MS (ESI) *m*/*z*: calcd for $C_{45}H_{53}NNaO_{22}$ $[M+Na]^+$: 982.3; found: 982.6; elemental analysis calcd (%) for $C_{45}H_{53}NO_{22}$ (959.90): C 56.31, H 5.57, N 1.46; found: C 56.15, H 5.68, N 1.41.

Ethyl (benzyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosynate)-(2→3)-2,4-di-*O*-acetyl-6-*O*-benzoyl-1-thio-β-D-galacto pyranoside (1.2).

3.14 (1.71 g, 1.78 mmol) and ethanethiol (0.20 mL, 2.70 mmol) were dissolved in anhydrous CH_2Cl_2 (40 mL) under argon. Freshly distilled $BF_3{\cdot}Et_2O$ (0.47 mL, 3.74 mmol) was added dropwise and the reaction was stirred at r.t. for 2 h. After dilution with CH_2Cl_2 (60 mL), the reaction mixture was washed with satd. aqueous $NaHCO_3$ (100 mL) and brine (100 mL). The aqueous layers were extracted with CH_2Cl_2 (2 ·100 mL) and the combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure. Column chromatography on silica (MTBE) afforded **1.2** as white foam (1.51 g, 1.57 mmol, 88%).
^{1}H NMR (500.1 MHz, $CDCl_3$): δ 8.10-7.32 (m, 10H, Ar-H), 5.52 (ddd, ^3J = 2.7, 5.4, 8.5Hz, 1H, Sia-H8), 5.36-5.31 (m, 2H, Sia-H7, PhCH_2), 5.20 (d, ^3J = 3.0Hz, 1H, Gal-H4), 5.11 (dd, ^3J =^3J = 9.9Hz, 1H, Gal-H2), 4.97 (B of AB, J = 11.4Hz, 1H, PhCH_2), 4.94 (d, ^3J = 10.4Hz,

1H, Sia-NH) 4.84 (ddd, ^3J = 4.6, 12.4Hz, 1H, Sia-H4), 4.71 (d, ^3J = 7.6Hz, 1H, Gal-H1), 4.69 (dd, ^3J = 3.0, 9.9Hz, 1H, Gal-H3), 4.43 (dd, ^3J = 6.8Hz, ^2J = 11.2Hz, 1H, Gal-H6a), 4.33 (dd, ^3J = 2.7Hz, ^2J =12.5Hz, 1H, Sia-H9a), 4.22 (dd, ^3J = 6.8, ^2J =11.2Hz, 1H, Gal-H6b), 4.09-4.03 (m, 2H, Gal-H5, Sia-H5), 3.99 (dd, ^3J = 5.4Hz, ^2J =12.5Hz, 1H, Sia-H9b), 3.48 (dd, 1H, ^3J = 2.7, 10.8Hz, Sia-H6), 2.83-2.68 (m, 2H, SCH_2CH$_3$), 2.62 (dd, ^3J = 4.6Hz, ^2J =12.4Hz, 1H, Sia-H3^{eq}), 2.22, 2.13, 2.10, 2.06, 2.04, 1.98, 1.83 (7s, 21H, 7 COCH_3), 1.70 (dd, ^2J =^3J =12.4Hz, 1H, Sia-H3^{ax}), 1.30 (t, ^3J = 7.4Hz, 3H, SCH$_2$CH_3); ^{13}C NMR (125.8 MHz, $CDCl_3$): δ 170.7, 170.6, 170.4, 170.0, 169.8 (7C, *C*OCH$_3$), 167.5 (Sia-C1), 166.0 (Ar*C*O), 134.9, 133.4, 129.9, 129.0, 128.8, 128.8, 128.5 (12C, Ar-C), 96.7 (Sia-C2), 83.9 (Gal-C1), 74.3 (Gal-C5), 72.5 (Gal-C3), 72.2 (Sia-C6), 69.4 (Sia-C4), 68.5 (Ph*C*H$_2$), 68.4 (Gal-C2), 68.0 (Gal-C4, Sia-C8), 67.1 (Sia C7), 62.5 (Sia-C9), 62.2 (Gal-C6), 49.1 (Sia-C5), 37.6 (Sia-C3), 24.7 (S-*C*H$_2$CH$_3$), 23.3, 21.6, 21.1, 20.9, (7C, CO*C*H$_3$), 15.2 (S-CH$_2$*C*H$_3$); $[\alpha]_D$ 1.2° (*c* 0.70, $CHCl_3$); MS (ESI) *m*/*z*: calcd for $C_{45}H_{55}NNaO_{20}S$ $[M+Na]^+$: 984.3; found: 984.6; elemental analysis calcd (%) for $C_{45}H_{55}NO_{20}S$ (961.98): C 56.19, H 5.76, N 1.46; found: C 56.14, H 5.79, N 1.33.

Benzyl 5-acetamido-2,4,7,8,9-penta-*O*-acetyl-3,5-dideoxy-D-*glycero*-β-D-*galacto*-2-nonulopyranosynate (3.17).

N-Acetyl neuraminic acid **3.15** (2.00 g, 6.5 mmol) and DMAP (cat.) were dissolved in anhydrous pyridine (10 mL) under argon at 0°C. Acetic anhydride (5.0 mL, 6.2 mmol) was added slowly and the mixture was stirred at r.t. for 16 h. After completion of the reaction (TLC: CH_2Cl_2/MeOH/H_2O 13:7:1.6), it was quenched with MeOH (4 mL) and stirred for 1 h. Evaporation of volatiles yielded crude **3.16** (3.91 g), which was used without further purification.

The crude product was dissolved in anhydrous DMF (35 mL) and KF (932 mg, 16.0 mmol) and BnBr (1.14 mL, 9.6 mmol) were added. After stirring for 21 h, DMF was evaporated under reduced pressure, the residue was dissolved in CH_2Cl_2, filtered and washed with water. Column chromatography on silica (CH_2Cl_2/MeOH) yielded **3.17** as a white foam (72%). Analytical data were in accordance with literature.[10]

***O*-Ethyl *S*-(benzyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosynate)dithiocarbonate (1.4).**

3.17 (2.0 g, 3.28 mmol) was dissolved in anhydrous CH_2Cl_2 (12 mL) in a sealed vessel and cooled to -20°C. Acetyl chloride (4.0 mL, 56.3 mmol) and concentrated HCl (0.50 mL) were

added dropwise under stirring. After stirring at r.t. for 27 h, the mixture was cooled to -20°C again, diluted with CH_2Cl_2 and washed with water, satd. aq $NaHCO_3$, and brine. The organic layer was dried (Na_2SO_4), filtered and concentrated under reduced pressure to yield the chloro derivative **3.18** as white foam (1.92 g, 3.28 mmol, quant.), which was directly used in the next step.

A solution of satd. aqueous $NaHCO_3$ (10 ml) was slowly added to a stirred solution of the chloro intermediate (1.0 g, 1.71 mmol), potassium ethyl xanthogenate (0.33 g, 2.06 mmol) and TBAHS (0.58 g, 1.71 mmol) in EtOAc (10 mL). The resulting mixture was stirred at r.t. for 3 h, diluted with EtOAc and washed with water and brine. The organic layer was dried (Na_2SO_4), filtered and concentrated under reduced pressure. Column chromatography on silica (PE/EtOAc 1/1 to 0/1) afforded **1.4** as white foam (0.96 g, 1.03 mmol, 60%). Analytical data were in accordance with literature.[6]

2,3,4,6-Tetra-*O*-acetyl-α-D-galactopyranosyl bromide (3.20).

HBr in acetic acid (33% solution, 90 mL) was added to a stirred solution of pentaacetyl-β-D-galactose **3.19** (10.0 g, 25.6 mmol) in anhydrous CH_2Cl_2 (40 mL) at 0°C over the course of 1 h. The mixture was stirred at 0°C for another 1.5 h and subsequently poured on an ice/water mixture. The aqueous layer was extracted with CH_2Cl_2 and the organic layers were washed with satd. aqueous $NaHCO_3$ and brine. The combined organic layers were dried over Na_2SO_4, filtered and concentrated. Column chromatography on silica (PE/EtOAc) afforded **3.20** as white solid (9.66 g, 23.5 mmol, 92%). Analytical data were in accordance with literature.[11]

2-(Trimethylsilyl)ethyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside (3.21).

A suspension of **3.20** (9.18 g, 22.3 mmol), HgO (4.84 g, 22.3 mmol), $HgBr_2$ (40mg, 0.11 mmol), powdered activated molecular sieves 3 Å (10 g) and 2-(trimethylsilyl)ethanol (6.50 mL, 45.0 mmol) in CH_2Cl_2 was stirred with light exclusion at r.t. for 12 h. The mixture was filtered (Celite) and the Celite was washed with CH_2Cl_2. The filtrate was concentrated and the resulting residue was purified by column chromatography on silica (PE/EtOAc 1/0 to 1/1) to afford **3.21** as white foam (8.50 g, 19.0 mmol, 85%). Analytical data were in accordance with literature.[12]

2-(Trimethylsilyl)ethyl β-D-galactopyranoside (3.22).

To a stirred solution of **3.21** (1.34 g, 2.99 mmol) in anhydrous MeOH (14 mL) was added a freshly prepared solution of NaOMe in MeOH (1M, 1.4 mL) under argon at r.t. The mixture

was stirred for 2 h, neutralized with Amberlyst 15 ion exchange resin and filtered (Celite). Celite was washed with MeOH and the combined filtrates were concentrated to afford **3.22** (0.836 g, 2.98 mmol, quant.) as white foam, which was used in the next step without further purification. Analytical data were in accordance with literature.[13]

2-(Trimethylsilyl)ethyl 3-*O*-benzyl-β-D-galactopyranoside (3.23).

A suspension of **3.22** (0.62 g, 2.21 mmol) and Bu_2SnO (0.83 g, 3.33 mmol) in anhydrous toluene (15 mL) was stirred under argon at 80°C for 4 h. TBAB (0.36 g, 1.12 mmol) and BnBr (3.20 mL, 26.90 mmol) were added and the solution was stirred at 60°C for 4 h. Concentration under reduced pressure followed by column chromatography on silica (PE/EtOAc 1/1 to 0/1) afforded **3.23** as sticky white solid (0.67 g, 1.81 mmol, 82%). Analytical data were in accordance with literature.[14]

2-(Trimethylsilyl)ethyl 6-*O*-benzoyl-3-*O*-benzyl-β-D-galactopyranoside (3.24).

A solution of benzoyl chloride (0.088 mL, 0.76 mmol) in anhydrous CH_2Cl_2 (10 mL) was slowly added to a stirred solution of **3.23** (0.20 g, 0.54 mmol) in anhydrous pyridine (2 mL) and anhydrous CH_2Cl_2 (8 mL) at -50°C under argon. After 1 h, the reaction mixture was warmed to -20°C, quenched with MeOH (0.5 mL) and stirred at -20°C for 0.5 h. The reaction was warmed to r.t., volatiles were evaporated, the mixture was redissolved in CH_2Cl_2 and washed with water. The organic layer was dried (Na_2SO_4), filtered, and concentrated. Column chromatography on silica (PE/EtOAc) afforded **3.24** (0.154 g, 0.32 mmol, 60%) as white crystalline solid. Analytical data were in accordance with literature.[14]

2-(Trimethylsilyl)ethyl 6-*O*-benzoyl-β-D-galactopyranoside (1.5).

A suspension of **3.24** (5.0 g, 10.5 mmol) and $Pd(OH)_2/C$ (0.20 g, 10% $Pd(OH)_2$) in dioxane/water (4/1, 50 mL) was hydrogenated at (4 bar H_2) at r.t. After 12 h, the mixture was filtered (Celite) and concentrated to afford **1.5** (4.05 g, 10.5 mmol, quant.) as white foam. Analytical data were in accordance with literature.[14]

2,3,4-Tri-*O*-benzyl-α-L-fucopyranosyl-(1→2)-(*R*,*R*)-cyclohexane-1,2-diol (1.3a).

Bromine (0.113 mL, 2.20 mmol), was slowly added to a stirred solution of fucose donor **1.6**[18] (0.96 g, 2.00 mmol) in anhydrous CH_2Cl_2 (70 mL) and DMF (3 mL) at 0°C under argon. After 10 min, excess bromine was quenched by addition of cyclohexene. Powdered activated molecular sieves 4 Å (6.0 g), TBAB (1.93 g, 5.99 mmol), and (*R*,*R*)-cyclohexane-

1,2-diol (0.70 g, 6.03 mmol) were added and the mixture was stirred at 0°C for 2 h before warming to r.t. and stirring at r.t. for 12 h The reaction mixture was filtered (celite) and washed with water and brine. The organic layer was dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Column chromatography on silica (CH_2Cl_2/MTBE 40/1) afforded **1.3a** as clear oil (0.716 g, 1.34 mmol, 67%). Analytical data were in accordance with literature.[18]

2.3.2.4 References

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2.4 P-selectin specific replacements for sialic acid

2.4.1 Towards a new class of non-charged, sialic acid free P-selectin antagonist

Author contributions: F.P.C. Binder: design and synthesis of selectin antagonists, manuscript; M. Smieško: design of selectin antagonists, molecular modeling studies; K. Lemme: biological characterization of selectin antagonists.

Manuscript

Towards a new class of non-charged, sialic acid free P-selectin antagonist

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Keywords

Glycomimetics, P-selectin, sialic acid replacement, sialyl Lewis^x,

Abstract

A small series of amides and sulfonamides was synthesized to target a hitherto unexplored binding pocket of P-selectin. The structurally simplified and non-charged mimetics of the lead sialyl Lewis^x (sLe^x) exhibited up to threefold higher binding affinities than sLe^x.

Introduction

Numerous chronic and acute inflammatory diseases like asthma, psoriasis, and rheumatoid arthritis are characterized by an excessive influx of leukocytes into inflamed tissue.[1] Selectins are lectins that mediate the initial step of leukocyte recruitment to sites of inflammation and consequently became attractive targets for the development of anti-inflammatory agents.[2] The minimum carbohydrate motif recognized by all selectins is the tetrasaccharide sialyl Lewis^x (sLe^x, **1**, Figure 1),[3] which has been the lead structure for the design of most selectin antagonists.

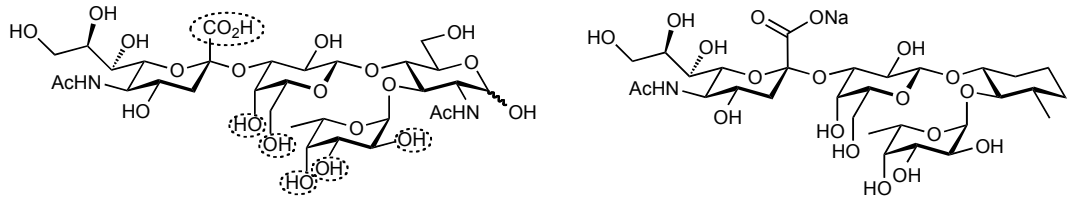


Figure 1. Left: the tetrasaccharide sialyl Lewis^x (**1**) and its pharmacophoric groups: hydroxyl groups of L-fucose[4,5], hydroxyl groups in 4- and 6- position of D-galactose[6] and the carboxylic acid residue of sialic acid[7]. Right: Exchange of Glc*N*Ac by (1*R*,2*R*,3*S*)-3-methyl-cyclohexane-1,2-diol enhances binding affinity on E- and P-selectin 30- and 20-fold, respectively.

Since sLe^x itself is binding only weakly to the selectins and suffers from poor pharmacokinetic properties as well as complex synthesis, huge efforts were made both by academic and industrial research groups to improve the drug-likeness of sLe^x.[8] A common and successful strategy is the stepwise replacement of single or several carbohydrate residues by mimics.[9] The replacement of *N*-acetyl-D-glucosamine (D-Glc*N*Ac), which merely serves as a spacer between L-fucose (L-Fuc) and D-galactose (D-Gal),[5,10] with (1*R*,2*R*,3*S*)-3-methyl-cyclohexane-1,2-diol for instance enhanced binding affinity towards E- and P-selectin 30-fold and 20-fold, respectively (**2**, Figure 1).[11] The comparable effect of this modification on the affinity to both selectins was rationalized by the almost identical conformation of the Lewis^x core upon binding to P- and E-selectin and the high degree of similarity of the core binding sites of both selectins. A major difference however exists in the sialic acid (*N*-acetyl-D-neuraminic acid, D-Neu5Ac) binding site formed by the loop of amino acids 94 to 100 (Figure 2).[12] In the complex with E-selectin, the carboxylate of sLe^x forms a salt bridge with Arg97 and a hydrogen bond with Tyr48, interactions of utmost importance for the affinity of the antagonists. In P-selectin, Arg97 is replaced with Ser97 and the salt bridge is no longer possible. Instead, a water-mediated hydrogen bond with Ser97 is formed. The additional H-

bond to Tyr48 contributes similarly to binding as in E-selectin. In line with this binding mode, we could recently show that the negative charge of the carboxylic acid residue of sialic acid is no prerequisite for affinity to P-selectin, while it is essential for binding to E-selectin.[11]

The side chains of Glu98, Lys99 and Asp100 in E-selectin form a rather wide and lipophilic binding pocket, which is partially occluded by the guanidinium moiety of Arg97 (Figure 2). In contrast, Ser97, Pro98, Ser99, and Ala100 of P-selectin form a rigid groove, stabilized by a highly ordered H-bond network. As this well defined binding pocket is only occupied by water, we reasoned that D-Neu5Ac could be replaced with appropriate mimics to directly target the pocket and gain additional interactions.

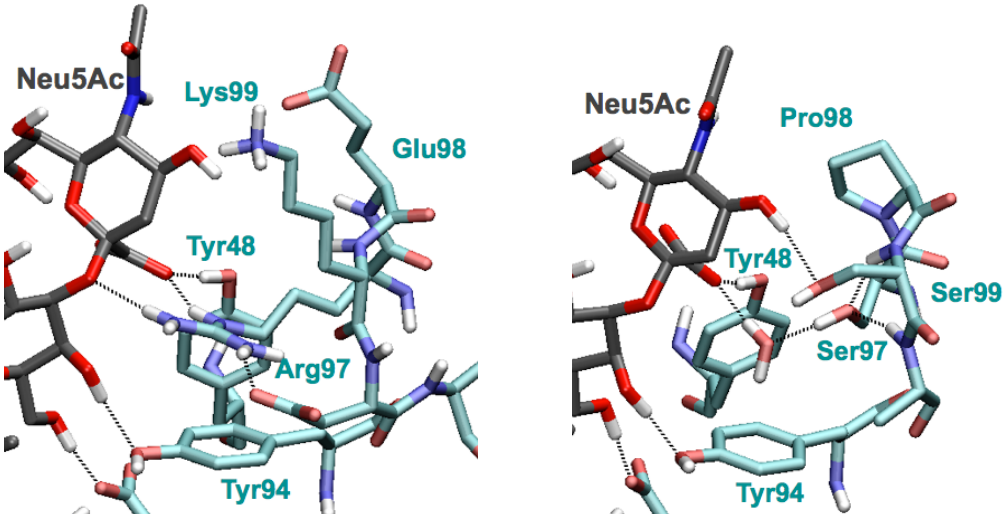


Figure 2. Sialic acid residue of sLe^x binding to E-selectin (left) and to P-selectin (right).[5]

Results and discussion

1. Design

A closer look at the crystal structures of apo-P-selectin (1g1q) and P-selectin co-crystallized with a fragment of the physiological P-selectin ligand PSGL-1 (SGP-3, pdb-code: 1g1s) reveals that a water molecule positioned above Tyr94 is present in both crystal structures.[5] In apo-P-selectin, it binds to Ser97, in the P-selectin SGP-3 complex, it mediates a hydrogen bond between Ser97 and the carboxy group of D-Neu5Ac. Replacement of this structure water with a properly positioned heteroatom consequently offers the possibility to increase the affinity of P-selectin antagonists, especially since this water molecule is only weakly anchored in the binding pocket. Additional affinity might also result from lipophilic interactions with the aromatic side chain of Tyr94. Based on the core of **2**, two small series of

mimetics were designed to target the D-Neu5Ac binding pocket of P-selectin (Table 1). Amides **12** and **13** were designed to replace the water molecule with the side chain oxygen, while amide **16** served to evaluate the effect of the amide moiety itself. Since molecular modeling studies indicated that sulfonamides were better suited to target the aromatic moiety of Tyr48, sulfonamide **15** was synthesized as well. Finally, sulfonamides **18** to **20** were designed to target both Tyr94 and Ser97 (Figure 3). Methyl sulfonamide **14** served as a control to study the effect of the sulfonamide moiety itself.

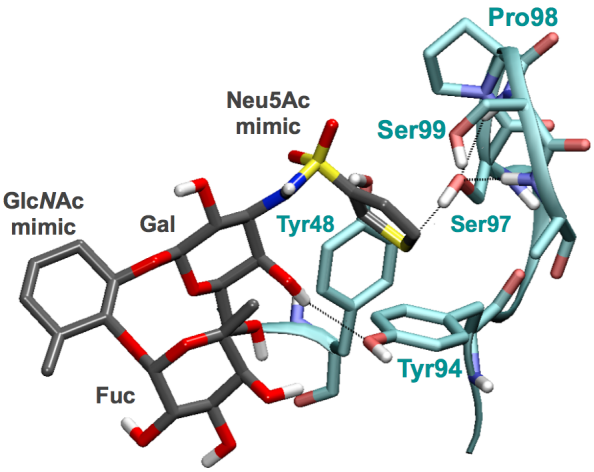
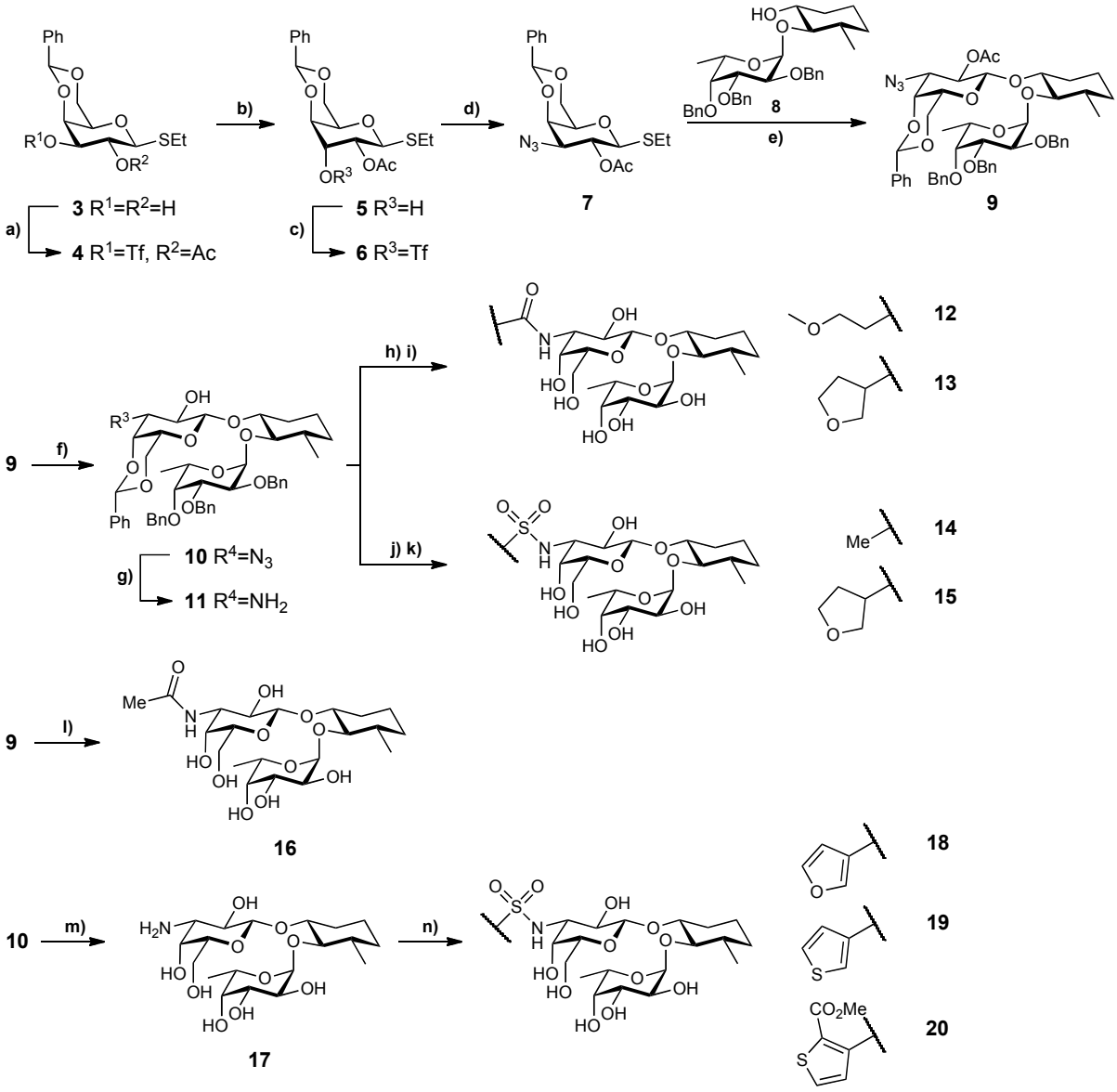


Figure 3. Predicted binding mode of sulfonamide **19** designed to occupy the pocket formed by Tyr48, Tyr94, Ser97, Pro98, and Ser99.

2. Synthesis

Azide **7** was obtained from **3** in analogy to a double inversion route developed by Öberg *et al*. (Scheme 1).[13] The 4,6-benzylidene acetal of ethyl thio-β-D-galactoside (**3**) was successively treated with triflic anhydride in pyridine/CH_2Cl_2 and acetyl chloride to yield triflate **4**. Nitrite mediated inversion of **4** afforded guloside **5** in 55% yield, which was again activated as triflate **6**. A second inversion using tetrabutylammonium azide under microwave conditions afforded 3-azido-3-deoxy-β-D-galactoside **7**, which upon DMTST[14] promoted coupling to pseudo disaccharide **8** provided **9**. Subsequent hydrolysation of the acetate followed by reduction of azide **10** to amine **11** proceeded in excellent yields.

Pseudotrisaccharide **11** was coupled to carboxylic acids using standard coupling conditions. Subsequent debenzylation by hydrogenolysis afforded **12** and a 1:1 diastereomeric mixture of **13**. Sulfonylation of **11** with methansulfonyl chloride and furan-3-sulfonyl chloride at 0°C yielded the corresponding sulfonamides, which were directly deprotected under reducing conditions to afford **14** and a diastereomeric mixture (58 : 42) of **15**.



Scheme 1. a) i. Tf_2O, pyridine, CH_2Cl_2, -20°C, ii. AcCl; b) *n*-Bu_4NNO_2, DMF, 50°C, 55% from **3**; c) Tf_2O, pyridine, CH_2Cl_2, -20°C; d) *n*-Bu_4NN_3, DMF, mw 80°C, 46% from **5**; e) DMTST, CH_2Cl_2, MS 4 Å, r.t., 81%; f) aq. NaOH, dioxane, r.t., 94%; g) $Pd(OH)_2/C$, H_2, dioxane, H_2O, r.t., 92%; h) RCO_2H, HBtU, HOBt, DIPEA, CH_2Cl_2, r.t.; i) $Pd(OH)_2/C$, H_2, CH_2Cl_2/MeOH/H_2O/ HAc, r.t., (**12**: 51%, **13**: 51% from **11**); j) RSO_2Cl, DIPEA, DCE, 0°C to r.t.; k) $Pd(OH)_2/C$, H_2, CH_2Cl_2/MeOH/H_2O/HAc, r.t., (**14**: 56%, **15**: 28% from **11**); l) $Pd(OH)_2/C$, H_2, CH_2Cl_2/MeOH/H_2O/HAc, r.t., 26%; m) $Pd(OH)_2/C$, H_2, CH_2Cl_2/MeOH/H_2O/HAc, r.t., 93%; n) RSO_2Cl, DIPEA, DCE, DMF, -78°C to r.t., **18**: 39% ; or RSO_2Cl, MgO, DIPEA, THF/H_2O, r.t., (**19**: 30%, **20**: 31%).

Initially, we planned to study the influence of an acetyl ester in 2-*O* position of D-galactose in addition to modifications in 3-*O* position. The reaction conditions for the combined reduction/debenzylation of **9** however caused acetyl group migration and directly provided acetamide **16**.

The aromatic sulfonamides **18**, **19**, and **20** were obtained from deprotected **17**, as the applied conditions for debenzylation were considered to be incompatible with furan and thiophene

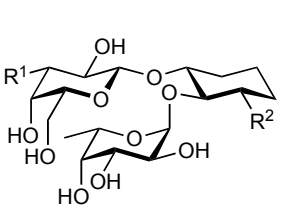
moieties. Chemoselective sulfonylation of **17** was achieved either by low temperature reaction with the sulfonyl chlorides in presence of DIPEA in a CH_2Cl_2/DMF mixture or by a modified version of the magnesium oxide mediated procedure reported by Kang *et al.*[15].

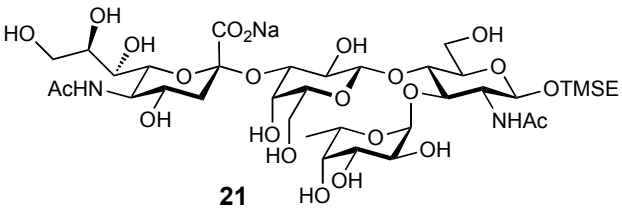
3. Biological Evaluation

The affinity of selectin antagonists to E- and P-selectin was evaluated in a competitive binding assay, utilizing a polyacrylamide-type glycoconjugate as synthetic ligand for immobilized E-/P-selectin.[16] Briefly, microtiter plates were coated with either E-selectin/IgG, or P-selectin/IgG, blocked with BSA, and incubated with a fixed concentration of sLe^a-polyacrylamide (sLe^a-PAA) either in presence or absence of the antagonists. The binding reaction was revealed by the addition of TMB substrate reagent and quantified spectrophotometrically at 450 nm. The IC_{50} defines than the molar concentration of the test compound that reduces the maximal specific binding of sLe^a-PAA polymer to E-selectin/P-selectin by 50%. To ensure comparability of different antagonists, the reference compounds **22** (P-selectin) / **23** (E-selectin) were tested in parallel on each individual microtiter plate. The affinities are reported relative to **21** as rIC_{50} in Table 1. The relative IC_{50} (rIC_{50}) is the ratio of the IC_{50} of the test compound to the IC_{50} of **21**.

The biological results can nicely be correlated to the modeling studies. As expected, none of the compounds bound to E-selectin up to 15 mM (Table 1). Neither the free amine in **17** (entry 4), nor the amide or sulfonamide moiety alone (**14** entry 8, **16** entry 5) supported binding up to 15 mM on P-selectin. This is in accordance with the expected binding mode for **14** (entry 8) and **16** (entry 5), which predicts no direct interaction of these moieties with the protein. In contrast, compound **12** (entry 6), which bears an oxygen atom in the side chain to replace the water molecule, was four-fold more potent than sLe^x. Its rigid analogues **13** (entry 7), a 1:1 mixture of diastereomers, bound slightly worse than **12** (entry 6), which can be explained by the unfavorable configuration of one of the two diastereomers. The same holds true for sulfonamide analogue **15** (entry 9), a 6:4 mixture of diastereomers. Thiophene derivatives **19** (entry 11) and **20** (entry 12) bound in the same range as **12** (entry 6). The weaker hydrogen bond between the thiophene sulfur and a hydroxyl group was obviously compensated by packing of the thiophene sulfur against Tyr94.

Table 1. Relative IC_{50} values (rIC_{50}) of P-selectin antagonists **12** to **20**. IC_{50} values were measured using **22** (P-sel) and **23** (E-sel) as reference compounds on each microtiter plate and are scaled on TMSE-sLe^x **21** (rIC_{50}= 1.0); n.b. = no binding observed up to 15 mM; n.d. = binding affinity not determined.





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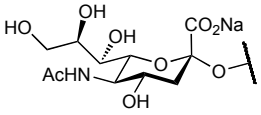
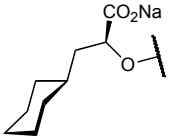
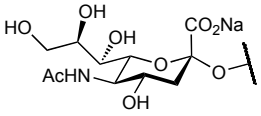
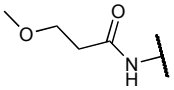
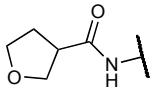
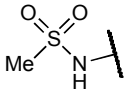
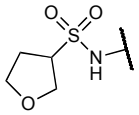
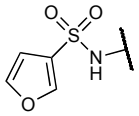
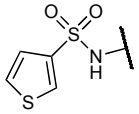
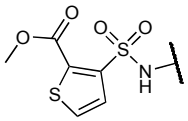
Entry	Compound	R^1	R^2	rIC_{50} (P-sel)	rIC_{50} (E-sel)
1	**22**		H	0.3	0.4
2	**23 (CGP69669)**		H	n.b.	0.08
3	**2**		Me	0.05	0.03
4	**17**		Me	n.b.	n.b.
5	**16**		Me	n.b.	n.b.
6	**12**		Me	0.3	n.b.
7	**13**		Me	1.1	n.b.
8	**14**		Me	n.b.	n.b.
9	**15**		Me	1.0	n.d.
10	**18**		Me	n.d.	n.d.
11	**19**		Me	0.3	n.b.
12	**20**		Me	0.3	n.d.

Conclusion

A small series of amides and sulfonamides, designed to target a potential binding pocket of P-selectin, was synthesized and evaluated in a competitive binding assay. Though it was not possible to fully replace sialic acid, the affinity of these structurally simplified and non-charged antagonists was improved up to three-fold compared to sLe^x.

Experimental Part

NMR spectra were recorded on a Bruker Avance DMX-500 (500 MHz) spectrometer. Assignment of ^1H and ^{13}C NMR spectra was achieved using 2D methods (COSY, HSQC, HMQC, HMBC). Chemical shifts are given in ppm and were assigned in relation to the solvent signals on the δ-scale[17] or to tetramethylsilane (0 ppm) as internal standard. Coupling constants J are given in Hertz (Hz). Multiplicities were specified as follows: s (singlet), d (doublet), dd (doublet of a doublet), t (triplet), q (quartet), m (multiplet). For assignment of resonance signals to the appropriate nuclei the following abbreviations were used: Fuc (fucose), Gal (galactose), MeCy (3-methylcyclohexane-1,2-diol), THF (tetrahydrofuran), Thio (thiophene), Fur (furan). Reactions were monitored by TLC using glass plates coated with silica gel 60 F_{254} (Merck) and visualized by using UV light and/or by charring with a molybdate solution (a 0.02 M solution of ammonium cerium sulfate dihydrate and ammonium molybdate tetrahydrate in aqueous 10% H_2SO_4). Column chromatography was performed manually using silica gel 60 (40-63 µm) from Fluka or using automated systems (RediSep Companion or RediSep Rf) from Teledyne Isco with normal phase RediSep columns from the same manufacturer or reversed-phase columns containing LiChroprep RP-18 (40-63 µm) from Merck KGaA, Darmstadt, Germany. LC-MS separations were carried out using Sunfire C_{18} columns (19 x 150 mm, 5.0 µm) on a Waters 2525 LC, equipped with Waters 2996 photodiode array and Waters micromass ZQ MS for detection. HRMS analysis were carried out using a Agilent 1100 LC equipped with a photodiode array detector and a Micromass QTOF I equipped with a 4 GHz digital-time converter. Size exclusion chromatography was performed with Bio-Gel® P-2 Gel (45-90 mm) from Bio-Rad. Solvents were purchased from Sigma-Aldrich or Acros. Solvents were dried prior to use where indicated. Dichloromethane (CH_2Cl_2) and dichlorethane (DCE) were dried by filtration over Al_2O_3 (Fluka, type 5016 A basic). DMF was dried by distillation from calcium hydride. Optical rotations were measured using a Perkin-Elmer Polarimeter 341. Electron spray ionization mass spectra (ESI-MS) were obtained on a Waters micromass ZQ. IR spectra were recorded on a Perkin Elmer Spectrum One FT-IR spectrometer as KBr pellets. Microanalysis

was performed at the Institute of Organic Chemistry at the University of Basel, Switzerland. Compound purity was determined on an Agilent 1100 HPLC; detector ELS, Waters 2420; column: Waters Atlantis dC18, 3 μm, 4.6 x 75 mm; eluents: A: water + 0.1% TFA; B: 90% acetonitrile + 10% water + 0.1% TFA; depending on the polarity of analytes, gradients were applied as indicated. A) linear gradient: 0 - 1 min 5% B; 1 - 20 min 5 to 70% B; flow: 0.5 mL/min; B) linear gradient: 0 - 1 min 5% B; 1-20 min 5 to 50% B; flow: 0.5 mL/min; C) linear gradient: 0 - 1 min 5% B; 1 - 20 min 5 to 40% B; flow: 0.5 mL/min.

Ethyl 2-*O*-acetyl-4,6-*O*-benzylidene-3-*O*-trifluoromethansulfonyl-1-thio-β-D-galactopyranoside 4.

Ethyl 4,6-*O*-benzylidene-1-thio-β-D-galactopyranoside **3** (9.00 g, 28.8 mmol) was dissolved in anhydrous CH_2Cl_2 (140 mL) and anhydrous pyridine (4.7 mL, 58.1 mmol) under argon. The solution was cooled to -20°C and Tf_2O (5.60 mL, 33.2 mmol) was slowly added. After 2.5 h, AcCl (2.25 mL, 31.7 mmol) was added and the reaction was slowly warmed to r.t. Additional pyridine (2.33 mL, 28.8 mmol) and AcCl (1.64 mL, 23.1 mmol) were added after 1 h and stirring was continued for another 1 h. The solution was diluted with CH_2Cl_2 (150 mL) and washed with aqueous HCl (5%, 200 mL), aqueous satd. $NaHCO_3$ (200 mL) and brine (200 mL). The organic layer was dried over Na_2SO_4, filtered, and concentrated under reduced pressure to give crude **4** as orange sticky solid, which was directly used in the next step.

Ethyl 2-*O*-acetyl-4,6-*O*-benzylidene-1-thio-β-D-gulopyranoside 5.

To a stirred solution of crude **4** (≤ 28.8 mmol) in anhydrous DMF (100 mL) at 50°C under argon was added tetrabutylammonium nitrite (25.0 g, 86.7 mmol). After 24 h, volatiles were evaporated and the residue was dissolved in CH_2Cl_2 (350 mL) and washed with aqueous HCl (5%, 350 mL), aqueous satd. $NaHCO_3$ (350 mL) and brine (350 mL). The organic layer was dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Column chromatography on silica (PE/EtOAc) afforded **5** as white foam (5.69 g, 16.1 mmol, 56%).
^{1}H NMR (500.1 MHz, $CDCl_3$): δ 7.53-7.33 (m, 5H, Ar-H), 5.53 (s, 1H, C*H*Ph), 5.31 (dd, ^3J = 3.0, 10.2Hz, 1H, H-2), 4.89 (d, ^3J = 10.2Hz, 1H, H-1), 4.34 (d, ^2J = 12.5, 1H, H-6a), 4.20 (dd, ^3J = 3.0Hz, 1H, H-3), 4.13 (d, ^3J = 3.0Hz, 1H, H-4), 4.03 (dd, ^3J = 1.5, ^2J =12.5Hz, 1H, H6b), 3.83 (s, 1H, H-5), 2.85-2.71 (m, 2H, SCH_2CH$_3$), 2.14 (s, 3H, COCH_3), 1.29 (t, ^3J = 7.5Hz, 3H, SCH$_2$CH_3); ^{13}C NMR (125.8 MHz, $CDCl_3$): δ 169.2 (CH$_3$*C*O), 137.8 (Ar-C^i),

129.4, 128.5, 126.5 (5C, Ar-CH), 101.5 (Ph*C*H), 79.0 (C1), 76.1 (C4), 69.6 (C6), 68.7 (C3), 68.4 (C2), 67.6 (C5), 23.0 (S*C*$H_2$$CH_3$), 21.2 (CO*C*$H_3$), 15.0 ($SCH_2$*C*$H_3$); $[\alpha]_D$ -76.4 (*c* 1.44, $CHCl_3$); MS (ESI) *m/z*: calcd for $C_{17}H_{22}NaO_6S$ $[M+Na]^+$: 377.1 ; found: 377.1; elemental analysis calcd (%) for $C_{17}H_{22}O_6S$ (354.42): C 57.61, H 6.26; found: C 57.75, H 6.38.

Ethyl 2-*O*-acetyl-4,6-*O*-benzylidene-3-*O*-trifluoromethansulfonyl-1-thio-β-D-gulopyranoside 6.

To a stirred solution of **5** (2.37 g, 6.72 mmol) in anhydrous CH_2Cl_2 (30 mL) and anhydrous pyridine (1.08 mL, 13.3 mmol) at -20°C under argon was slowly added Tf_2O (1.35 mL, 8.02 mmol). After 6 h, the solution was warmed to r.t., diluted with CH_2Cl_2 (60 mL) and washed with aqueous HCl (5%, 100 mL), aqueous satd. $NaHCO_3$ (100 mL) and brine (100 mL). The organic layer was dried over Na_2SO_4, filtered, and concentrated under reduced pressure to afford crude **6** as slightly orange foam (2.93 g), which was directly used in the next step.

Ethyl 2-*O*-acetyl-3-azido-4,6-*O*-benzylidene-3-deoxy-1-thio-β-D-galactopyranoside 7.

Crude **6** (1.00 g, 2.06 mmol) and tetrabutylammonium azide (1.17 g, 4.71 mmol) were dissolved in anhydrous DMF (6.0 mL) under argon in a sealed microwave vial. After microwave irradiation at 200W and 80°C for 1 h, volatiles were removed under reduced pressure. Column chromatography on silica (PE/EtOAc) afforded **7** as white foam (0.40 g, 1.05 mmol, 46%).

^{1}H NMR (500.1 MHz, $CDCl_3$): 7.54-7.31 (m, 5H, Ar-H), 5.59 (s, 1H, C*H*Ph), 5.46 (dd, ^3J = 9.9Hz, 1H, H-2), 4.44 (dd, ^3J = 9.9Hz, 1H, H-1), 4.38-4.33 (m, 2H, H-4, H-6a), 4.06 (m, 1H, H6b), 3.52 (s, 1H, H-5), 3.40 (dd, ^3J = 3.2, 9.9Hz, H-3), 2.91-2.67 (m, 2H, SC$H_2$$CH_3$), 2.15 (s, 3H, COC$H_3$), 1.28 (t, ^3J = 7.5Hz, 3H, SCH_2CH_3); ^{13}C NMR (125.8 MHz, $CDCl_3$): δ 169.6 (CH_3*C*O), 137.3 (Ar-C^i), 129.3, 128.4, 126.3 (5C, Ar-CH), 101.4 (Ph*C*H), 83.2 (C1), 75.6 (C4), 70.5 (C5), 69.4 (C6), 67.1 (C2), 62.7 (C3), 23.0 (S*C*$H_2$$CH_3$), 21.1 (CO*C*$H_3$), 14.9 ($SCH_2$*C*$H_3$); $[\alpha]_D$ + 2.6 (*c* 0.88, $CHCl_3$); IR (KBr): 2111 (s, N_3), 1730 (s, C=O) cm^{-1}; MS (ESI) *m/z*: calcd for $C_{17}H_{21}N_3NaO_5S$ $[M+Na]^+$: 402.11; found: 402.06; elemental analysis calcd (%) for $C_{17}H_{21}N_3O_5S$ (379.43): C 53.81, H 5.58, N 11.07; found: C 54.00, H 5.66, N 10.88.

2-*O*-Acetyl-3-azido-4,6-*O*-benzylidene-3-deoxy-β-D-galactopyranosyl-(1→1)-[2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl-(1→2)]-(1*R*,2*R*,3*S*)-3-methyl-cyclohexane-1,2-diol 9.

Compounds **9** (533 mg, 1.40 mmol) and **8** (640 mg, 1.17 mmol) were dissolved in anhydrous CH_2Cl_2 (25 mL). Powdered activated molecular sieves 4 Å (2.5 g) were added and the mixture was stirred at r.t. under argon. After 3.5 h a solution of DMTST (725 mg, 2.81 mmol) in anhydrous CH_2Cl_2 (15 mL) that had been stirred with molecular sieves 4 Å (1.5 g) for 3.5 h, was added. After stirring for 26 h, the solution was filtered and the filtrate was concentrated under reduced pressure. Column chromatography on silica (PE/EtOAc) afforded **9** as white foam (820 mg, 0.95 mmol, 81%).

^{1}H NMR (500.1 MHz, $CDCl_3$): δ 7.56-7.07 (m, 20H, Ar-H), 5.63 (s, 1H, C*H*Ph), 5.28 (dd, ^3J = 7.8, 10.8Hz, 1H, Gal-H2), 4.89 (d, ^3J = 3.3Hz, 1H, Fuc-H1), 4.82 (q, ^3J = 6.5Hz, 1H, Fuc-H5), 4.74 (A of AB, J = 11.6Hz, 1H, PhCH_2), 4.63 (B of AB, J = 11.6Hz, 1H, PhCH_2), 4.56-4.49 (m, 2H, PhCH_2), 4.44 (d, ^3J = 7.8Hz, 1H, Gal-H1), 4.29 (d, ^2J = 12.2Hz, 1H, Gal-H6a), 4.25 (d, ^3J = 3.2Hz, 1H, Gal-H4), 4.13 (A of AB , J = 11.3Hz, 1H, PhCH_2), 4.05 (d, ^2J = 12.2Hz, 1H, Gal-H6b), 3.91-3.82 (m, 2H, Fuc-H2, Fuc-H3), 3.53 (B of AB , J = 11.3Hz, 1H, PhCH_2), 3.50 (m, 1H, MeCy-H1), 3.36 (s, 1H, Gal-H5), 3.20-3.11 (m, 3H, Fuc-H4, Gal-H3, MeCy-H2), 2.05 (s, 3H, COCH_3), 1.90 (m, 1H, MeCy), 1.60-1.50 (m, 3H, MeCy), 1.21-1.13 (m, 2H, MeCy), 1.09 (d, ^3J = 6.5Hz, 3H, Fuc-H6), 1.00 (d, ^3J = 6.4Hz, 3H, MeCy-CH_3), 0.94 (m, 1H, MeCy); ^{13}C NMR (125.8 MHz, $CDCl_3$): δ 168.1 (CH_3*C*O), 138.6, 138.5, 137.7, 136.4 (4C, Ar-C^i), 127.9, 127.6, 127.2, 127.1, 126.8, 126.5, 126.4, 126.1, 125.8, 124.8 (20C, Ar-CH), 99.4 (Ph*C*H), 98.6 (Gal-C1), 97.4 (Fuc-C1), 80.6 (MeCy-C2), 79.6 (MeCy-C1), 78.7 (Fuc-C3), 78.0 (Fuc-C4), 75.0 (Gal-C4), 74.6 (Fuc-C2), 73.9, 73.5, 70.3 (3C, Ph*C*H$_2$), 68.4 (Gal-C6), 67.5 (Gal-C2), 65.8 (Gal-C5), 65.2 (Fuc-C5), 60.6 (Gal-C3), 38.5 (MeCy-C3), 32.6 (MeCy-C4), 30.2 (MeCy-C6), 22.3 (MeCy-C5), 19.8 (*C*H_3CO), 17.7 (MeCy-*C*H_3), 15.2 (Fuc-C6); $[\alpha]_D$ -71.3 (*c* 1.20, $CHCl_3$); IR (KBr): 2102 (s, N_3), 1756 (s, C=O) cm^{-1}; MS (ESI) *m*/*z*: calcd for $C_{49}H_{57}N_3NaO_{11}$ $[M+Na]^+$: 886.4; found: 886.5; elemental analysis calcd (%) for $C_{49}H_{57}N_3O_{11}$ (863.99): C 68.12, H 6.65, N 4.86; found: C 68.03, H 6.85, N 4.79.

3-Azido-4,6-*O*-benzylidene-3-deoxy-β-D-galactopyranosyl-(1→1)-[2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl-(1→2)]-(1*R*,2*R*,3*S*)-3-methyl-cyclohexane-1,2-diol 10.

A solution of **9** (600 mg, 0.69 mmol) in aqueous NaOH (1 M, 7.0 mL) and dioxane (14 mL) was stirred at r.t. for 16 h. Dioxane was evaporated under reduced pressure, the mixture was diluted with CH_2Cl_2 (30 mL), and washed with brine (30 mL). The aqueous layer was extracted with CH_2Cl_2 (2 · 25 mL) and the combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure. Column chromatography on silica (PE/EtOAc) afforded **10** as white foam (540 mg, 0.66 mmol, 95%).

^{1}H NMR (500.1 MHz, $CDCl_3$): δ 7.70-7.13 (m, 20H, Ar-H), 5.66 (s, 1H, C*H*Ph), 4.97 (d, ^3J = 2.2Hz, 1H, Fuc-H1), 4.85-4.79 (m, 2H, Fuc-H5, PhCH_2), 4.71 (B of AB, J = 11.6Hz, 1H, PhCH_2), 4.63-4.58 (m, 2H, PhCH_2), 4.38 (d, ^3J = 7.6Hz, 1H, Gal-H1), 4.34 (dd, ^3J = 1.0Hz, ^2J = 12.2Hz, 1H, Gal-H6a), 4.23 (d, ^3J = 3.2Hz, 1H, Gal-H4), 4.11 (dd, ^3J = 1.5, ^2J = 12.2Hz, 1H, Gal-H6b), 4.01 (dd, ^3J = 7.6, 10.5Hz, Gal-H2), 3.98-3.92 (m, 2H, Fuc-H2, Fuc-H3), 3.70-3.62 (m, 2H, MeCy-H1, PhCH_2), 3.45 (s, 1H, Gal-H5), 3.32 (dd, ^3J = 3.2, 10.5Hz, Gal-H3), 3.26-3.16 (m, 2H, Fuc-H4, MeCy-H2), 2.07 (m, 1H, MeCy), 1.71-1.54 (m, 3H, MeCy), 1.39-1.16 (m, 2H, MeCy), 1.09 (d, ^3J = 6.5Hz, 3H, MeCy-CH_3), 1.08-0.97 (m, 4H, Fuc-H6, MeCy); ^{13}C NMR (125.8 MHz, $CDCl_3$): δ 139.7, 139.5, 138.7, 137.8 (4C, Ar-C^i), 129.0, 128.8, 128.4, 128.3, 128.2, 128.1, 127.7, 127.6, 127.5, 127.3, 127.1, 125.9 (20C, Ar-CH), 101.2 (Gal-C1), 100.4 (Ph*C*H), 98.7 (Fuc-C1), 82.6 (MeCy-C2), 80.0, 79.8 (MeCy-C1, Fuc-C3), 78.8 (Fuc-C4), 75.6, 75.5 (Fuc-C2, Gal-C4), 75.0, 74.7, 71.6 (3C, Ph*C*H$_2$), 69.6 (Gal-C6), 68.4 (Gal-C2), 67.2 (Gal-C5), 66.3 (Fuc-C5), 62.6 (Gal-C3), 39.6 (MeCy-C3), 33.8 (MeCy-C4), 31.5 (MeCy-C6), 22.4 (MeCy-C5), 18.9 (MeCy-*C*H$_3$), 16.7 (Fuc-C6); $[\alpha]_D$ -94.6 (*c* 0.72, $CHCl_3$); MS (ESI) *m*/*z*: calcd for $C_{47}H_{55}N_3NaO_{10}$ $[M+Na]^+$: 844.4; found: 844.5; elemental analysis calcd (%) for $C_{47}H_{55}N_3O_{10}$ (821.95): C 68.68, H 6.74, N 5.11; found: C 68.71, H 6.56, N 5.13;

3-Amino-4,6-*O*-benzylidene-3-deoxy-β-D-galactopyranosyl-(1→1)-[2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl-(1→2)]-(1*R*,2*R*,3*S*)-3-methyl-cyclohexane-1,2-diol 11.

10 (190 mg, 0.23 mmol) was dissolved in dioxane/H_2O (4/1, 3.5 mL) under argon. $Pd(OH)_2$/C (40 mg, 10% $Pd(OH)_2$) was added and the resulting mixture was hydrogenated (4 bar H_2) at r.t. After 3 h, the mixture was filtered and the solvent removed under reduced pressure. The residue was dissolved in CH_2Cl_2 (25 mL) and washed with satd. aqueous $NaHCO_3$ (2 · 25 mL). The organic layer was dried over Na_2SO_4, filtered and concentrated under reduced pressure to give **11** (170 mg) as slightly grey solid, which was directly used in the next step.

3-(3-Methoxy-propanamido)-3-deoxy-β-D-galactopyranosyl-(1→1)-[α-L-fucopyranosyl-(1→2)]-(1*R*,2*R*,3*S*)-3-methyl-cyclohexane-1,2-diol 12.

To a solution of HOBt (14 mg, 0.10 mmol) and HBtU (33 mg, 0.09 mmol) in anhydrous DMF (1.0 mL) under argon was added DIPEA (0.035 mL, 0.02 mmol) and 3-methoxy propionic acid (0.010 mL, 0.11 mmol). After 10 min, a solution of **11** (27 mg, 0.034 mmol) in CH_2Cl_2/DMF (1/1, 1.0 mL) was added and the resulting solution was stirred for 1 h. The

solution was concentrated under reduced pressure, roughly purified *via* column chromatography (PE/EtOAc) and directly used in the next step. The residue was dissolved in CH_2Cl_2/MeOH/H_2O/HAc (1/1/2/2, 2.0 mL) under argon. $Pd(OH)_2$/C (10 mg, 10% $Pd(OH)_2$) was added and the resulting mixture was hydrogenated (5 bar H_2) at r.t. After 42 h, the mixture was filtered and the solvent removed under reduced pressure. Purification *via* RP chromatography (H_2O/MeOH) and lyophilization from water afforded **12** as white fluffy solid (9.0 mg, 0.017 mmol, 51%).

^{1}H NMR (500.1 MHz, CD_3OD): δ 5.00 (d, ^3J = 4.0Hz, 1H, Fuc-H1), 4.93-4.86 (m, 1H, Fuc-H5), 4.62 (s, 1H, NH), 4.35 (d, ^3J = 7.6Hz, 1H, Gal-H1), 3.86-3.82 (m, 3H, Fuc-H3, Gal-H3, Gal-H4), 3.74 (dd, ^3J = 4.0, 10.3Hz, 1H, Fuc-H2), 3.71-3.61 (m, 6H, $COCH_2CH_2OCH_3$, Fuc-H4, Gal H6a, Gal-H6b, MeCy-H1), 3.56 (dd, ^3J = 7.6, 10.3Hz, 1H, Gal-H2), 3.50 (t, ^3J = 6.0Hz, 1H, Gal-H5), 3.35 (s, 3H, $COCH_2CH_2OCH_3$), 3.20 (t, ^3J = 9.3Hz, 1H, MeCy-H2), 2.53 (m, 2H, $COCH_2CH_2OCH_3$), 2.13 (m, 1H, MeCy), 1.72-1.54 (m, 3H, MeCy), 1.39-1.25 (m, 2H, MeCy), 1.20 (d, ^3J = 6.6Hz, 3H, Fuc-H6), 1.13 (d, ^3J = 6.3Hz, 3H, MeCy-CH_3), 1.07 (m, 1H, MeCy); ^{13}C NMR (125.8 MHz, CD_3OD): δ 174.1 (*C*ONH), 103.0 (Gal-C1), 100.4 (Fuc-C1), 84.7 (MeCy-C2), 80.0 (MeCy-C1), 77.3 (Gal-C5), 73.9 (Fuc-C4), 71.4 (Fuc-C3), 70.4 (Fuc-C2), 69.8 (2C, $COCH_2CH_2OCH_3$, Gal-C2), 68.4 (Gal-C4), 67.5 (Fuc-C5), 63.0 (Gal-C6), 58.9 ($COCH_2CH_2OCH_3$), 56.7 (Gal-C3), 40.4 (MeCy-C3), 37.3 ($COCH_2CH_2CH_3$), 34.9 (MeCy-C4), 31.9 (MeCy-C6), 24.2 (MeCy-C5), 19.6 (MeCy-CH_3), 16.8 (Fuc-C6); $[\alpha]_D$ -48.5 (*c* 1.12, MeOH); HR-MS (ESI) *m*/*z*: calcd for $C_{23}H_{41}NNaO_{12}$ $[M+Na]^+$: 546.2521; found: 546.2532; HPLC-purity: > 99.5% (A).

3-(Tetrahydrofuran-3-carboxamido)-3-deoxy-β-D-galactopyranosyl-(1→1)-[α-L-fucopyranosyl-(1→2)]-(1*R*,2*R*,3*S*)-3-methyl-cyclohexane-1,2-diol 13.

To a solution of HOBt (14 mg, 0.10 mmol) and HBtU (33 mg, 0.09 mmol) in anhydrous DMF (1.0 mL) under argon was added DIPEA (0.035 mL, 0.02 mmol) and tetrahydro-3-furoic acid (0.010 mL, 0.10 mmol). After 10 min, a solution of **11** (27 mg, 0.034 mmol) in CH_2Cl_2/DMF (1/1, 1.0 mL) was added and the resulting solution was stirred for 1 h. The solution was concentrated under reduced pressure, roughly purified *via* column chromatography (PE/EtOAc), and directly used in the next step. The residue was dissolved in CH_2Cl_2/MeOH/H_2O/HAc (1/1/2/2, 2.0 mL) under argon. $Pd(OH)_2$/C (50 mg, 10% $Pd(OH)_2$) was added and the resulting mixture was hydrogenated (1 bar H_2) at r.t. After 2 d, the mixture was filtered and the solvent removed under reduced pressure. Purification *via* RP

chromatography (H_2O/MeOH) and lyophilization from water/acetonitrile afforded **13** as white fluffy solid (9.0 mg, 0.017 mmol, 51%).

^{1}H NMR (500.1 MHz, CD_3OD): δ 5.00 (d, ^3J = 4.0Hz, 1H, Fuc-H1), 4.95-4.85 (m, 1H, Fuc-H5), 4.62 (s, 1H, NH), 4.35 (2d, ^3J = 7.6Hz, 1H, Gal-H1), 3.96 (td, J = 3.9, 8.2Hz, 1H, THF), 3.89 (m, 1H, THF), 3.86-3.76 (m, 5H, Fuc-H3, Gal-H3, Gal-H4, THF), 3.74 (dd, ^3J = 4.0, 10.3Hz, 1H, Fuc-H2), 3.71-3.55 (m, 5H, Fuc-H4, Gal-H2, Gal-H6a, Gal-H6b, MeCy-H1), 3.50 (t, ^3J = 5.9Hz, 1H, Gal-H5), 3.21 (t, ^3J = 9.3Hz, 1H, MeCy-H2), 3.13 (m, 1H, THF), 2.17-2.09 (m, 3H, MeCy, THF), 1.71-1.59 (m, 3H, MeCy), 1.39-1.24 (m, 2H, MeCy), 1.21 (d, ^3J = 6.6Hz, 3H, Fuc-H6), 1.13 (d, ^3J = 6.3Hz, 3H, MeCy-CH_3), 1.06 (m, 1H, MeCy); ^{13}C NMR (125.8 MHz, CD_3OD): δ 176.3 (*C*ONH), 103.1 (Gal-C1), 100.4 (Fuc-C1), 84.7 (MeCy-C2), 80.1 (MeCy-C1), 77.4 (Gal-C5), 73.9 (Fuc-C4), 72.1, 72.0 (1C, THF), 71.4 (Fuc-C3), 70.4 (Fuc-C2), 69.7 (Gal-C2), 69.5 (THF), 68.5, 68.4 (Gal-C4), 67.5 (Fuc-C5), 63.0 (Gal-C6), 56.7 (Gal-C3), 45.9, 45.8 (1C, THF), 40.3 (MeCy-C3), 34.9 (MeCy-C4), 31.9 (MeCy-C6), 31.5 (THF), 24.2 (MeCy-C5), 19.6 (MeCy-*C*H$_3$), 16.8 (Fuc-C6); $[\alpha]_D$ -49.5 (*c* 0.86, MeOH); HR-MS (ESI) *m*/*z*: calcd for $C_{24}H_{41}NNaO_{12}$ $[M+Na]^+$: 558.2521; found: 558.2522; HPLC-purity: > 99.5%; diastereomeric ratio: 1:1 (B).

3-(Methylsulfonamido)-3-deoxy-β-D-galactopyranosyl-(1→1)-[α-L-fucopyranosyl-(1→2)]-(1*R*,2*R*,3*S*)-3-methyl-cyclohexane-1,2-diol 14.

To a solution of **11** (20 mg, 0.025 mmol) and DIPEA (0.008 mL, 0.046 mmol) in anhydrous DCE (0.40 mL) under argon was added a solution of methansulfonyl chloride (0.005 mL, 4.4 mg, 0.038 mmol) in anhydrous DCE (0.06 mL) at 0°C. After 30 min, the reaction was quenched with MeOH (2 mL) and concentrated under reduced pressure to yield a white foam, which was directly used in the next step. The residue was dissolved in CH_2Cl_2/MeOH/H_2O/HAc (1/1/2/2, 2.5 mL) under argon. $Pd(OH)_2$/C (30 mg, 10% $Pd(OH)_2$) was added and the resulting mixture was hydrogenated (5 bar H_2) at r.t. After 48 h, the mixture was filtered and the solvent removed under reduced pressure. Purification *via* HPLC and lyophilization afforded **14** as white solid (7.2 mg, 0.014 mmol, 56%).

^{1}H NMR (500.1 MHz, CD_3OD): δ 5.00 (d, ^3J = 4.0Hz, 1H, Fuc-H1), 4.95-4.83 (m, 1H, Fuc-H5), 4.63 (s, 1H, NH), 4.32 (d, ^3J = 7.6Hz, 1H, Gal-H1), 3.83 (dd, ^3J = 3.3, 10.4Hz, 1H, Fuc-H3), 3.81 (d, ^3J = 2.8Hz, 1H, Gal-H4), 3.73 (dd, ^3J = 4.0, 10.4Hz, 1H, Fuc-H2), 3.71-3.66 (m, 3H, Fuc-H4, Gal H6a, MeCy-H1), 3.64 (dd, ^3J = 5.1Hz, ^2J = 11.3Hz, 1H, Gal-H6b), 3.52-3.46 (m, 2H, Gal-H2, Gal-H5), 3.31 (m, 1H, Gal-H3), 3.20 (t, ^3J = 9.3Hz, 1H, MeCy-H2), 3.06 (s, 3H, $NHSO_2CH_3$), 2.15 (m, 1H, MeCy), 1.73-1.57 (m, 3H, MeCy), 1.41-1.23 (m,

2H, MeCy), 1.19 (d, ^3J = 6.6Hz, 3H, Fuc-H6), 1.13 (d, ^3J = 6.3Hz, 3H, MeCy-CH_3), 1.06 (m, 1H, MeCy); ^{13}C NMR (125.8 MHz, CD_3OD): δ 103.1 (Gal-C1), 100.4 (Fuc-C1), 84.7 (MeCy-C2), 80.0 (MeCy-C1), 77.2 (Gal-C5), 73.9 (Fuc-C4), 71.4 (Fuc-C3), 70.7, 70.5, 70.3 (Fuc-C2, Gal-C2, Gal-C4), 67.5 (Fuc-C5), 62.9 (Gal-C6), 60.8 (Gal-C3), 41.8 ($NHSO_2CH_3$), 40.4 (MeCy-C3), 34.8 (MeCy-C4), 32.0 (MeCy-C6), 24.2 (MeCy-C5), 19.6 (MeCy-CH_3), 16.8 (Fuc-C6); $[\alpha]_D$ -61.9 (*c* 0.44, MeOH); HR-MS (ESI) *m*/*z*: calcd for $C_{20}H_{37}NNaO_{12}S$ $[M+Na]^+$: 538.1929 ; found: 538.1936; HPLC-purity: > 99.5% (B).

3-(Tetrahydrofuran-3-sulfonamido)-3-deoxy-β-D-galactopyranosyl-(1→1)-[α-L-fucopyranosyl-(1→2)]-(1*R*,2*R*,3*S*)-3-methyl-cyclohexane-1,2-diol 15.

To a solution of **11** (25 mg, 0.031 mmol) and DIPEA (0.006 mL, 0.035 mmol) in anhydrous DCE (0.40 mL) under argon was added a solution of furan-3-sulfonyl chloride (5.8 mg, 0.035 mmol) in anhydrous DCE (0.075 mL) at 0°C. After 30 min, the reaction was quenched with MeOH (1.0 mL) and concentrated under reduced pressure to yield a white solid, which was directly used in the next step. The residue was dissolved in CH_2Cl_2/MeOH/H_2O/HAc (1/1/2/2, 6 mL) under argon. $Pd(OH)_2$/C (25 mg, 10% $Pd(OH)_2$) was added and the resulting mixture was hydrogenated (5 bar H_2) at r.t. After 2 d, the mixture was filtered and the solvent removed under reduced pressure. Purification *via* HPLC and lyophilization afforded **15** as white solid (5.0 mg, 0.009 mmol, 28%).

^{1}H NMR (500.1 MHz, CD_3OD): δ 5.00 (d, ^3J = 3.9Hz, 1H, Fuc-H1), 4.94-4.84 (m, 1H, Fuc-H5), 4.63 (s, 1H, NH), 4.30, 4.29 (2d, ^3J = 7.5Hz, 1H, Gal-H1), 4.16-3.88 (m, 4H, THF), 3.86-3.62 (m, 5H, Fuc-H4, Gal-H4, Gal-H6a, MeCy-H1, THF), 3.83 (dd, ^3J = 2.9, 10.3Hz, 1H, Fuc-H3), 3.74 (dd, ^3J = 4.0, 10.3Hz, 1H, Fuc-H2), 3.64 (dd, ^3J = 5.3Hz, ^2J = 11.3Hz, 1H, Gal-H6b), 3.52-3.44 (m, 2H, Gal-H2, Gal-H5), 3.34-3.24 (m, 1H, Gal-H3), 3.19 (t, ^3J = 9.3Hz, 1H, MeCy-H2), 2.30 (m, 2H, THF), 2.13 (m, 1H, MeCy), 1.74-1.57 (m, 3H, MeCy), 1.42-1.22 (m, 2H, MeCy), 1.19 (d, ^3J = 6.5Hz, 3H, Fuc-H6), 1.13 (d, ^3J = 6.3Hz, 3H, MeCy-CH_3), 1.06 (m, 1H, MeCy); ^{13}C NMR (125.8 MHz, CD_3OD): δ 103.2 (Gal-C1), 100.4 (Fuc-C1), 84.7 (MeCy-C2), 80.1 (MeCy-C1), 77.2 (Gal-C5), 73.9 (Fuc-C4), 71.4 (Fuc-C3), 70.7, 70.3, 70.1, 69.5, 69.2, 69.0 (5C, Fuc-C2, Gal-C2, Gal-C4, THF), 67.5 (Fuc-C5), 62.9 (Gal-C6), 62.6, 62.1 (1C, THF), 60.7, 60.6 (Gal-C3), 40.4 (MeCy-C3), 34.9 (MeCy-C4), 32.0 (MeCy-C6), 29.9, 29.1 (1C, THF), 24.2 (MeCy-C5), 19.6 (MeCy-CH_3), 16.8 (Fuc-C6); $[\alpha]_D$ -46.5 (*c* 0.55, MeOH); HR-MS (ESI) *m*/*z*: calcd for $C_{23}H_{41}NNaO_{13}S$ $[M+Na]^+$: 594.2191; found: 594.2196; HPLC-purity: > 99.5%; diastereomeric ratio 58:42 (C).

3-Acetamido-3-deoxy-β-D-galactopyranosyl-(1→1)-[α-L-fucopyranosyl-(1→2)]-(1*R*,2*R*,3*S*)-3-methyl-cyclohexane-1,2-diol 16.

9 (68 mg, 0.08 mmol) was dissolved in CH_2Cl_2/MeOH/H_2O/HAc (1/1/2/2, 4 mL) under argon. $Pd(OH)_2$/C (80 mg, 10% $Pd(OH)_2$) was added and the resulting mixture was hydrogenated (2.5 bar H_2) at r.t. After 14 h, the mixture was filtered and the solvent removed under reduced pressure. Purification *via* HPLC and lyophilization from water/acetonitrile afforded **16** as white solid (10.0 mg, 0.02 mmol, 26%).

^{1}H NMR (500.1 MHz, CD_3OD): δ 5.00 (d, ^3J = 3.9Hz, 1H, Fuc-H1), 4.93-4.86 (m, 1H, Fuc-H5), 4.62 (s, 1H, NH), 4.35 (d, ^3J = 7.6Hz, 1H, Gal-H1), 3.86-3.79 (m, 3H, Fuc-H3, Gal-H3, Gal-H4), 3.74 (dd, ^3J = 3.9, 10.5Hz, 1H, Fuc-H2), 3.71-3.65 (m, 3H, Fuc-H4, Gal H6a, MeCy-H1), 3.63 (dd, ^3J = 5.9Hz, ^2J = 11.4Hz, 1H, Gal-H6b), 3.57 (dd, ^3J = 7.6, 10.3Hz, 1H, Gal-H2), 3.50 (t, ^3J = 5.9Hz, 1H, Gal-H5), 3.21 (t, ^3J = 9.3Hz, 1H, MeCy-H2), 2.14 (m, 1H, MeCy), 2.00 (s, 3H, COC*H*$_3$), 1.72-1.57 (m, 3H, MeCy), 1.41-1.23 (m, 2H, MeCy), 1.20 (d, ^3J = 6.5Hz, 3H, Fuc-H6), 1.13 (d, ^3J = 6.3Hz, 3H, MeCy-C*H*$_3$), 1.06 (m, 1H, MeCy); ^{13}C NMR (125.8 MHz, CD_3OD): δ 173.7 (*C*ONH), 103.0 (Gal-C1), 100.3 (Fuc-C1), 84.7 (MeCy-C2), 80.0 (MeCy-C1), 77.3 (Gal-C5), 73.9 (Fuc-C4), 71.4 (Fuc-C3), 70.3 (Fuc-C2), 69.7 (Gal-C2), 68.4 (Gal-C4), 67.5 (Fuc-C5), 63.0 (Gal-C6), 56.7 (Gal-C3), 40.3 (MeCy-C3), 34.9 (MeCy-C4), 31.9 (MeCy-C6), 24.2 (MeCy-C5), 22.6 (CO*C*H$_3$), 19.6 (MeCy-*C*H$_3$), 16.8 (Fuc-C6); $[\alpha]_D$ -60.4 (*c* 0.94, MeOH); HR-MS (ESI) *m*/*z*: calcd for $C_{21}H_{37}NNaO_{11}$ $[M+Na]^+$: 502.2259; found: 502.2260; HPLC-purity: > 99.5% (A).

3-Amino-3-deoxy-β-D-galactopyranosyl-(1→1)-[α-L-fucopyranosyl-(1→2)]-(1*R*,2*R*,3*S*)-3-methyl-cyclohexane-1,2-diol 17.

10 (250 mg, 0.30 mmol) was dissolved in CH_2Cl_2/MeOH/H_2O/HAc (1/1/2/2, 6 mL) under argon. $Pd(OH)_2$/C (250 mg, 10% $Pd(OH)_2$) was added and the resulting mixture was hydrogenated (4 bar H_2) at r.t. After 48 h, the mixture was filtered and the solvent removed under reduced pressure. Purification *via* HPLC and lyophilization from water/acetonitrile afforded **17** as white solid (123 mg, 0.28 mmol, 93%).

^{1}H NMR (500.1 MHz, CD_3OD): δ 4.99 (d, ^3J = 3.9Hz, 1H, Fuc-H1), 4.96-4.84 (m, 1H, Fuc-H5), 4.35 (d, ^3J = 7.6Hz, 1H, Gal-H1), 3.98 (d, ^3J = 2.8Hz, 1H, Gal-H4) 3.82 (dd, ^3J = 3.2, 10.3Hz, 1H, Fuc-H3), 3.74 (dd, ^3J = 3.9, 10.3Hz, 1H, Fuc-H2), 3.72-3.61 (m, 5H, Fuc-H4, Gal-H2, Gal H6a, Gal-H6b, MeCy-H1), 3.51 (t, ^3J = 6.1Hz, 1H, Gal-H5), 3.23-3.13 (m, 2H, Gal-H3, MeCy-H2), 2.12 (m, 1H, MeCy), 1.72-1.57 (m, 3H, MeCy), 1.40-1.23 (m, 2H, MeCy), 1.20 (d, ^3J = 6.6Hz, 3H, Fuc-H6), 1.14 (d, ^3J = 6.3Hz, 3H, MeCy-C*H*$_3$), 1.06 (m, 1H,

MeCy);^{13}C NMR (125.8 MHz, CD_3OD): δ 102.5 (Gal-C1), 100.5 (Fuc-C1), 84.7 (MeCy-C2), 80.4 (MeCy-C1), 76.8 (Gal-C5), 73.9 (Fuc-C4), 71.4 (Fuc-C3), 70.3 (Fuc-C2), 68.7 (Gal-C2), 67.5 (Gal-C4), 66.5 (Fuc-C5), 62.2 (Gal-C6), 57.0 (Gal-C3), 40.4 (MeCy-C3), 34.9 (MeCy-C4), 32.0 (MeCy-C6), 24.2 (MeCy-C5), 19.6 (MeCy-*C*H_3), 16.7 (Fuc-C6); $[\alpha]_D$ -72.4 (*c* 1.04, MeOH); HR-MS (ESI) *m/z*: calcd for $C_{19}H_{35}NNaO_{10}$ $[M+Na]^+$: 460.2153; found: 460.2161; HPLC-purity: > 99.5% (A).

3-(Furan-3-sulfonamido)-3-deoxy-β-D-galactopyranosyl-(1→1)-[α-L-fucopyranosyl-(1→2)]-(1*R*,2*R*,3*S*)-3-methyl-cyclohexane-1,2-diol 18.

To a solution of **17** (10 mg, 0.023 mmol) in DIPEA (0.30 mL), THF (0.60 mL) and DMF (0.30 mL) was added a solution of furan-3-sulfonyl chloride (5.7 mg, 0.034 mmol) in DCE (0.075 mL) at -78°C under argon. The solution was stirred at -78°C for 10 min and slowly warmed to r.t. Concentration under reduced pressure and purification *via* HPLC afforded **18** as white solid (5.0 mg, 0.009 mmol, 39%).

^{1}H NMR (500.1 MHz, CD_3OD): δ 8.11 (s, 1H, Fur-H2), 7.61 (t, *J* = 1.7Hz, 1H, fur-H5), 6.75 (d, *J* = 1.7Hz, Fur-H4), 4.99 (d, ^3J = 3.9Hz, 1H, Fuc-H1), 4.86 (m, 1H, Fuc-H5), 4.63 (s, 1H, NH), 4.28 (d, ^3J = 7.6Hz, 1H, Gal-H1), 3.82 (dd, ^3J = 3.3, 10.3Hz, 1H, Fuc-H3), 3.79 (d, ^3J = 2.8Hz, 1H, Gal-H4), 3.73 (dd, ^3J = 3.9, 10.3Hz, 1H, Fuc-H2), 3.70-3.62 (m, 3H, Fuc-H4, Gal H6a, MeCy-H1), 3.60 (dd, ^3J = 6.0Hz, ^2J = 11.3Hz, 1H, Gal-H6b), 3.46 (dd, ^3J = 7.6, 10.5Hz, 1H, Gal-H2), 3.42 (t, ^3J = 6.0Hz, 1H, Gal-H5), 3.22 (dd, ^3J = 2.8, 10.5Hz, 1H, Gal-H3), 3.18 (t, ^3J = 9.3Hz, 1H, MeCy-H2), 2.10 (m, 1H, MeCy), 1.70-1.54 (m, 3H, MeCy), 1.37-1.20 (m, 2H, MeCy), 1.18 (d, ^3J = 6.6Hz, 3H, Fuc-H6), 1.12 (d, ^3J = 6.3Hz, 3H, MeCy-C*H*$_3$), 1.05 (m, 1H, MeCy); ^{13}C NMR (125.8 MHz, CD_3OD): δ 146.7 (Fur-C2), 145.8 (fur-C5), 130.2 (fur-C3), 109.7 (fur-C4), 102.8 (Gal-C1), 100.4 (Fuc-C1), 84.7 (MeCy-C2), 79.9 (MeCy-C1), 76.9 (Gal-C5), 73.8 (Fuc-C4), 71.4 (Fuc-C3), 70.3 (Fuc-C2), 69.8 (Gal-C2), 69.5 (Gal-C4), 67.5 (Fuc-C5), 62.9 (Gal-C6), 60.8 (Gal-C3), 40.3 (MeCy-C3), 34.9 (MeCy-C4), 31.9 (MeCy-C6), 24.2 (MeCy-C5), 19.6 (MeCy-*C*H_3), 16.8 (Fuc-C6); $[\alpha]_D$ -51.6 (*c* 0.34, MeOH); HR-MS (ESI) *m/z*: calcd for $C_{23}H_{37}NNaO_{13}S$ $[M+Na]^+$: 590.1878; found: 590.1885; HPLC-purity: > 99.5% (B).

3-(Thiophene-3-sulfonamido)-3-deoxy-β-D-galactopyranosyl-(1→1)-[α-L-fucopyranosyl-(1→2)]-(1*R*,2*R*,3*S*)-3-methyl-cyclohexane-1,2-diol 19.

To a suspension of **17** (10 mg, 0.023 mmol) and MgO (4 mg, 0.10 mmol) in THF/H_2O (4/1, 0.30 mL) that had been stirred at r.t. for 40 min, was added a solution of 3-thiophenesulfonyl

chloride (4 mg, 0.022 mmol) in THF (0.04 mL). After 1 h, DIPEA (0.005 mL, 0.03 mmol) was added and the mixture was stirred for 22 h. Subsequent filtration (celite), concentration under reduced pressure, and purification *via* HPLC afforded **19** as white solid (4.0 mg, 0.007 mmol, 30%).

^{1}H NMR (500.1 MHz, CD_3OD): δ 8.14 (dd, *J* = 1.2, 3.0Hz, 1H, Thio-H5), 7.54 (dd, *J* = 3.0, 5.1Hz, 1H, Thio-H2), 7.43 (dd, *J* = 1.2, 5.1Hz, Thio-H3), 4.98 (d, ^3J = 3.9Hz, 1H, Fuc-H1), 4.85 (dd, ^3J = 6.6, 13.3Hz, 1H, Fuc-H5), 4.63 (s, 1H, NH), 4.27 (d, ^3J = 7.6Hz, 1H, Gal-H1), 3.81 (dd, ^3J = 3.3, 10.3Hz, 1H, Fuc-H3), 3.72 (dd, ^3J = 3.9, 10.3Hz, 1H, Fuc-H2), 3.69 (d, ^3J = 2.8Hz, 1H, Gal-H4), 3.68-3.60 (m, 3H, Fuc-H4, Gal H6a, MeCy-H1), 3.57 (dd, ^3J = 6.0Hz, ^2J = 11.3Hz, 1H, Gal-H6b), 3.46 (dd, ^3J = 7.6, 10.4Hz, 1H, Gal-H2), 3.39 (t, ^3J = 6.0Hz, 1H, Gal-H5), 3.22 (dd, ^3J = 2.8, 10.4Hz, 1H, Gal-H3), 3.17 (t, ^3J = 9.3Hz, 1H, MeCy-H2), 2.09 (m, 1H, MeCy), 1.71-1.56 (m, 3H, MeCy), 1.37-1.20 (m, 2H, MeCy), 1.17 (d, ^3J = 6.6Hz, 3H, Fuc-H6), 1.12 (d, ^3J = 6.3Hz, 3H, MeCy-CH_3), 1.05 (m, 1H, MeCy); ^{13}C NMR (125.8 MHz, CD_3OD): δ 143.1 (Thio-C4), 131.0 (Thio-C5), 128.8 (Thio-C3), 126.9 (Thio-C2), 102.8 (Gal-C1), 100.3 (Fuc-C1), 84.6 (MeCy-C2), 79.9 (MeCy-C1), 76.9 (Gal-C5), 73.8 (Fuc-C4), 71.4 (Fuc-C3), 70.3 (Fuc-C2), 69.9 (Gal-C2), 69.5 (Gal-C4), 67.5 (Fuc-C5), 62.9 (Gal-C6), 60.8 (Gal-C3), 40.3 (MeCy-C3), 34.9 (MeCy-C4), 31.9 (MeCy-C6), 24.2 (MeCy-C5), 19.6 (MeCy-CH_3), 16.8 (Fuc-C6); $[\alpha]_D$ -44.0 (*c* 0.22, MeOH); HR-MS (ESI) *m*/*z*: calcd for $C_{23}H_{37}NNaO_{12}S_2$ $[M+Na]^+$: 606.1649; found: 606.1649; HPLC-purity: > 99.5% (B).

3-(2-(Methoxycarbonyl)thiophene-3-sulfonamido)-3-deoxy-β-D-galactopyranosyl-(1→1)-[α-L-fucopyranosyl-(1→2)]-(1*R*,2*R*,3*S*)-3-methyl-cyclohexane-1,2-diol 20.

To a suspension of **17** (15 mg, 0.034 mmol), DIPEA (0.012 mL, 0.07 mmol) and MgO (6 mg, 0.15 mmol) in THF/H_2O (4/1, 0.30 mL) that had been stirred at r.t. for 90 min, was added a solution of 2-carboxymethoxy3-thiophenesulfonyl chloride (6 mg, 0.025 mmol) in THF (0.04 mL). After 5 h, the mixture was filtered (celite), concentrated under reduced pressure and purified *via* HPLC to yield **20** as white solid (5.0 mg, 0.008 mmol, 31%).

^{1}H NMR (500.1 MHz, CD_3OD): δ 7.77 (d, *J* = 5.2Hz, 1H, Thio-H5), 7.52 (d, *J* = 5.2Hz, 1H, Thio-H4), 4.96 (d, ^3J = 3.9Hz, 1H, Fuc-H1), 4.81 (q, ^3J = 6.4Hz, 1H, Fuc-H5), 4.63 (s, 1H, NH), 4.21 (d, ^3J = 7.5Hz, 1H, Gal-H1), 3.92 (s, 3H, CO_2CH_3), 3.80 (dd, ^3J = 3.3, 10.3Hz, 1H, Fuc-H3), 3.76 (d, ^3J = 2.9Hz, 1H, Gal-H4), 3.72 (dd, ^3J = 3.9, 10.3Hz, 1H, Fuc-H2), 3.69-3.55 (m, 4H, Fuc-H4, Gal H6a, Gal-H6b, MeCy-H1), 3.40-3.34 (m, 2H, Gal-H2, Gal-H5), 3.25 (dd, ^3J = 3.1, 10.4Hz, 1H, Gal-H3), 3.13 (t, ^3J = 9.3Hz, 1H, MeCy-H2), 2.04 (m, 1H, MeCy), 1.68-1.53 (m, 3H, MeCy), 1.36-1.18 (m, 2H, MeCy), 1.15 (d, ^3J = 6.6Hz, 3H,

Fuc-H6), 1.11 (d, ^{3}J = 6.3Hz, 3H, MeCy-CH_3), 1.04 (m, 1H, MeCy); ^{13}C NMR (125.8 MHz, CD_3OD): δ 162.5 (CO_2CH_3), 147.4 (Thio-C2, Thio-C3), 131.9 (Thio-C5), 131.2 (Thio-C4), 102.4 (Gal-C1), 100.5 (Fuc-C1), 84.8 (MeCy-C2), 79.6 (MeCy-C1), 76.9 (Gal-C5), 73.8 (Fuc-C4), 71.4 (Fuc-C3), 70.6, 70.3 (Fuc-C2, Gal-C2), 69.5 (Gal-C4), 67.5 (Fuc-C5), 62.8 (Gal-C6), 61.0 (Gal-C3), 53.5 (CO_2CH_3), 40.3 (MeCy-C3), 34.9 (MeCy-C4), 31.7 (MeCy-C6), 24.2 (MeCy-C5), 19.5 (MeCy-CH_3), 16.7 (Fuc-C6); $[\alpha]_D$ -8.9 (*c* 0.08, MeOH); HR-MS (ESI) *m*/*z*: calcd for $C_{25}H_{39}NNaO_{14}S_2$ $[M+Na]^+$: 664.1704; found: 664.1715; HPLC-purity: > 99.5% (B).

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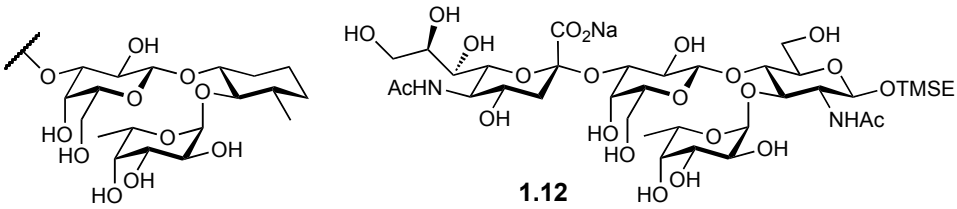
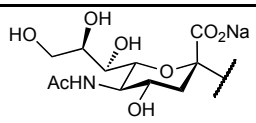
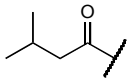
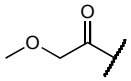
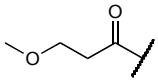
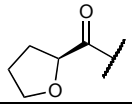
2.4.2 Replacement of sialic acid with esters

To initially validate the concept of sialic acid free antagonists described in the previous section, a small library of carboxylic acid esters was synthesized and tested (Table 2.4.1). In contrast to amides and sulfonamides, these esters could be synthesized from readily available building blocks and gave a first impression of suitable substituents.

Table 2.4.1. Relative IC_{50} values (rIC_{50}) of P-selectin antagonists **4.1a-4.1d** (determined by Katrin Lemme). IC_{50} values are scaled on TMSE-sLe^x **1.12** (rIC_{50}=1); n.b. = no binding observed up to 15 mM.



1.12

entry	Compound	R	rIC_{50} (P-selectin)
1	**1.1b**		0.05
2	**4.1a**		n.b.
3	**4.1b**		2.1
4	**4.1c**		0.4
5	**4.1d**		1.3

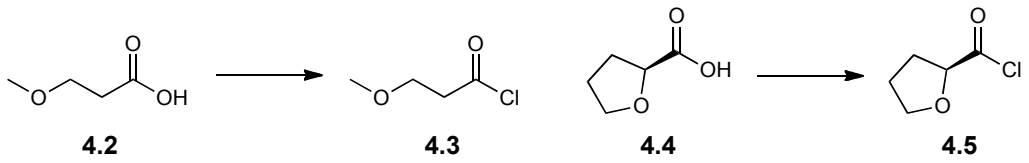
2.4.2.1 Biological evaluation

The results of this first small library (Table 2.4.1) nicely agreed with the results of molecular modeling studies. Compound **4.1a** (entry 2), which lacks a heteroatom essential for interaction with P-selectin, did not bind up to 15 mM. Introduction of an oxygen atom increased the binding affinity dramatically as it allows interaction with either Ser97 or Ser99. In addition, affinity depended on the position of the heteroatom. As expected from docking studies, **4.1c** (entry 4) was more potent than **4.1b** (entry 3), since it was better suited to properly position the oxygen atom. The cyclic analogue of **4.1b** (entry 3), **4.1d** (entry 5) bound slightly stronger which can be explained by a higher degree of pre-organization, or the

reduced number of rotatable bonds. Though it was not possible to fully replace sialic acid (→ **1.1b**) with any of the ester side chains, the results obtained for this series strongly supported our concept and laid the basis for the P-selectin antagonists presented in the previous section.

2.4.2.2 Synthesis

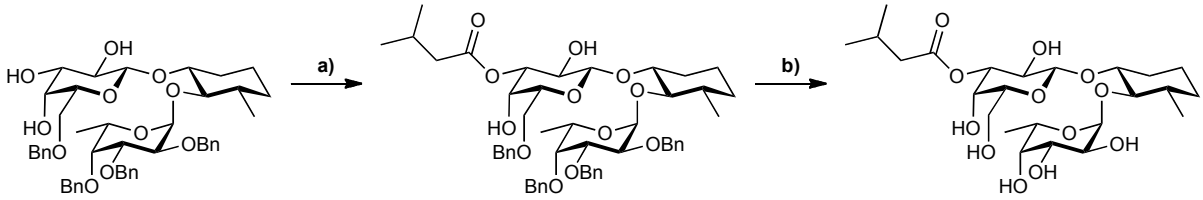
Esters **4.1a** to **4.1d** were synthesized by acylation of tin acetal activated building blocks **1.13** or **1.14** with acyl chlorides and subsequent hydrogenolytic debenzylation (Scheme 2.4.2 and Scheme 2.4.3). Acyl chlorides not commercially available were synthesized from the corresponing acids *via* DMF catalyzed chlorination with oxalyl dichloride (Scheme 2.4.1).



4.2 **4.3** **4.4** **4.5**

Scheme 2.4.1 $(COCl)_2$, DMF, CH_2Cl_2, r.t., 0.5 h, (**4.3**: 57%, **4.5**: 43%).

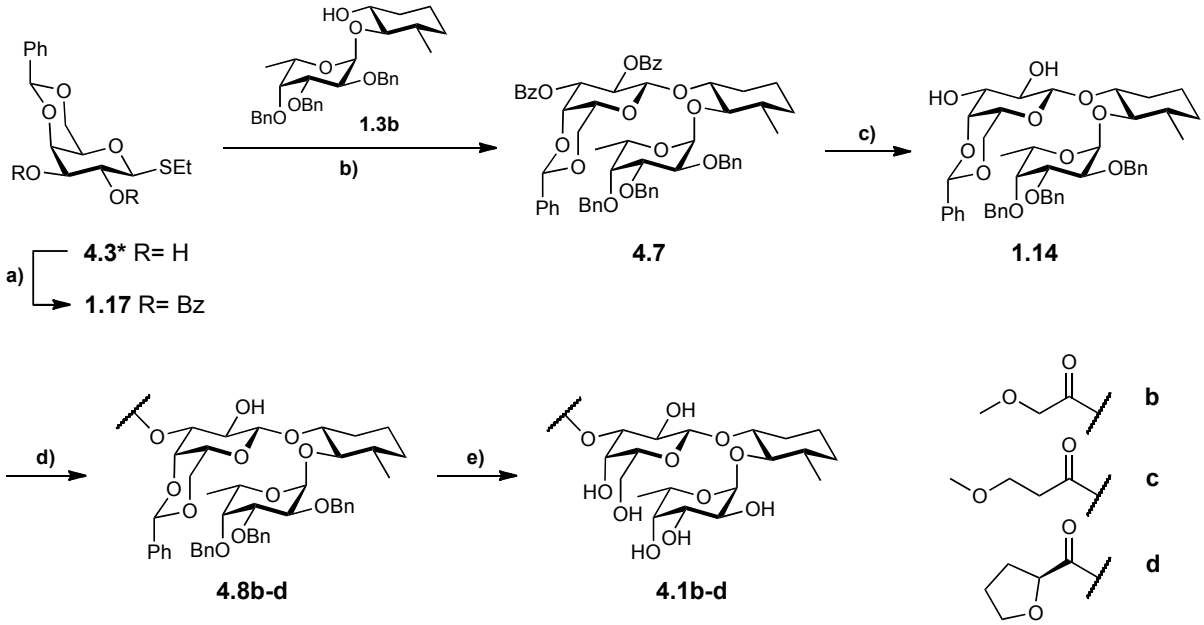
Acylation of the tin acetal of **1.13** with 3-methylbutyryl chloride repeatedly proceeded with poor regioselectivity even at -78°C, leading to very poor yields of **4.1a** (Scheme 2.4.2).



1.13 **4.6** **4.1a**

Scheme 2.4.2 a) i. Bu_2SnO, toluene, MS 4 Å, 80°C, 12 h; ii. 3-methylbutyryl chloride, toluene, -78°C, 2 h, 32%; b) $Pd(OH)_2/C$, H_2, dioxane, H_2O, 9 h, 45%.

As it is known, that the tin acetal of 4,6 benzylidene-galactosides can be benzoylated with high regioselectivity in 3-position,[1] esters **4.1b** to **4.1d** were synthesized from building block **1.14**, which was available from **4.3*** in three steps (Scheme 2.4.3). Though acylation of tin acetal activated **1.14** proceeded quite smoothly then, overall yields were still poor. To increase the regioselectivity of the acylation reaction and the stability of the final antagonists, as well as to broaden the range of substituents, amide and sulfonamide analogues were synthesized (see section 2.4.1).



Scheme 2.4.3. a) BzCl, pyridine, r.t., 2 h, 96%; b) DMTST, CH_2Cl_2, MS 4 Å, r.t., 16 h, (**4.7**: 76%, **1.3b**: 22%); c) NaOMe, MeOH, r.t., 22 h, 82%; d) i. Bu_2SnO, toluene, MS 4 Å, 80°C, 12 h; ii. RCOCl, toluene, r.t., (**4.8b**:77%, **4.8c**: 40%, **4.8d**: 69%); b) $Pd(OH)_2/C$, H_2, dioxane, H_2O, (**4.1b**: 32%, **4.1c**: 25%, **4.1d**: 21%).

2.4.2.3 Experimental

General experimental conditions are described in section 2.4.1.

3-Methoxypropanoyl chloride 4.3.

To a stirred solution of 3-methoxypropionic acid (2.0 mL, 21.1 mmol) in anhydrous CH_2Cl_2 (30 ml) under argon were added oxalyl dichloride (4.0 mL, 46.6 mmol) and DMF (cat.). The solution was stirred at r.t. for 30 min, concentrated and distilled to yield **4.3** (1.47 g, 12.0 mmol, 57%) as clear oil, which was directly used in the next step.

(*R*)-Tetrahydrofuran-2-carbonyl chloride 4.5.

To a stirred solution of (*S*)-tetrahydrofuroic acid (2.0 mL, 20.7 mmol) in anhydrous CH_2Cl_2 (30 ml) under argon were added oxalyl dichloride (4.0 mL, 46.6 mmol) and DMF (cat.). The solution was stirred at r.t. for 30 min, concentrated and distilled to yield **4.5** (1.20 g, 8.9 mmol, 43%) as clear oil, which was directly used in the next step.

6-*O*-Benzyl-3-*O*-(3-methylbutanoyl)-β-D-galactopyranosyl-(1→1)-[2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl-(1→2)]-(1*R*,2*R*,3*S*)-3-methyl-cyclohexane-1,2-diol 4.6.

A suspension of **1.13** (120 mg, 0.150 mmol), dibutyltin oxide (56 mg, 0.225 mmol), and activated powdered molecular sieves 4 Å (0.2 g) in anhydrous toluene (2.0 mL) was stirred in

a sealed vessel under argon at 80°C for 19 h. The mixture was cooled to -78°C and isovaleroyl chloride (0.030 mL, 0.244 mmol) was added slowly. After 2 h, the reaction was quenched with MeOH, the mixture was filtered (celite) and concentrated under reduced pressure. Column chromatography on silica afforded (PE/EtOAc) **4.6** as white solid (43 mg, 0.049 mmol, 32%), which was directly used in the next step.

General procedure A for the hydrogenolytic debenzylation

The corresponding intermediate was dissolved in dioxane/water (4/1, 5.0 mL) under argon. $Pd(OH)_2/C$ (25 mg, 10% $Pd(OH)_2$) was added and the resulting mixture was hydrogenated (3.5 bar H_2) at r.t. After 9 h, the mixture was filtered and the solvent removed under reduced pressure. Purification *via* HPLC and lyophilization from water/acetonitrile afforded the corresponding products.

3-*O*-(3-methylbutanoyl)-β-D-galactopyranosyl-(1→1)-[α-L-fucopyranosyl-(1→2)]-(1*R*,2*R*,3*S*)-3-methyl-cyclohexane-1,2-diol 4.1a.

According to general procedure **A**, **4.6** (43 mg, 0.049 mmol) was hydrogenated and purified to give **4.1a** as a white fluffy solid (12 mg, 0.023 mmol, 45% from **1.13**).

^{1}H NMR (500.1 MHz, CD_3OD): δ 5.00 (d, ^3J = 4.0Hz, 1H, Fuc-H1), 4.93-4.86 (m, 1H, Fuc-H5), 4.72 (dd, ^3J = 3.2, 10.2Hz, 1H, Gal-H3), 4.36 (d, ^3J = 7.7Hz, 1H, Gal-H1), 3.97 (d, ^3J = 2.9Hz, 1H, Gal-H4), 3.84 (dd, ^3J = 3.3, 10.2Hz, 1H, Fuc-H3), 3.77-3.60 (m, 6H, Fuc-H2, Fuc-H4, Gal-H2, Gal-H6a, Gal-H6b, MeCy-H1), 3.49 (t, ^3J = 6.0Hz, 1H, Gal-H5), 3.20 (t, ^3J = 9.3Hz, 1H, MeCy-H2), 2.37-2.20 (m, 2H, $(CH_3)_2CHCH_2CO$), 2.16-2.07 (m, 2H, MeCy, $(CH_3)_2CHCH_2CO$), 1.73-1.57 (m, 3H, MeCy), 1.41-1.23 (m, 2H, MeCy), 1.20 (d, ^3J = 6.6Hz, 3H, Fuc-H6), 1.13 (d, ^3J = 6.3Hz, 3H, MeCy-CH_3), 1.10-1.03 (m, 1H, MeCy), 0.99, 0.98 (2s, 6H, $(CH_3)_2CHCH_2CO$); ^{13}C NMR (125.8 MHz, CD_3OD): δ 174.5 ($(CH_3)_2CHCH_2CO$), 102.5 (Gal-C1), 100.4 (Fuc-C1), 84.6 (MeCy-C2), 80.1 (MeCy-C1), 77.1 (Gal-C3), 76.1 (Gal-C5), 73.8 (Fuc-C4), 71.4 (Fuc-C3), 70.4 (Fuc-C2), 69.7 (Gal-C2), 67.8 (Gal-C4), 67.5 (Fuc-C5), 62.7 (Gal-C6), 44.2 ($(CH_3)_2CHCH_2CO$), 40.4 (MeCy-C3), 34.9 (MeCy-C4), 31.9 (MeCy-C6), 26.8 ($(CH_3)_2CHCH_2CO$), 24.2 (MeCy-C5), 22.8 (2C, $(CH_3)_2CHCH_2CO$), 19.6 (MeCy-CH_3), 16.7 (Fuc-C6); $[\alpha]_D$ -57.4 (*c* 0.87, MeOH); HR-MS (ESI) *m*/*z*: calcd for $C_{24}H_{42}NaO_{12}$ $[M+Na]^+$: 545.2568 ; found: 545.2569.

Ethyl 2,3-di-*O*-benzoyl-4,6-*O*-benzylidene-1-thio-β-D-galactopyranoside 1.17.

A solution of ethyl 4,6-*O*-benzylidene-1-thio-β-D-galactopyranoside **4.3*** (1.40 g, 4.48 mmol)

and benzoyl chloride (1.24 mL, 10.69 mmol) in anhydrous pyridine (15 mL) was stirred for 2 h at r.t. under argon. The solution was concentrated under reduced pressure and the resulting residue was redissolved in CH_2Cl_2 (50 mL) and washed with cold aqueous HCl (1 N, 50 mL), aqueous satd. $NaHCO_3$ (5%, 50 mL) and brine (50 mL). The aqueous layers were extracted with CH_2Cl_2 (3 · 50 mL) and the combined organic layers were dried over Na_2SO_4, filtered, and concentrated. Column chromatography on silica afforded **1.17** as white solid (2.23 g, 4.28 mmol, 96%).

^{1}H NMR (500.1 MHz, $CDCl_3$): δ 7.97, 7.50, 7.37 (3m, 15H, Ar-H), 5.96 (t, ^3J = 9.9Hz, 1H, H2), 5.54 (s, 1H, C*H*Ph), 5.40 (dd, ^3J = 3.5, 9.9Hz, 1H, H3), 4.74 (d, ^3J = 9.9Hz, 1H, H1), 4.63 (d, ^3J = 3.5 Hz, 1H, H-4), 4.42 (d, ^2J = 11.3Hz, 1H, H-6a), 4.09 (d, ^2J = 11.3Hz, 1H, H-6b), 3.73 (s, 1H, H-5), 2.95, 2.81 (2m, 2H, SCH_2CH$_3$), 1.30 (m, 3H, SCH$_2$CH_3); ^{13}C NMR (125.8 MHz, $CDCl_3$): δ 166.3, 165.5 (2C, *C*OC_6H_5), 137.8, 133.5, 133.3, 130.1, 129.9, 129.7, 129.2, 128.5, 128.3, 126.4 (18C, Ar-C), 101.2 (*C*HPh), 83.1 (C-1), 74.0 (2C, C-3, C-4), 70.1 (C-5), 69.4 (C-6), 67.4 (C-2), 23.1 (S*C*H$_2$CH$_3$), 15.0 (SCH$_2$*C*H$_3$); $[\alpha]_D$ 103.0 (*c* 0.82, $CHCl_3$); MS (ESI) *m*/*z*: calcd for $C_{29}H_{28}NaO_7S^+$ $[M+Na]^+$: 543.14; found: 543.10; elemental analysis calcd (%) for $C_{29}H_{28}O_7S$ + 0.25 H_2O (525.10): C 66.33, H 5.47; found: C 66.45, H 5.40.

2,3-Di-*O*-benzoyl-4,6-*O*-benzylidene-β-D-galactopyranosyl-(1→1)-[2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl-(1→2)]-(1*R*,2*R*,3*S*)-3-methyl-cyclohexane-1,2-diol 4.7.

Powdered activated molecular sieves 4 Å (3.0 g) were added to a solution of **1.3b** (0.70 g, 1.28 mmol) and galactoside **1.17** (0.89 g, 1.71 mmol) in anhydrous CH_2Cl_2 (30 ml) and the mixture was stirred at r.t. under argon for 4 h. DMTST (1.29 g, 5.00 mmol) was dissolved in anhydrous CH_2Cl_2 (10 ml), powdered activated molecular sieves 4 Å (1.0 g) were added, and the suspension was stirred at r.t. under argon for 4 h as well. Subsequently, the two suspensions were combined and stirred at r.t. under argon for 16 h. The mixture was diluted with CH_2Cl_2 (30 mL), filtered (celite), and washed with satd. aqueous $NaHCO_3$ (100 mL) and brine (100 mL). The aqueous layers were extracted with CH_2Cl_2 (3 · 50 mL) and the combined organic layers were dried over Na_2SO_4 and concentrated. Column chromatography on silica (PE/EtOAc 4/1 to 2/1) afforded **4.7** as white foam (0.98 g, 0.98 mmol, 76%). **1.3b** was recovered in 22% (0.16 g, 0.29 mmol).

^{1}H NMR (500.1 MHz, $CDCl_3$): δ 7.98, 7.91, 7.51, 7.44 (4m, 8H, Ar-H), 7.37-7.04 (m, 22H, Ar-H), 5.80 (dd, ^3J = 8.1, 10.4Hz, Gal-H2), 5.55 (s, 1H, C*H*Ph), 5.25 (dd, ^3J = 3.5, 10.4Hz, Gal-H3), 4.96 (q, ^3J = 6.4, 6.5Hz, Fuc-H5) 4.90 (d, ^3J = 3.8Hz, 1H, Fuc-H1), 4.78-4.70 (m, 2H, Gal-H1, CH_2Ph), 4.63 (B of AB, ^2J = 11.7Hz, 1H, CH_2Ph), 4.57-4.51 (m, 3H, Gal-H4,

CH_2Ph), 4.34 (m, 1H, Gal-H6a), 4.17 (A of AB, ^2J = 11.3Hz, 1H, CH_2Ph), 4.08 (m, 1H, Gal-H6b), 3.93 (dd, ^3J = 2.6, 10.4Hz, Fuc-H3), 3.87 (dd, ^3J = 3.8, 10.4Hz, Fuc-H2), 3.61-3.53 (m, 3H, Gal-H5, MeCy-H1, CH_2Ph), 3.23 (m, 1H, Fuc-H4), 3.12 (t, ^3J = 9.6Hz, MeCy-H2), 1.89 (m, 1H, MeCy), 1.67-1.37 (m, 3H, MeCy), 1.24 (d, ^3J = 6.5Hz, 3H, Fuc-H6), 1.20-1.01 (m, 2H, MeCy), 0.97 (d, ^3J = 6.5Hz, 3H, MeCy-CH_3), 0.84 (m, 1H, MeCy); δ ^{13}C NMR (125.8 MHz, $CDCl_3$): δ 166.3, 165.2 (2C, *C*OC_6H_5), 139.9, 139.7, 138.9, 138.0, 133.6, 133.2, 130.1, 129.9, 129.8, 129.3, 129.0, 128.8, 128.6, 128.5, 128.3, 128.2, 128.0, 127.7, 127.5, 127.2, 127.0, 125.9 (36C, Ar-C), 99.8 (*C*HPh), 99.6 (Gal-C1), 98.5 (Fuc-C1), 81.6 (MeCy-C2), 80.9, (MeCy-C1), 79.9 (Fuc-C3), 79.2 (Fuc-C4), 75.8 (Fuc-C2), 75.1, 74.6 (2C, *C*H_2Ph), 73.7 (Gal-C4), 73.2 (Gal-C3), 71.4 (*C*H_2Ph), 69.4 (Gal-C6), 69.1 (Gal-C2), 66.4, 66.3 (2C, Fuc-C5, Gal-C5), 39.6 (MeCy-C3), 33.6 (MeCy-C4), 31.3 (MeCy-C6), 23.5 (MeCy-C5), 18.8 (MeCy-*C*H_3), 16.6 (Fuc-C6); $[\alpha]_D$ -40.6 (*c* 1.50, $CHCl_3$); MS (ESI) *m*/*z*: calcd for $C_{61}H_{64}NaO_{13}^+$ $[M+Na]^+$: 1027.4; found: 1027.5; elemental analysis calcd (%) for $C_{61}H_{64}O_{13}$: C 72.89, H 6.42; found: C 72.81, H 6.37.

4,6-*O*-Benzylidene-β-D-galactopyranosyl-(1→1)-[2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl-(1→2)]-(1*R*,2*R*,3*S*)-3-methyl-cyclohexane-1,2-diol 1.14.

A freshly prepared solution of NaOMe in anhydrous MeOH (3 N, 0.47 mL) was slowly added to a solution of **4.7** (920 mg, 0.92 mmol) in anhydrous MeOH (20 mL) under argon at r.t. After 22 h, the mixture was neutralized with HCl in MeOH (3 N, 0.47 mL) and concentrated under reduced pressure. Column chromatography on silica (EtOAc) afforded **1.14** as white foam (600 mg, 0.75 mmol, 82%).

^{1}H NMR (500.1 MHz, $CDCl_3$): δ 7.56-6.93 (m, 20H, Ar-H), 5.49 (s, 1H, C*H*Ph), 4.90 (d, ^3J = 2.6Hz, 1H, Fuc-H1), 4.80 (q, ^3J = 6.3Hz, Fuc-H5), 4.74 (A of AB, ^2J = 11.6Hz, 1H, CH_2Ph), 4.63 (B of AB, ^2J = 11.6Hz, 1H, CH_2Ph), 4.56-4.49 (m, 2H, CH_2Ph), 4.62 (A of AB , ^2J = 11.3Hz, 1H, CH_2Ph), 4.22-4.16 (m, 2H, Gal-H1, Gal-H6a), 4.04 (d, ^3J = 2.9Hz, 1H, Gal-H4), 3.93 (m, 1H, Gal-H6b), 3.96-3.90 (m, 2H, Fuc-H2, Fuc-H3), 3.69-3.59 (m, 3H, CH_2Ph, Gal-H2, Gal-H3), 3.55 (m, 1H, MeCy-H1), 3.24 (m, 1H, Fuc-H4), 3.21 (m, 1H, Gal-H5), 3.13 (t, ^3J = 9.5Hz, MeCy-H2), 1.96 (m, 1H, MeCy), 1.61-1.42 (m, 3H, MeCy), 1.32-1.05 (m, 2H, MeCy), 1.04-0.85 (m, 7H Fuc-H6, MeCy); ^{13}C NMR (125.8 MHz, $CDCl_3$): δ 139.6, 139.4, 138.7, 138.0, 128.9, 128.7, 128.3, 128.2, 128.1, 128.0, 127.5, 127.4, 127.2, 125.8 (24C, Ar-C), 101.0 (Gal-C1), 100.0 (C*H*Ph), 98.5 (Fuc-C1), 82.4 (MeCy-C2), 80.1 (MeCy-C1), 79.7 (Fuc-C3), 78.8 (Fuc-C4), 75.6, 75.5 (2C, Fuc-C2, Gal-C4), 74.9, 74.5 (2C, *C*H_2Ph), 72.6 (Gal-C3), 71.4, 71.3 (Gal-C2, *C*H_2Ph), 69.5 (Gal-C6), 66.4, 66.22 (Fuc-C5,

Gal-C5), 39.5 (MeCy-C3), 33.7 (MeCy-C4), 31.4 (MeCy-C6), 23.4 (MeCy-C5), 19.9 (MeCy-*C*H_3), 16.7 (Fuc-C6); $[\alpha]_D$ -83.0 (*c* 1.18, $CHCl_3$); MS (ESI) *m*/*z*: calcd for $C_{47}H_{56}NaO_{11}^+$ $[M+Na]^+$: 819.4; found: 819.4; elemental analysis calcd (%) for $C_{47}H_{56}O_{11}$ + 0.5 H_2O (805.95): C 70.04, H 7.13; found: C 69.80, H 7.00.

General procedure B for acylations of 1.14

A suspension of **1.14**, dibutyltin oxide, and activated powdered molecular sieves 4 Å in anhydrous toluene was stirred in a sealed vessel under argon at 80°C for 19 h. The mixture was cooled to r.t. and the corresponding acyl chloride was added slowly. After 30 min, the reaction was quenched with MeOH, the mixture was filtered (celite) and concentrated under reduced pressure. Column chromatography on silica afforded the corresponding products esters.

4,6-*O*-Benzylidene-2-*O*-(2-methoxyacetyl)-β-D-galactopyranosyl-(1→1)-[2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl-(1→2)]-(1*R*,2*R*,3*S*)-3-methyl-cyclohexane-1,2-diol 4.8b.

According to general procedure **B**, **1.14** (80 mg, 0.100 mmol), dibutyltin oxide (33 mg, 0.133 mmol) and molecular sieves (0.05 g) were reacted in anhydrous toluene (0.5 mL). Methoxyacetyl chloride (17 mg, 0.157 mmol) was added. Column chromatography on silica afforded **4.8b** as white solid (67 mg, 0.077 mmol, 77%), which was directly used in the next step.

4,6-*O*-Benzylidene-3-*O*-(3-methoxypropanoyl)-β-D-galactopyranosyl-(1→1)-[2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl-(1→2)]-(1*R*,2*R*,3*S*)-3-methyl-cyclohexane-1,2-diol 4.8c.

According to general procedure **B**, **1.14** (70 mg, 0.088 mmol), dibutyltin oxide (33 mg, 0.133 mmol) and molecular sieves (0.05 g) were reacted in anhydrous toluene (0.5 mL). **4.3** (16 mg, 0.131 mmol) was added. Column chromatography on silica afforded **4.8c** as white solid (31 mg, 0.035 mmol, 40%).

^{1}H NMR (500.1 MHz, $CDCl_3$): δ 7.57-7.04 (m, 20H, Ar-H), 5.50 (s, 1H, C*H*Ph), 4.92-4.85 (m, 2H, Fuc-H1, Gal-H3), 4.80 (q, ^3J = 6.4Hz, Fuc-H5), 4.75 (A of AB, ^2J = 11.6Hz, 1H, CH_2Ph), 4.64 (B of AB, ^2J = 11.6Hz, 1H, CH_2Ph), 4.56-4.52 (m, 2H, CH_2Ph), 4.33 (d, ^3J = 7.8Hz, 1H, Gal-H1), 4.30 (d, ^3J = 3.4Hz, 1H, Gal-H4), 4.28-4.20 (2H, m, Gal-H6a, CH_2Ph), 4.02-3.98 (m, 1H, Gal-H6b), 3.94-3.84 (m, 3H, Fuc-H2, Fuc-H3, Gal-H2), 3.69-3.52 (m, 4H, $CH_2CH_2OCH_3$, MeCy-H1, CH_2Ph), 3.40 (s, 1H, Gal-H5), 3.25 (s, 3H, $CH_2CH_2OCH_3$), 3.18 (s, 1H, Fuc-H4), 3.14 (t, ^3J = 9.5Hz, MeCy-H2), 2.66 (td, *J* =1.8, 6.2Hz, 2H, C$H_2CH_2OCH_3$),

2.00 (m, 1H, MeCy), 1.65-1.43 (m, 3H, MeCy), 1.20-1.17 (m, 2H, MeCy), 1.06-0.90 (m, 7H Fuc-H6, MeCy); ^{13}C NMR (125.8 MHz, $CDCl_3$): δ 171.6 (*C*$OCH_2CH_2OCH_3$), 139.7, 139.5, 138.7, 138.1, 128.9, 128.8, 128.3, 128.2, 128.0, 127.6, 127.5, 127.2, 127.0, 125.9 (24C, Ar-C), 101.2 (Gal-C1), 99.7 (C*H*Ph), 98.6 (Fuc-C1), 82.4 (MeCy-C2), 80.2 (MeCy-C1), 79.7 (Fuc-C3), 78.8 (Fuc-C4), 75.6 (Fuc-C2), 74.9, 74.6 (2C, *C*H_2Ph), 73.9 (Gal-C3), 73.6 (Gal-C4), 71.4 (*C*H_2Ph), 69.4 (Gal-C6), 68.3 (Gal-C2), 68.1 (CH_2*C*H_2OCH_3), 66.3, 66.2 (Fuc-C5, Gal-C5), 58.9 (CH_2CH_2O*C*H_3), 39.6 (MeCy-C3), 35.2 (*C*$H_2CH_2OCH_3$), 33.8 (MeCy-C4), 31.5 (MeCy-C6), 23.4 (MeCy-C5), 18.9 (MeCy-*C*H_3), 16.7 (Fuc-C6); MS (ESI) *m*/*z*: calcd for $C_{51}H_{62}NaO_{13}^+$ $[M+Na]^+$: 905.41; found: 905.47.

4,6-*O*-Benzylidene-3-*O*-((*R*)-tetrahydrofuran-2-carboxyl)-β-D-galactopyranosyl-(1→1)-[2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl-(1→2)]-(1*R*,2*R*,3*S*)-3-methyl-cyclohexane-1,2-diol 4.8d.

According to general procedure **B**, **1.14** (70 mg, 0.088 mmol), dibutyltin oxide (33 mg, 0.133 mmol) and molecular sieves (0.05 g) were reacted in anhydrous toluene (0.5 mL). **4.5** (15 mg, 0.111 mmol) was added. Column chromatography on silica (CH_2Cl_2/MeOH) afforded **4.8d** as white foam (54 mg, 0.060 mmol, 69%).

^{1}H NMR (500.1 MHz, $CDCl_3$): δ 7.56-7.00 (m, 20H, Ar-H), 5.49 (s, 1H, C*H*Ph), 4.93-4.85 (m, 2H, Fuc-H1, Gal-H3), 4.78 (m, 1H, Fuc-H5), 4.75 (A of AB, ^2J = 11.6Hz, 1H, CH_2Ph), 4.64 (B of AB, ^2J = 11.6Hz, 1H, CH_2Ph), 4.59-4.51 (m, 3H, CH_2Ph, THF-H1), 4.33-4.29 (m, 2H, Gal-H1, Gal-H4), 4.28-4.22 (2H, m, Gal-H6a, CH_2Ph), 4.06-3.97 (m, 2H, Gal-H6b, THF-H4a), 3.95-3.83 (m, 4H, Fuc-H2, Fuc-H3, Gal-H2, THF-H4b), 3.64-3.54 (m, 2H, MeCy-H1, CH_2Ph), 3.41 (s, 1H, Gal-H5), 3.19 (s, 1H, Fuc-H4), 3.13 (t, ^3J = 9.5Hz, MeCy-H2), 2.26 (m, 1H, THF-H2a), 2.05 (m, 1H, THF-H2b), 1.99 (m, 1H, MeCy), 1.95-1.80 (m, 2H, THF-H3a,b), 1.62-1.47 (m, 3H, MeCy), 1.31-1.09 (m, 2H, MeCy), 1.06-0.86 (m, 7H Fuc-H6, MeCy); ^{13}C NMR (125.8 MHz, $CDCl_3$): δ 173.7 (THF-*C*O), 139.6, 139.5, 138.7, 138.1, 128.9, 128.8, 128.3, 128.2, 128.0, 127.6, 127.5, 127.2, 127.0, 125.8 (24C, Ar-C), 101.2 (Gal-C1), 99.7 (C*H*Ph), 98.6 (Fuc-C1), 82.5 (MeCy-C2), 80.3 (MeCy-C1), 79.7 (Fuc-C3), 78.8 (Fuc-C4), 76.7 (THF-C1), 75.6 (Fuc-C2), 74.9, 74.6 (2C, *C*H_2Ph), 73.8, 73.6 (2C, Gal-C3, Gal-C4), 71.5 (*C*H_2Ph), 69.7 (THF-C4), 69.4 (Gal-C6), 68.4 (Gal-C2), 66.3, 66.2 (Fuc-C5, Gal-C5), 39.6 (MeCy-C3), 33.7 (MeCy-C4), 31.5 (MeCy-C6), 30.7 (THF-C2), 29.8 (THF-C3), 23.4 (MeCy-C5), 18.9 (MeCy-*C*H_3), 16.7 (Fuc-C6); MS (ESI) *m*/*z*: calcd for $C_{52}H_{62}NaO_{13}^+$ $[M+Na]^+$: 917.4; found: 917.5.

3-*O*-(2-methoxyacetyl)-β-D-galactopyranosyl-(1→1)-[α-L-fucopyranosyl-(1→2)]-(1*R*,2*R*,3*S*)-3-methyl-cyclohexane-1,2-diol 4.1b.

According to general procedure **A**, **4.8b** (53 mg) was hydrogenated and purified to give **4.1b** as a white fluffy solid (10 mg, 0.020 mmol, 32%).

^{1}H NMR (500.1 MHz, CD_3OD): δ 5.00 (d, ^3J = 4.0Hz, 1H, Fuc-H1), 4.94-4.86 (m, 1H, Fuc-H5), 4.79 (dd, ^3J = 2.8, 10.1Hz, 1H, Gal-H3), 4.39 (d, ^3J = 7.7Hz, 1H, Gal-H1), 4.16 (s, 2H, $COCH_2OCH_3$), 4.02 (d, ^3J = 2.8Hz, 1H, Gal-H4), 3.84 (dd, ^3J = 3.3, 10.3Hz, 1H, Fuc-H3), 3.77-3.64 (m, 6H, Fuc-H2, Fuc-H4, Gal-H2, Gal H6a, Gal-H6b, MeCy-H1), 3.52 (t, ^3J = 6.0Hz, 1H, Gal-H5), 3.44 (s, 3H, $COCH_2OCH_3$), 3.20 (t, ^3J = 9.3Hz, 1H, MeCy-H2), 2.14 (m, 1H, MeCy), 1.72-1.57 (m, 3H, MeCy), 1.40-1.22 (m, 2H, MeCy), 1.19 (d, ^3J = 6.6Hz, 3H, Fuc-H6), 1.14 (d, ^3J = 6.3Hz, 3H, MeCy-CH_3), 1.11-1.02 (m, 1H, MeCy); ^{13}C NMR (125.8 MHz, CD_3OD): δ 171.9 ($COCH_2OCH_3$), 102.3 (Gal-C1), 100.5 (Fuc-C1), 84.6 (MeCy-C2), 80.1 (MeCy-C1), 78.0 (Gal-C3), 75.9 (Gal-C5), 73.8 (Fuc-C4), 71.4 (Fuc-C3), 70.3 (2C, $COCH_2OCH_3$, Fuc-C2), 69.6 (Gal-C2), 67.7 (Gal-C4), 67.5 (Fuc-C5), 62.6 (Gal-C6), 59.5 ($COCH_2OCH_3$), 40.4 (MeCy-C3), 34.9 (MeCy-C4), 31.9 (MeCy-C6), 24.2 (MeCy-C5), 19.6 (MeCy-CH_3), 16.7 (Fuc-C6); $[\alpha]_D$ -62.2 (*c* 0.60, MeOH); HR-MS (ESI) *m*/*z*: calcd for $C_{22}H_{38}NaO_{13}$ $[M+Na]^+$: 533.2205 ; found: 533.2205.

3-*O*-(3-Methoxypropanoyl)-β-D-galactopyranosyl-(1→1)-[α-L-fucopyranosyl-(1→2)]-(1*R*,2*R*,3*S*)-3-methyl-cyclohexane-1,2-diol 4.1c.

According to general procedure **A**, **4.8c** (30 mg, 0.034 mmol) was hydrogenated and purified to give **4.1c** as a white fluffy solid (4.5 mg, 0.009 mmol, 25%).

^{1}H NMR (500.1 MHz, CD_3OD): δ 4.99 (d, ^3J = 4.0Hz, 1H, Fuc-H1), 4.97-4.86 (m, 1H, Fuc-H5), 4.71 (dd, ^3J = 3.1, 10.1Hz, 1H, Gal-H3), 4.37 (d, ^3J = 7.7Hz, 1H, Gal-H1), 3.99 (d, ^3J = 2.6Hz, 1H, Gal-H4), 3.84 (dd, ^3J = 3.3, 10.3Hz, 1H, Fuc-H3), 3.79-3.62 (m, 8H, Fuc-H2, Fuc-H4, Gal-H2, Gal H6a, Gal-H6b, MeCy-H1, $COCH_2CH_2OCH_3$), 3.50 (t, ^3J = 6.0Hz, 1H, Gal-H5), 3.34 (s, 3H, $COCH_2CH_2OCH_3$), 3.20 (t, ^3J = 9.3Hz, 1H, MeCy-H2), 2.68 (t, 2H, $COCH_2CH_2OCH_3$), 2.12 (m, 1H, MeCy), 1.72-1.57 (m, 3H, MeCy), 1.39-1.22 (m, 2H, MeCy), 1.19 (d, ^3J = 6.6Hz, 3H, Fuc-H6), 1.13 (d, ^3J = 6.3Hz, 3H, MeCy-CH_3), 1.06 (m, 1H, MeCy); ^{13}C NMR (125.8 MHz, CD_3OD): δ 172.9 ($COCH_2CH_2OCH_3$), 102.4 (Gal-C1), 100.5 (Fuc-C1), 84.6 (MeCy-C2), 80.1 (MeCy-C1), 77.8 (Gal-C3), 76.0 (Gal-C5), 73.9 (Fuc-C4), 71.4 (Fuc-C3), 70.4, 69.7 (2C, Fuc-C2, Gal-C2), 69.1 ($COCH_2CH_2OCH_3$), 67.6, 67.5 (2C, Fuc-C5, Gal-C4), 62.6 (Gal-C6), 59.0 ($COCH_2CH_2OCH_3$), 40.4 (MeCy-C3), 35.7 ($COCH_2CH_2OCH_3$), 34.9 (MeCy-C4), 31.9 (MeCy-C6), 24.2 (MeCy-C5), 19.6 (MeCy-CH_3),

16.7 (Fuc-C6); $[\alpha]_D$ -63.1 (*c* 0.76, MeOH); HR-MS (ESI) *m/z*: calcd for $C_{23}H_{40}NaO_{13}$ $[M+Na]^+$: 547.2361; found: 547.2365.

3-*O*-((*R*)-tetrahydrofuran-2-carboxyl)-β-D-galactopyranosyl-(1→1)-[α-L-fucopyranosyl-(1→2)]-(1*R*,2*R*,3*S*)-3-methyl-cyclohexane-1,2-diol 4.1d.

According to general procedure **A**, **4.8d** (43 mg, 0.048 mmol) was hydrogenated and purified to give **4.1d** as white fluffy solid (5.4 mg, 0.010 mmol, 21%).

^{1}H NMR (500.1 MHz, CD_3OD): δ 4.99 (d, ^3J = 4.0Hz, 1H, Fuc-H1), 4.95-4.86 (m, 1H, Fuc-H5), 4.76 (dd, ^3J = 3.2, 10.1Hz, 1H, Gal-H3), 4.57 (dd, ^3J = 5.1, 8.6Hz, 1H, THF-H1), 4.37 (d, ^3J = 7.7Hz, 1H, Gal-H1), 4.04-3.95 (m, 2H, Gal-H4, THF-H4a), 3.92-3.86 (m, 1H, THF-H4b), 3.83 (dd, ^3J = 3.3, 10.2Hz, 1H, Fuc-H3), 3.77-3.62 (m, 6H, Fuc-H2, Fuc-H4, Gal-H2, Gal H6a, Gal-H6b, MeCy-H1), 3.51 (t, ^3J = 6.1Hz, 1H, Gal-H5), 3.20 (t, ^3J = 9.3Hz, 1H, MeCy-H2), 2.35-2.24 (m, 1H, THF-H2a), 2.19-2.08 (m, 2H, MeCy, THF-H2b), 2.01-1.87 (m, 2H, THF-H3a,3b), 1.73-1.57 (m, 3H, MeCy), 1.38-1.23 (m, 2H, MeCy), 1.19 (d, ^3J = 6.6Hz, 3H, Fuc-H6), 1.13 (d, ^3J = 6.3Hz, 3H, MeCy-C*H*$_3$), 1.10-1.02 (m, 1H, MeCy); ^{13}C NMR (125.8 MHz, CD_3OD): δ 174.8 (THF-*C*O), 102.5 (Gal-C1), 100.4 (Fuc-C1), 84.6 (MeCy-C2), 80.2 (MeCy-C1), 77.8 (2C, Gal-C3, THF-C1), 76.0 (Gal-C5), 73.8 (Fuc-C4), 71.4 (Fuc-C3), 70.4 (2C, Fuc-C2, THF-C4), 69.8 (Gal-C2), 67.8 (Gal-C4), 67.5 (Fuc-C5), 62.6 (Gal-C6), 40.4 (MeCy-C3), 34.9 (MeCy-C4), 32.0 (MeCy-C6), 31.2 (THF-C2), 26.0 (THF-C3), 24.2 (MeCy-C5), 19.6 (MeCy-*C*H$_3$), 16.7 (Fuc-C6); $[\alpha]_D$ -68.7 (*c* 0.87, MeOH); HR-MS (ESI) *m/z*: calcd for $C_{24}H_{40}NaO_{13}$ $[M+Na]^+$: 559.2361; found: 559.2365.

References

[1] S. David, A. Thieffry, *J. Chem. Soc. Perkin 1* **1979**, 1568-1573; S. David, A. Malleron, *Carbohydr. Res.* **2000**, *329*, 215-218.

2.5 Derivatives of 2,2-dialkyl-2-*O*-glycosyl glycolic acid as E-selectin antagonists

2.5.1 Design, synthesis, and characterization of 2,2-dialkyl-2-*O*-glycosyl glycolic acid derivatives as E-selectin antagonists

Author contributions: F.P.C. Binder: design and synthesis, manuscript; M. Smieško: molecular modeling studies; K. Lemme: static binding assay.

Manuscript

Design, Synthesis, and Characterization of 2,2-Dialkyl-2-*O*-glycosyl Glycolic Acid Derivatives as E-Selectin Antagonists

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Keywords: Antagonist, carbohydrate, glycomimetic, pre-organization, selectin,

Abstract

Selectins have been recognized as promising targets for the development of anti-inflammatory drugs. Since the carbohydrate lead structure sialyl Lewis^x (sLe^x) exhibits poor pharmacokinetic and pharmacodynamic properties and requires a complex synthesis, efforts were directed to replace carbohydrate residues with less complex and less polar mimics. For E-selectin antagonists, mostly derivatives of (*S*)-lactic acid were used to replace the sialic acid moiety of sLe^x. As molecular dynamics simulations indicated that derivatives of 2,2-dialkyl glycolic acid might be suited as replacements as well, two glycomimetic derivatives were synthesized and tested for their ability to block E-selectin in a competitive binding assay.

1. Introduction

Selectins, namely E-, P-, and L-selectin, are Ca^{2+}-dependent carbohydrate binding proteins that mediate the initial step of leukocyte recruitment to sites of inflammation. Blocking the interaction of selectins with their physiological ligands is consequently considered a promising therapeutic approach to tackle chronic and acute inflammatory diseases like stroke, psoriasis or reperfusion injuries.[1] Most drug discovery programs started from the tetrasaccharide sialyl Lewis^x (**1,** Figure 1), as it is the common carbohydrate binding epitope recognized by all three selectins.[2] However, the development of carbohydrate derived drugs is strongly hampered by the intrinsic properties of carbohydrates, as these typically exhibit poor pharmacokinetic and pharmacodynamic properties and require complex synthesis. A typical strategy to overcome these drawbacks is the stepwise replacement of carbohydrate residues with (carbocyclic) mimics. In the ideal case, this leads to glycomimetics of reduced complexity, increased affinity, and improved pharmacokinetic properties. This strategy was also applied to the rational design of selectin antagonists.[3]

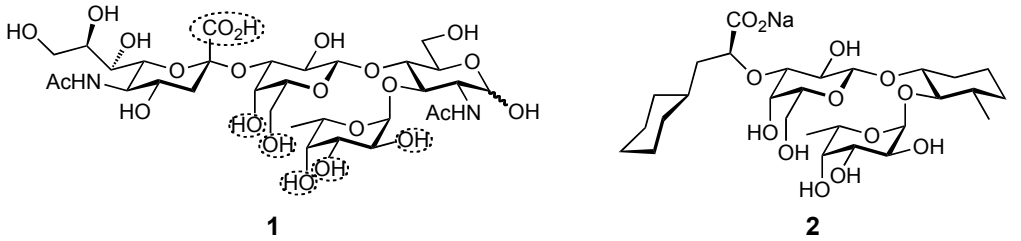


Figure 1 Left: the tetrasaccharide sialyl Lewis^x (**1**) and its pharmacophoric groups: hydroxyl groups of L-Fuc[4], hydroxyl groups in 4- and 6- position of D-Gal[5] and the carboxylic acid residue of sialic acid[6]. Right: E-selectin antagonist **2**.[7]

N-Acetyl-D-glucosamine (D-Glc*N*Ac), which does not bear pharmacophoric groups, but rather acts as a spacer between L-fucose (L-Fuc) and D-galactose (D-Gal), was replaced by numerous linkers.[8,9] It was shown that the affinity of the resulting mimetics correlated with the ability of the linker to pre-organize the Lewis^x core.[9] Conformationally restricted linkers like (*R,R*)-cyclohexane-1,2-diol were better suited than more flexible ones like ethane-1,2-diol. Sialic acid, which contributes to binding mainly with its carboxy group, [6,10] was typically replaced by glycolic acid, lactic acid, or derivatives thereof.[11] Pre-organization also turned out to be essential for the carboxy group of sialic acid, *i.e.* derivatives of (*S*)-lactic acid were superior to derivatives of (*R*)-lactic acid and to glycolic acid. Efforts to replace both D-Glc*N*Ac and sialic acid simultaneously resulted in antagonist **2** (Figure 1), which showed a 80-fold improvement of affinity compared to the lead structure sLe^x (**1**).[7]

Though derivatives of lactic acid have been studied extensively as mimics of sialic acid,[12,13] no replacements with 2,2-dialkyl substituted glycolic acid or derivatives thereof have been reported. Given the fact that neither the glycerol side chain nor the acetamide moiety of sialic acid contribute to binding,[6,10] we wondered if sialic acid could be replaced by a simple carbocyclic mimic (→ **3**, Figure 2) as well. In this short communication, we report on the design, synthesis, and biological evaluation of 2,2-dialkyl substituted glycolic acids as replacements for sialic acid.

2. Results and Discussion

The ability to properly orient the carboxylic acid residue of sialic acid in the bioactive conformation is a key prerequisite for novel sialic acid mimics. Consequently, we used a molecular modeling tool developed by Ernst and Kolb[13] to assess the degree of pre-organization of potential sialic acid replacements.

Based on a Monte Carlo (jumping between wells)/stochastic dynamics [MC(JBW)/SD] simulation,[13] this tool allows to compare the calculated conformational preference of the mimetic with the experimentally observed conformation of sLe^x bound to E-selectin,[14] which defines the bioactive window. For a graphical presentation, two internal coordinates, the acid orientation and the core conformation were defined and the relative population of the conformations was indicated with a color code (Figure 2). Mimetics populating the bioactive window are predicted to be better selectin ligands than those outside the window.

To rule out conformational bias caused by a ring flip of **3**, we also planned the synthesis of non-cyclic **4**. According to the conformational preferences found for **3** and **4**, both compounds should be very well pre-organized (Figure 2).

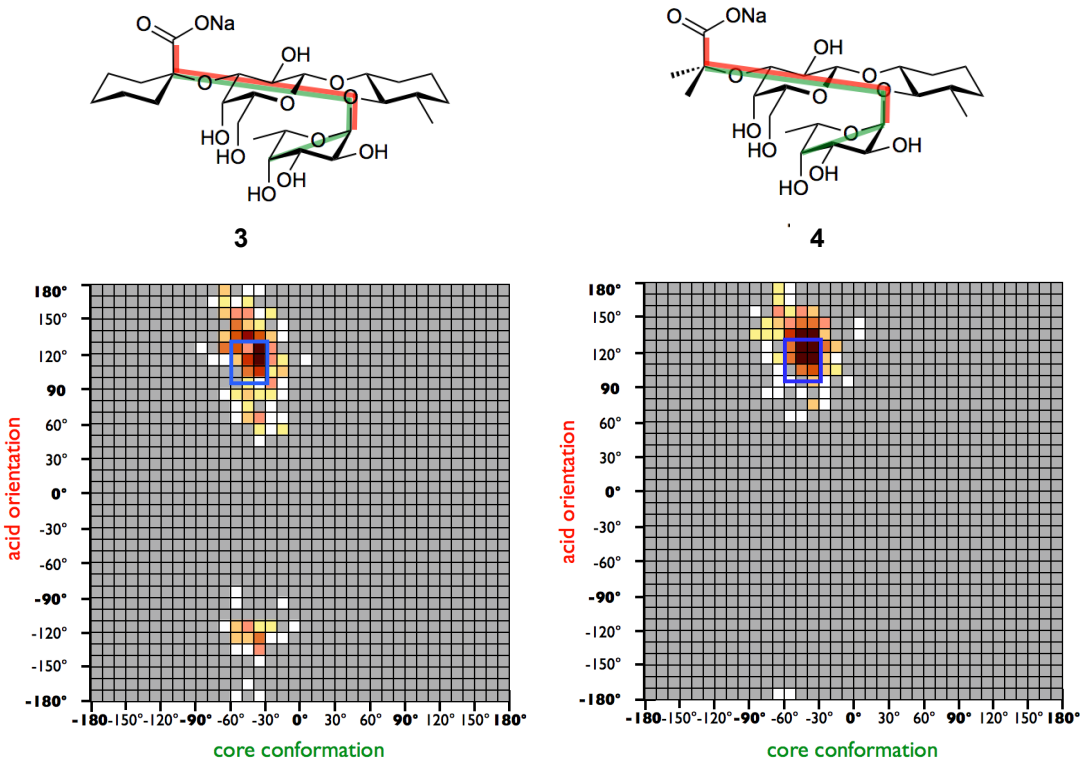


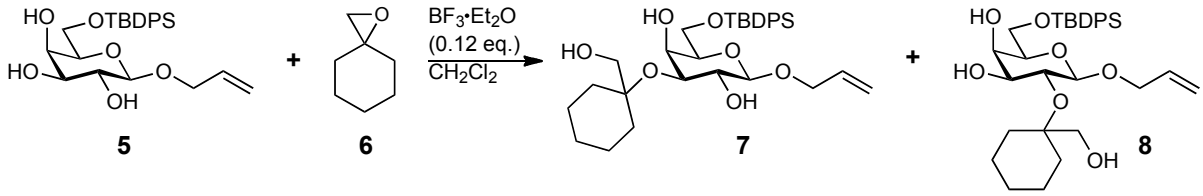
Figure 2 The conformational preferences calculated for **3** (left) and **4** (right) nicely superimpose with the experimentally observed conformation of sLe^x bound to E-selectin (blue rectangle). Relative population of the conformations is indicated with a color code ranging from white to dark red. The darker the color, the higher is the population of the conformation.

Synthesis

Glycomimetics of type **2** are typically synthesized by S_N2-type alkylation of an appropriately protected trisaccharide mimic with a lactic acid derivative, activated as triflate. However, this strategy could not be applied to the synthesis of compounds **3** and **4**, as the sterically demanding tertiary triflates are not suited for S_N2-type reactions. Since, to the best of our knowledge, no method is described for the synthesis of 2,2-dialkyl-2-*O*-glycosyl-glycolic acids, and the harsh conditions usually applied for the ether formation with *tert*-alcohols were considered incompatible with carbohydrate chemistry, an alternative route had to be found (Table 1). Alkylation of galactoside **5**[15] with spiroepoxide **6**[16] was planned to give **7** regio- and chemoselectively under acidic conditions. The newly formed primary hydroxy group should serve as precursor for the carboxy group. To reduce steric constraints and thereby increase the reactivity of the galactoside, the 2-*O* and 4-*O* position of galactoside **5** were not protected. $BF_3{\cdot}Et_2O$ catalyzed alkylation of **5** with spiroepoxide **6** was repeated several times with slightly modified conditions (Table 1). Unfortunately, the rate of conversion was rather low and several attempts to increase it failed. Higher concentrations of **5** did not affect the

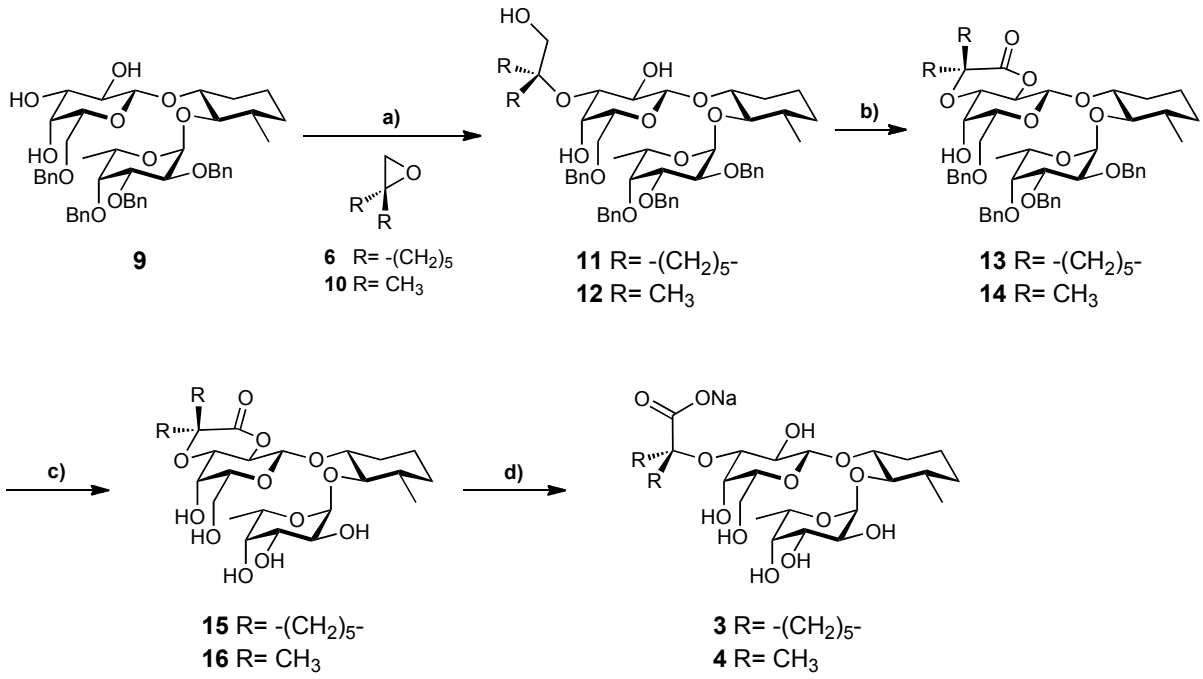
conversion, and higher temperatures, stronger Lewis acids, or increased concentrations of **6** led to the enhanced formation of side products (results not shown). Multiple alkylation and hemiacetal formation due to hydride shift were observed as main side reactions.

Table 1 Alkylation of **5** with spiroepoxide **6** using different reaction conditions.



Entry	c(6) [M]	ratio 5/6	T [°C]	t [min]	Recovered 5	Yield 7 + 8	Ratio 7/8 (NMR)
A	2.9	2/1	-15 to 0	55	75%	7%	1.0/1.2
B	2.2	1.7/1	-15	50	84%	7%	1.0/1.4
C	2.3	1.4/1	-15	55	67%	5%	1.0/1.0
D	0.4	0.7/1	-18	135	72%	8%	1.0/1.5

Finally, the reaction conditions shown in entry **D** were successfully applied to the synthesis of the tetrasaccharide mimetics **11** and **12** (Scheme 1). Yields and conversion of the alkylation reactions were comparable to the alkylation of **5** and proved the general applicability of the reaction conditions to the synthesis of more complex glycomimetics. TEMPO mediated chemoselective oxidation[17] of **11** and **12** provided lactones **13** and **14**. The oxidation led to a significant low-field shift of the proton in 2-position of D-Gal ($\Delta\delta$= 0.8 ppm), confirming the identity of **13** and **14**. Debenzylation by hydrogenolysis finally yielded the lactones **15** and **16**, which were hydrolyzed to give **3** and **4**. The identity of compounds **3** and **4** was unambiguously confirmed by HMBC, as a crosspeak between the tertiary carbon of the sialic acid mimic and H-3 of D-Gal was observed.



Scheme 1 a) **6** or **10**, $BF_3 \cdot Et_2O$, CH_2Cl_2, -18°C, (**11**: 4%, **9**: 74%), (**12**: 6%, **9**: 56%); b) NaOCl, TEMPO, NaBr, Bu_4NBr, $NaHCO_3$, CH_2Cl_2, H_2O, 0°C to r.t., 45 min to 80 min, (**13**: 68%, **14**: 67%); c) H_2, $Pd(OH)_2/C$, dioxane, H_2O, r.t., 8 h, (**15**: 81%, **16**: 77%); d) NaOH, dioxane, H_2O, r.t., 5 h, (**3**: 70%, **4**: 70%).

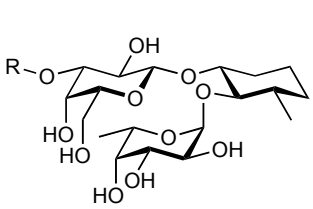
Evaluation of 3 and 4 as E-selectin antagonists

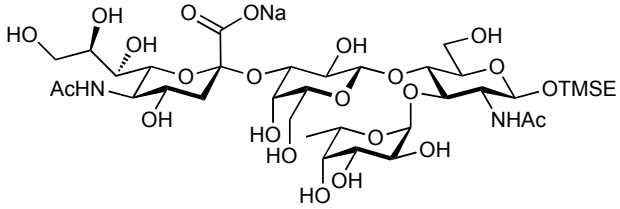
The affinity of selectin antagonists to E-selectin was evaluated in a competitive binding assay, utilizing a polyacrylamide-type glycoconjugate as synthetic ligand for immobilized E-selectin.[18] Briefly, microtiter plates were coated with E-selectin/IgG, blocked with BSA, and incubated with a fixed concentration of sLe^a-polyacrylamide (sLe^a-PAA) either in presence or absence of the antagonists. The binding reaction was revealed by the addition of TMB substrate reagent and quantified spectrophotometrically at 450 nm. The IC_{50} defines the molar concentration of the test compound that reduces the maximal specific binding of sLe^a-PAA polymer to E-selectin by 50%. The affinities are reported relative to TMSE protected sLe^x (**17**) as rIC_{50} in Table 2. The relative IC_{50} (rIC_{50}) is the ratio of the IC_{50} of the test compound to the IC_{50} of **17**. To assess the impact of the sialic acid exchange, affinity data is also provided for compounds **2** and **18**.

Antagonists **3** (entry C) and **4** (entry D) showed a dramatic loss in affinity compared to cyclohexyllactic acid containing analogue **2** (entry A) and sialic acid containing **18** (entry B, Table 2). The affinity of **3** was 50 fold weaker than the one of **18**. The low affinity of **3** could be the consequence of a ring flip of the cyclohexane moiety, resulting in an equatorial orientation of the carboxy group. In the bioactive conformation, this orientation leads to a

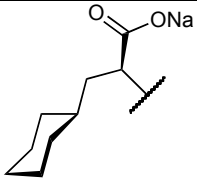
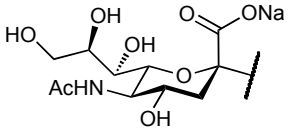
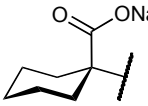
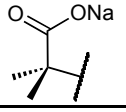
steric clash of the cyclohexane moiety and D-Gal. However, non-cyclic **4** bound in the same range as **3**, arguing against this hypothesis. Furthermore, both mimetics should be perfectly pre-organized and no steric clash of the alkyl substituents with the protein is to be expected according to MD simulations. A possible explanation for the unexpectedly poor binding affinities might be a binding mode different from the one of sLe^x, which had been the basis for our modeling studies.

Table 2 Relative IC_{50} values (rIC_{50}) of compounds **3**, **4** and reference compounds **2** and **18**. IC_{50} are scaled on TMSE-sLe^x **17** (rIC_{50}=1; IC_{50} = 0.88 mM).





17

Entry	Compound	R	rIC_{50} (E-sel)
A	**2**		0.01
B	**18**		0.03
C	**3**		1.8
D	**4**		1.2

3. Conclusion

Two derivatives of glycolic acid were explored as replacements for sialic acid in E-selectin antagonists. According to MD simulations, both mimics should be able to properly pre-organize the carboxylic acid moiety. Nevertheless, a significant drop in affinity was observed compared to sialic acid or (*S*)-cyclohexyllactic acid bearing derivatives.

Experimental Part

NMR spectra were recorded on a Bruker Avance DMX-500 (500 MHz) spectrometer. Assignment of ^{1}H and ^{13}C NMR spectra was achieved using 2D methods (COSY, HSQC, HMQC, HMBC). Chemical shifts are given in ppm and were assigned in relation to the solvent signals on the δ-scale[19] or to tetramethylsilane (0 ppm) as internal standard. Coupling constants *J* are given in Hertz (Hz). Multiplicities were specified as follows: s (singlet), d (doublet), dd (doublet of a doublet), t (triplet), q (quartet), m (multiplet). For assignment of resonance signals to the appropriate nuclei the following abbreviations were used: Cy (cyclohexyl), Fuc (fucose), Gal (galactose), MeCy (3-methylcyclohexane-1,2-diol).

Reactions were monitored by TLC using glass plates coated with silica gel 60 F_{254} (Merck) and visualized by using UV light and/or by charring with a molybdate solution (a 0.02 M solution of ammonium cerium sulfate dihydrate and ammonium molybdate tetrahydrate in aqueous 10% H_2SO_4.

Column chromatography was performed using automated systems (RediSep Companion) from Teledyne Isco with normal phase RediSep columns from the same manufacturer or reversed-phase columns containing LiChroprep RP-18 (40-63 μm) from Merck KGaA, Darmstadt, Germany. LC-MS separations were carried out using Sunfire C_{18} columns (19 x 150 mm, 5.0 μm) on a Waters 2525 LC, equipped with Waters 2996 photodiode array and Waters micromass ZQ MS for detection.

Solvents were purchased from Sigma-Aldrich or Acros. Solvents were dried prior to use where indicated. Tetrahydrofurane (THF) was dried by refluxing with sodium/benzophenone and distilled immediately before use. Dichloromethane (CH_2Cl_2) and dimethoxyethane (DME) were dried by filtration over Al_2O_3 (Fluka, type 5016 A basic). Methanol was dried by distillation from sodium methoxide, DMF by distillation from calcium hydride. Optical rotations were measured using a Perkin-Elmer Polarimeter 341. Electron spray ionization mass spectra (ESI-MS) were obtained on a Waters micromass ZQ. HRMS analysis were carried out using a Agilent 1100 LC equipped with a photodiode array detector and a Micromass QTOF I equipped with a 4 GHz digital-time converter. Microanalysis was performed at the Institute of Organic Chemistry at the University of Basel, Switzerland.

Purity of final compounds was determined on an Agilent 1100 HPLC; detector ELS, Waters 2420; column: Waters Atlantis dC18, 3 μm, 4.6 x 75 mm; eluents: A: water + 0.1% TFA; B: 90% acetonitrile + 10% water + 0.1% TFA; depending on the polarity of analytes, gradients

were applied as indicated. A) linear gradient: 0 - 1 min 5% B; 1 - 20 min 5 to 95% B; flow: 0.5 mL/min; B) linear gradient: 0 - 1 min 5% B; 1 - 20 min 5 to 70% B; flow: 0.5 mL/min.

2-Propen-1-yl 3-*O*-(1-hydroxymethylcyclohexyl)-6-*O*-(*tert*-butyldiphenylsilyl)-β-D-galactopyranoside 7

and

2-Propen-1-yl 2-*O*-(1-hydroxymethylcyclohexyl)-6-*O*-(*tert*-butyldiphenylsilyl)-β-D-galactopyranoside 8 using condition D.

To a stirred solution of **5** (1.00 g, 2.18 mmol) and **6** (0.150 g, 1.34 mmol) in anhydrous CH_2Cl_2 (5.0 mL) under argon was slowly added freshly distilled BF_3 etherate (0.020 mL, 0.16 mmol) at -18°C. The solution was stirred at -18°C and after 40 min and after 90 min, additional **6** (0.100 g, 0.89 mmol) and BF_3 etherate (0.020 mL, 0.16 mmol) were added. After 2 h and 15 min, the reaction was quenched with satd. aqueous $NaHCO_3$ (2.0 mL) and brine (30 mL). The aqueous layer was extracted with CH_2Cl_2 (3 · 30 mL) and the combined organic layers were dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Column chromatography on silica (PE/EtOAc) yielded starting material **5** (720 mg, 1.57 mmol, 72%) and an impure mixture of **7** and **8** (140 mg), which was purified in a second chromatography (PE/MTBE) to give 3-O alkylated **7** (35 mg, 0.06 mmol, 3%) and 2-O alkylated **8** (60 mg, 0.11 mmol, 5%).

7: ^{1}H NMR (500.1 MHz, $CDCl_3$): δ 7.72-7.64, 7.46-7.34 (2m, 10H, Ar-H), 5.90 (dddd, *J* = 5.2, 6.6, 10.4, 17.1Hz, 1H, OCH_2CHCH_2), 5.27 (m, 1H, OCH_2CHCH_2), 5.19 (m, 1H, OCH_2CHCH_2), 4.35 (m, 1H, OCH_2CHCH_2), 4.27 (bd, ^3J = 7.8Hz, 2H, Gal-H1, Cy-O*H*), 4.08 (m, 1H, OCH_2CHCH_2), 3.99 (dd, ^3J = 6.6Hz, ^2J = 10.2Hz, 1H, Gal-H6a), 3.93 (d, ^3J = 3.1Hz, Gal-H4), 3.90 (dd, ^3J = 6.0Hz, ^2J = 10.2Hz, 1H, Gal-H6b), 3.76-3.66 (m, 2H, Gal-H2, Cy-CH_2OH), 3.60 (dd, ^3J = 3.1, 9.4Hz, 1H, Gal-H3), 3.51 (bt, ^3J = 6.3Hz, 1H, Gal-H5), 3.32 (dd, *J* = 10.3, 12.3 Hz, 1H, Cy-CH_2OH), 3.22 (s, 1H, Gal-C^2-O*H*), 2.71 (s, 1H, Gal-C^4-O*H*), 1.89 (m, 1H, Cy), 1.65-1.23 (m, 9H, Cy), 1.06 (s, 9H, $SiPh_2C(CH_3)_3$); ^{13}C NMR (125.8 MHz, $CDCl_3$): δ 135.7, 133.6, 133.4, 129.9, 127.8 (12C, Ar-C), 133.8 (OCH_2CHCH_2), 118.2 (OCH_2CHCH_2), 101.7 (Gal-C1), 78.9 (Cy-C_q), 75.0 (Gal-C5), 72.2 (Gal-C3), 71.3 (Gal-C2), 69.9 (OCH_2CHCH_2), 68.9 (Gal-C4), 65.1 (Cy-CH_2OH), 62.6 (Gal-C6), 33.9, 31.3 (2C, Cy), 26.9 (3C, $SiC(CH_3)_3$), 26.0, 22.4, 22.2 (3C, Cy), 19.4 ($SiC(CH_3)_3$); $[\alpha]_D$ -1.39° (*c* 0.90, $CHCl_3$); HR-MS (ESI) *m*/*z*: calcd for $C_{32}H_{46}NaO_7Si^+$ $[M+Na]^+$: 593.2905; found: 593.2904.

8: ^{1}H NMR (500.1 MHz, $CDCl_3$): δ 7.72-7.64, 7.46-7.34 (2m, 10H, Ar-H), 5.92 (m, 1H, OCH_2CHCH_2), 5.28 (m, 1H, OCH_2CHCH_2), 5.18 (m, 1H, OCH_2CHCH_2), 4.36 (m, 1H, OCH_2CHCH_2), 4.23 (d, ^{3}J = 7.5Hz, 1H, Gal-H1), 4.09-3.99 (m, 4H, Cy-O*H*, Gal-H4, Gal-C^3-O*H*, OCH_2CHCH_2), 3.95-3.86 (m, 2H, Gal-H6a, Gal-H6b), 3.70 (dd, ^{3}J = 7.8, 8.9Hz, 1H, Gal-H2), 3.64 (d, ^{2}J = 12.4Hz, 1H, Cy-CH_2OH), 3.56-3.50 (m, 2H, Cy-CH_2OH, Gal-H3), 3.47 (dd, ^{3}J = 5.6Hz, ^{2}J = 11.3 Hz, 1H, Gal-H5), 3.01 (d, ^{3}J = 4.3Hz, 1H, Gal-C^4-O*H*), 1.76-1.23 (m, 10H, Cy), 1.05 (s, 9H, $SiPh_2C(CH_3)_3$); ^{13}C NMR (125.8 MHz, $CDCl_3$): δ 135.7, 133.2, 133.0, 130.0, 127.9 (12C, Ar-C), 133.8 (OCH_2CHCH_2), 118.0 (OCH_2CHCH_2), 102.2 (Gal-C1), 79.2 (C_q), 74.8 (Gal-C3), 74.2 (Gal-C5), 71.8 (Gal-C2), 70.3 (OCH_2CHCH_2), 69.5 (Gal-C4), 64.1 (Cy-CH_2OH), 63.4 (Gal-C6), 33.9, 32.3 (2C, Cy), 26.9 (3C, $SiC(CH_3)_3$), 26.0, 23.2, 23.1 (3C, Cy), 19.3 ($SiC(CH_3)_3$); $[\alpha]_D$ -13.9 (*c* 2.22, $CHCl_3$); HR-MS (ESI) *m*/*z*: calcd for $C_{32}H_{46}NaO_7Si^+$ $[M+Na]^+$: 593.2905; found: 593.2904.

6-*O*-Benzyl-3-*O*-(1-(hydroxymethyl)cyclohexyl)-β-D-galactopyranosyl-(1→1)-[2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl-(1→2)]-(1*R*,2*R*,3*S*)-3-methylcyclohexane-1,2-diol 11.

To a stirred solution of **9** (0.265 g, 0.332 mmol) and **6** (0.022 g, 0.196 mmol) in anhydrous CH_2Cl_2 (0.8 mL) under argon was added BF_3 etherate (0.004 mL, 0.032 mmol) at -18°C, and the solution was stirred at -18°C. After 40 min, additional **6** (0.022 g, 0.196 mmol) and BF_3 etherate (0.004 mL, 0.032 mmol) were added. After another 60 min, the solution was quenched with satd. aqueous $NaHCO_3$ (1.0 mL) and brine (10 mL). The aqueous layer was extracted with CH_2Cl_2 (3 · 10 mL) and the combined organic layers were dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Column chromatography on silica (PE/EtOAc) afforded starting material **9** (0.195 g, 0.088 mmol, 74%) and impure **11** (25 mg), which was further purified *via* HPLC to yield pure **11** (13 mg, 0.014 mmol, 4%).

^{1}H NMR (500.1 MHz, $CDCl_3$): δ 7.33-7.12 (m, 20H, Ar-H), 5.04 (d, ^{3}J = 3.4Hz, 1H, Fuc-H1), 4.87 (A of AB, ^{2}J = 11.4Hz, 1H, CH_2Ph), 4.76-4.64 (m, 4H, CH_2Ph), 4.61 (B'' of A''B'', ^{2}J = 11.5Hz, 1H, CH_2Ph), 4.47 (s, 2H, CH_2Ph), 4.42 (q, ^{2}J = ^{3}J = 6.3Hz, 1H, Fuc-H5), 4.27 (m, 1H, Gal-H1), 3.98 (dd, ^{3}J = 3.4, 10.3Hz, 1H, Fuc-H2), 3.92 (dd, ^{3}J = 2.4, 10.3Hz, 1H, Fuc-H3), 3.75 (s, 1H, Gal-H4), 3.73-3.64 (m, 2H, Gal-H6a, MeCy-H1), 3.64-3.56 (m, 3H, Fuc-H4, Gal-H6b, Cy-CH_2OH), 3.55-3.51 (m, 2H, Gal-H2, Gal-H3), 3.49 (t, ^{3}J = 6.1Hz, Gal-H5), 3.25 (d, ^{2}J = 12.8Hz, Cy-CH_2OH), 3.17 (t, ^{3}J = 9.3Hz, 1H, MeCy-H2), 2.06 (m, 1H, MeCy), 1.76 (m, 1H, MeCy), 1.62-1.08 (14H, MeCy, Cy), 1.06 (d, ^{3}J = 6.4Hz, 3H, Fuc-H6), 1.02 (d, ^{3}J = 6.2Hz, 3H, MeCy-CH_3), 0.91 (m, 1H, MeCy); ^{13}C NMR (125.8 MHz, $CDCl_3$): δ 139.1, 138.5, 138.2, 128.6, 128.5, 128.4, 128.3, 127.9, 127.8, 127.6, 127.5

(24C, Ar-C), 99.8 (Gal-C1), 97.6 (Fuc-C1), 83.9 (MeCy-C2), 79.8 (Fuc-C3), 79.0 (Cy-C_q), 78.8, 78.4 (2C, Fuc-C4, MeCy-C1), 76.5 (Fuc-C2), 75.1, 74.2, 73.8 (3C, *C*H_2Ph), 73.4 (Gal-C5), 72.9 (*C*H_2Ph), 72.1 (Gal-C3), 70.6 (Gal-C2), 69.2 (Gal-C4), 69.1 (Gal-C6), 66.9 (Fuc-C5), 64.8 (Cy-*C*H_2OH), 38.6 (MeCy-C3), 34.1, 33.8, 31.5, 31.4, 26.0, 23.3, 22.6, 22.4 (8C, 5C Cy, 3C MeCy), 19.2 (MeCy-*C*H_3), 17.0 (Fuc-C6); $[\alpha]_D$ - 36.8 (*c* 0.50, $CHCl_3$); MS (ESI) *m*/*z*: calcd for $C_{54}H_{70}NaO_{12}^+$ $[M+Na]^+$: 933.48; found: 933.56; elemental analysis calcd (%) for $C_{54}H_{70}O_{12}$ (911.13): C 71.19 H 7.74; found: C 71.06 , H 7.76.

6-*O*-Benzyl-3-*O*-(1-hydroxy-2-methylpropan-2-yl)-β-D-galactopyranosyl-(1→1)-[2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl-(1→2)]-(1*R*,2*R*,3*S*)-3-methylcyclohexane-1,2-diol 12.

To a stirred solution of **9** (0.500 g, 0.626 mmol) and 2,2-dimethyloxirane **10** (0.040 mL, 0.450 mmol) in anhydrous CH_2Cl_2 (2.0 mL) under argon was added BF_3 etherate (0.009 mL, 0.072 mmol) at -18°C, and the solution was stirred at -18°C. After 40 min, additional 2,2-dimethyloxirane (0.040 mL, 0.450 mmol) and BF_3 etherate (0.009 mL, 0.072 mmol) were added. After another 25 min, the solution was quenched with satd. aqueous $NaHCO_3$ (2.0 mL) and brine (30 mL). The aqueous layer was extracted with CH_2Cl_2 (3 · 30 mL) and the combined organic layers were dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Column chromatography on silica (PE/MTBE) afforded starting material **9** (0.280 g, 0.350 mmol, 56%) and impure **12** (70 mg), which was further purified *via* HPLC to yield pure **12** (30 mg, 0.034 mmol, 6%).

^{1}H NMR (500.1 MHz, $CDCl_3$): δ 7.39-7.12 (m, 20H, Ar-H), 5.03 (d, ^3J = 3.5 Hz, 1H, Fuc-H1), 4.87 (A of AB, ^2J = 11.5Hz, 1H, C*H*$_2$Ph), 4.75-4.59 (m, 4H, C*H*$_2$Ph), 4.53 (B of AB, ^2J = 11.5Hz, 1H, C*H*$_2$Ph), 4.49-4.45 (m, 2H, C*H*$_2$Ph), 4.42 (q, ^3J = 6.2Hz, 1H, Fuc-H5), 4.28 (d, ^3J = 7.6Hz, 1H, Gal-H1), 3.98 (dd, ^3J = 3.5, 10.3Hz, 1H, Fuc-H2), 3.92 (dd, ^3J = 2.6, 10.3Hz, 1H, Fuc-H3), 3.75-3.46 (m, 9H, Fuc-H4, Gal-H2, Gal-H3, Gal-H4, Gal-H5, Gal-H6a,b, MeCy-H1, C(CH$_3$)$_2$C*H*$_2$OH), 3.20-3.13 (m, 2H, MeCy-H2, C(CH$_3$)$_2$C*H*$_2$OH), 2.06 (m, 1H, MeCy), 1.61-1.48 (3H, MeCy), 1.30-1.03 (m, 11H, Fuc-H6, MeCy, C(C*H*$_3$)$_2$CH$_2$OH), 1.00 (d, ^3J = 6.2Hz, 3H, MeCy-C*H*$_3$), 0.92 (m, 1H, MeCy); ^{13}C NMR (125.8 MHz, $CDCl_3$): δ 139.1, 138.5, 138.2, 128.6, 128.5, 128.4, 128.3, 127.9, 127.8, 127.7, 127.6, 127.5 (24C, Ar-C), 99.9 (Gal-C1), 97.7 (Fuc-C1), 83.9 (MeCy-C2), 79.8 (Fuc-C3), 78.5 (MeCy-C1), 78.4 (Fuc-C4), 77.8 (*C*(CH$_3$)$_2$CH$_2$OH), 76.5 (Fuc-C2), 75.1, 74.2, 73.8 (3C, *C*H$_2$Ph), 73.4 (Gal-C5), 72.9 (2C, *C*H$_2$Ph, Gal-C3), 70.4 (Gal-C2), 69.5 (Gal-C4), 69.2 (Gal-C6), 66.9 (2C, Fuc-C5, C(CH$_3$)$_2$*C*H$_2$OH), 38.6 (MeCy-C3), 33.8 (MeCy-C4), 31.5 (MeCy-C6), 25.9 (C(*C*H$_3$)$_2$CH$_2$OH), 23.3 (MeCy-C5), 22.6 (C(*C*H$_3$)$_2$CH$_2$OH), 19.2 (MeCy-*C*H$_3$), 17.0 (Fuc-

C6); $[\alpha]_D$ - 66.0 (*c* 1.28, MeOH); HR-MS (ESI) *m*/*z*: calcd for $C_{51}H_{66}NaO_{12}$ $[M+Na]^+$: 893.4446; found: 869.4454.

6-*O*-Benzyl-3-*O*-(carboxycyclohexyl)-2-*O*-lactone-β-D-galactopyranosyl-(1→1)-[2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl-(1→2)]-(1*R*,2*R*,3*S*)-3-methylcyclohexane-1,2-diol 13.

To a stirred suspension of **11** (100 mg, 0.110 mmol) in CH_2Cl_2 (3.0 mL) and H_2O (0.430 mL) at 0°C were added an aqueous solution of NaBr (1 M, 0.070 mL), an aqueous solution of Bu_4NBr (0.120 mL), TEMPO (5.0 mg, 0.032 mmol), and satd. aq $NaHCO_3$ (0.330 mL). Subsequently, an aqueous solution of NaOCl (0.220 mL, 10 to 15% NaOCl) was added and the mixture was stirred vigorously at 0°C. After 45 min, the mixture was warmed to r.t., diluted with H_2O (40 mL), and extracted with CH_2Cl_2 (2 · 40 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure. Column chromatography on silica (PE/EtOAc) afforded **13** (68 mg, 0.075 mmol, 68%) as white fluffy solid.

^{1}H NMR (500.1 MHz, $CDCl_3$): δ 7.37-7.10 (m, 20H, Ar-H), 5.00 (d, ^3J = 3.6Hz, 1H, Fuc-H1), 4.87 (A of AB, ^2J = 11.5Hz, 1H, CH_2Ph), 4.74 (A' of A'B', ^2J = 11.6Hz, 1H, CH_2Ph), 4.69-4.64 (m, 3H, CH_2Ph, Fuc-H5), 4.59 (B'' of A''B'', ^2J = 11.4Hz, 1H, CH_2Ph), 4.51 (B of AB, ^2J = 11.5Hz, 1H, CH_2Ph), 4.46-4.42 (m, 3H, CH_2Ph, Gal-H1), 4.25 (m, 1H, Gal-H2), 4.09 (d, ^3J = 2.7Hz, 1H, Gal-H4), 3.99 (dd, ^3J = 3.6, 10.3Hz, 1H, Fuc-H2), 3.94 (dd, ^3J = 2.6, 10.3Hz, 1H, Fuc-H3), 3.71 (dd, ^3J = 6.7Hz, ^2J = 8.9Hz, 1H, Gal-H6a), 3.65-3.55 (m, 4H, Fuc-H4, Gal-H5, Gal-H6b, MeCy-H1), 3.48 (dd, ^3J = 3.0, 9.5Hz, 1H, Gal-H3), 3.20 (t, ^3J = 9.2Hz, 1H, MeCy-H2), 1.96 (m, 1H, MeCy), 1.91-0.91 (16H, MeCy, Cy), 1.05 (d, ^3J = 6.5Hz, 3H, Fuc-H6), 1.03 (d, ^3J = 6.5Hz, 3H, MeCy-CH_3); ^{13}C NMR (125.8 MHz, $CDCl_3$): δ 172.0 (*C*O), 139.2, 139.1, 138.6, 137.7, 128.7, 128.5, 128.4, 128.3, 128.2, 128.0, 127.7, 127.6, 127.5, 127.4, 127.3 (24C, Ar-C), 98.3, 97.8 (Fuc-C1, Gal-C1), 82.8 (MeCy-C2), 80.3 (Fuc-C3), 79.5 (MeCy-C1), 79.4 (Cy-C_q), 78.4 (Fuc-C4), 76.4 (Fuc-C2), 75.9 (Gal-C2), 75.0, 74.4, 73.8 (3C, *C*H_2Ph), 73.1 (Gal-C5), 72.6 (*C*H_2Ph), 71.9 (Gal-C3), 68.5 (Gal-C6), 67.0 (Gal-C4), 66.2 (Fuc-C5), 39.1, 35.6, 33.5, 31.9, 30.7, 24.8, 22.9, 20.7, 20.6 (9C, 5C Cy, 4C MeCy), 19.0 (MeCy-*C*H_3), 16.9 (Fuc-C6); $[\alpha]_D$ - 77.9 (*c* 0.76, $CHCl_3$); HR-MS (ESI) *m*/*z*: calcd for $C_{54}H_{66}NaO_{12}^+$ $[M+Na]^+$: 929.4446; found: 929.4447.

6-*O*-Benzyl-3-*O*-(dimethylcarboxy)-2-*O*-lactone-β-D-galactopyranosyl-(1→1)-[2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl-(1→2)]-(1*R*,2*R*,3*S*)-3-methylcyclohexane-1,2-diol 14.

To a stirred suspension of **12** (30 mg, 0.034 mmol) in CH_2Cl_2 (0.8 mL) and H_2O (0.130 mL) at 0°C were added an aqueous solution of NaBr (1 M, 0.020 mL), an aqueous solution of Bu_4NBr (0.036 mL), TEMPO (2.0 mg 0.013 mmol), and satd. aq $NaHCO_3$ (0.100 mL). Subsequently, an aqueous solution of NaOCl (0.065 mL, 10 to 15% NaOCl) was added and the mixture was stirred vigorously at 0°C. After 45 min, the mixture was warmed to r.t., diluted with H_2O (10 mL), and extracted with CH_2Cl_2 (2 · 10 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure. Column chromatography on silica (PE/EtOAc) afforded **14** (20 mg, 0.023 mmol, 67%) as white fluffy solid.

^{1}H NMR (500.1 MHz, $CDCl_3$): δ 7.33-7.11 (m, 20H, Ar-H), 5.01 (d, ^3J = 3.6Hz, 1H, Fuc-H1), 4.86 (A of AB, ^2J = 11.5Hz, 1H, C*H*$_2$Ph), 4.74 (A' of A'B', ^2J = 11.6Hz, 1H, C*H*$_2$Ph), 4.70-4.62 (m, 3H, C*H*$_2$Ph, Fuc-H5), 4.59 (B'' of A''B'', ^2J = 11.5Hz, 1H, C*H*$_2$Ph), 4.50 (B of AB, ^2J = 11.5Hz, 1H, C*H*$_2$Ph), 4.46 (d, ^3J = 7.8Hz, 1H, Gal-H1), 4.43 (m, 2H, C*H*$_2$Ph), 4.30 (dd, ^3J = 7.9, 9.4Hz, 1H, Gal-H2), 4.06 (d, ^3J = 2.1Hz, 1H, Gal-H4), 4.00 (dd, ^3J = 3.6, 10.3Hz, 1H, Fuc-H2), 3.94 (dd, ^3J = 2.6, 10.3Hz, 1H, Fuc-H3), 3.71 (dd, ^3J = 6.7Hz, ^2J = 9.4Hz, 1H, Gal-H6a), 3.66-3.59 (m, 3H, Fuc-H4, Gal-H6b, MeCy-H1), 3.56 (m, 1H, Gal-H5), 3.51 (dd, ^3J = 3.0, 9.5Hz, 1H, Gal-H3), 3.21 (t, ^3J = 9.2Hz, 1H, MeCy-H2), 2.40 (s, 1H, Gal-C^{4}O*H*), 1.98 (m, 1H, MeCy), 1.63-1.47 (6H, MeCy, C(C*H*$_3$)$_2$CO$_2$), 1.45 (s, 3H, C(C*H*$_3$)$_2$CO$_2$), 1.33 (m, 1H, MeCy), 1.21-0.92 (m, 2H, MeCy), 1.06 (d, ^3J = 6.5Hz, 3H, Fuc-H6), 1.03 (d, ^3J = 6.5Hz, 3H, MeCy-C*H*$_3$); ^{13}C NMR (125.8 MHz, $CDCl_3$): δ 172.0 (C(CH$_3$)$_2$*C*O$_2$), 139.2, 138.7, 137.7, 128.7, 128.5, 128.4, 128.3, 128.2, 128.1, 127.7, 127.6, 127.5, 127.4 (24C, Ar-C), 98.4 (Fuc-C1), 97.7 (Gal-C1), 82.9 (MeCy-C2), 80.3 (Fuc-C3), 79.5 (MeCy-C1), 78.5 (Fuc-C4), 78.2 (*C*(CH$_3$)$_2$CO$_2$), 76.6, 76.4 (Fuc-C2, Gal-C2), 75.0, 74.4, 73.8 (3C, *C*H$_2$Ph), 73.1 (Gal-C5), 72.7 (*C*H$_2$Ph), 72.3 (Gal-C3), 68.8 (Gal-C6), 67.2 (Gal-C4), 66.3 (Fuc-C5), 39.1 (MeCy-C3), 33.5 (MeCy-C4), 30.6 (MeCy-C6), 28.0, 25.8 (2C, C(*C*H$_3$)$_2$CO$_2$) 22.9 (MeCy-C5), 19.0 (MeCy-*C*H$_3$), 16.9 (Fuc-C6); $[\alpha]_D$ - 75.0 (*c* 1.08, $CHCl_3$); HR-MS (ESI) *m*/*z*: calcd for $C_{51}H_{62}NaO_{12}^+$ $[M+Na]^+$: 889.4133; found: 889.4141.

3-*O*-(Carboxycyclohexyl)-2-*O*-lactone-β-D-galactopyranosyl-(1→1)-[α-L-fucopyranosyl-(1→2)]-(1*R*,2*R*,3*S*)-3-methylcyclohexane-1,2-diol 15.

13 (45 mg, 0.64 mmol) was dissolved in dioxane/water (4/1, 2.0 mL) under argon. Pd(OH)$_2$/C (5 mg, 10% Pd(OH)$_2$) was added and the resulting mixture was hydrogenated (1 bar H_2) at r.t. After 15 h, the mixture was filtered and the solvent was removed under reduced

pressure. Column chromatography on silica (CH_2Cl_2/i-propanol) afforded **15** as white fluffy solid (22 mg, 0.040 mmol, 81%).

^{1}H NMR (500.1 MHz, CD_3OD): δ 4.99 (d, ^3J = 4.0Hz, 1H, Fuc-H1), 4.83 (q, ^3J = 6.5Hz, 1H, Fuc-H5), 4.64 (d, ^3J = 7.9Hz, 1H, Gal-H1), 4.33 (dd, ^3J = 7.9, 9.6Hz, 1H, Gal-H2), 4.02 (d, ^3J = 2.4Hz, 1H, Gal-H4), 3.82 (dd, ^3J = 3.3, 10.3Hz, 1H, Fuc-H3), 3.78 (dd, ^3J = 2.4, 9.6Hz, 1H, Gal-H3), 3.76-3.67 (m, 5H, Fuc-H2, Fuc-H4, Gal-H6a,b, MeCy-H1), 3.60 (t, ^3J = 6.3Hz, Gal-H5), 3.22 (t, ^3J = 9.3Hz, MeCy-H2), 2.17-1.05 (23H, Cy, MeCy, Fuc-H6); ^{13}C NMR (125.8 MHz, CD_3OD): δ 174.6 (*C*O), 100.5 (Fuc-C1), 98.7 (Gal-C1), 84.3 (MeCy-C2), 80.0 (MeCy-C1), 79.9 (Cy, C_q), 78.0 (Gal-C2), 77.0 (Gal-C5), 73.8 (Fuc-C4), 72.7 (Gal-C3), 71.5 (Fuc-C3), 70.3 (Fuc-C2), 67.9 (Gal-C4), 67.4 (Fuc-C5), 62.4 (Gal-C6), 40.4, 36.8, 34.8, 32.6, 31.7, 26.1, 21.6, 21.5 (9C, 5C Cy, 4C MeCy), 19.5 (MeCy-*C*H$_3$), 16.7 (Fuc-C6); $[\alpha]_D$ - 99.3 (*c* 1.20, MeOH); HR-MS (ESI) *m*/*z*: calcd for $C_{26}H_{42}NaO_{12}^+$ $[M+Na]^+$: 569.5268; found: 569.2570; HPLC-purity: 97% (A).

3-*O*-(Dimethylcarboxy)-2-*O*-lactone-β-D-galactopyranosyl-(1→1)-[α-L-fucopyranosyl-(1→2)]-(1*R*,2*R*,3*S*)-3-methylcyclohexane-1,2-diol 16.

14 (20 mg, 0.023 mmol) was dissolved in dioxane (2.0 mL) under argon. Pd/C (10 mg, 10% Pd) was added and the resulting mixture was hydrogenated (1 bar H_2) at r.t. After 29 h, the mixture was filtered and the solvent was removed under reduced pressure. Column chromatography on silica (CH_2Cl_2/i-propanol) afforded **16** as white fluffy solid (9.0 mg, 0.018 mmol, 77%).

^{1}H NMR (500.1 MHz, CD_3OD): δ 5.00 (d, ^3J = 4.0Hz, 1H, Fuc-H1), 4.92-4.79 (m, 1H, Fuc-H5), 4.66 (d, ^3J = 7.8Hz, 1H, Gal-H1), 4.36 (dd, ^3J = 7.9, 9.5Hz, 1H, Gal-H2), 3.97 (d, ^3J = 2.5Hz, 1H, Gal-H4), 3.84-3.80 (m, 2H, Fuc-H3, Gal-H3), 3.77-3.67 (m, 5H, Fuc-H2, Fuc-H4, Gal-H6a,b, MeCy-H1), 3.60 (m, 1H, Gal-H5), 3.22 (t, ^3J = 9.3Hz, MeCy-H2), 2.12 (m, 1H, MeCy), 1.75-1.58 (m, 3H, MeCy), 1.52 (s, 6H, C(C*H*$_3$)$_2$CO$_2$), 1.40-1.23 (m, 2H, MeCy), 1.17 (d, ^3J = 6.6Hz, 3H, MeCy-C*H*$_3$), 1.14 (d, ^3J = 6.4Hz, 3H, Fuc-H6), 1.10 (m, 1H, MeCy); ^{13}C NMR (125.8 MHz, CD_3OD): δ 174.6 (C(CH$_3$)$_2$*C*O$_2$), 100.5 (Fuc-C1), 98.7 (Gal-C1), 84.4 (MeCy-C2), 80.0 (MeCy-C1), 78.8 (*C*(CH$_3$)$_2$CO$_2$), 78.5 (Gal-C2), 76.9 (Gal-C5), 73.8 (Fuc-C4), 73.0 (Gal-C3), 71.5 (Fuc-C3), 70.3 (Fuc-C2), 67.9 (Gal-C4), 67.4 (Fuc-C5), 62.4 (Gal-C6), 40.3 (MeCy-C3), 34.8 (MeCy-C4), 31.7 (MeCy-C6), 28.2, 25.8 (2C, C(*C*H$_3$)$_2$CO$_2$), 24.1 (MeCy-C5), 19.5 (MeCy-*C*H$_3$), 16.7 (Fuc-C6); $[\alpha]_D$ - 112.9 (*c* 0.40, MeOH); HR-MS (ESI) *m*/*z*: calcd for $C_{23}H_{38}NaO_{12}^+$ $[M+Na]^+$: 529.2255; found: 529.2258; HPLC-purity > 99.5 % (B).

3-*O*-(Sodium carboxycyclohexyl)-β-D-galactopyranosyl-(1→1)-[2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl-(1→2)]-(1*R*,2*R*,3*S*)-3-methylcyclohexane-1,2-diol 3.

A solution of **15** (12.0 mg, 0.020 mmol) in dioxane (1.0 mL) and aqueous 1 N NaOH (0.1 ml) was stirred at r.t. for 5 h. The solution was concentrated under reduced pressure and the crude product was purified *via* RP chromatography (H_2O/MeOH). Lyophilization from water afforded **3** as white fluffy solid (9.0 mg, 0.015 mmol, 70%).

^{1}H NMR (500.1 MHz, CD_3OD): δ 5.02 (d, ^3J = 4.0Hz, 1H, Fuc-H1), 4.95-4.85 m, 1H, Fuc-H5), 4.31 (d, ^3J = 7.8Hz, 1H, Gal-H1), 3.86 (dd, ^3J = 3.3, 10.2Hz, 1H, Fuc-H3), 3.83 (d, ^3J = 2.4Hz, 1H, Gal-H4), 3.76-3.62 (m, 6H, Fuc-H2, Fuc-H4, Gal-H2, Gal-H6a,b, MeCy-H1), 3.52 (dd, ^3J = 2.4, 9.7Hz, 1H, Gal-H3), 3.41 (m, 1H, Gal-H5), 3.23 (t, ^3J = 9.4Hz, MeCy-H2), 2.16-1.23 (16H, Cy, MeCy), 1.21 (d, ^3J = 6.6Hz, 3H, Fuc-H6), 1.12 (d, ^3J = 6.3Hz, 1H, MeCy-CH_3), 1.08 (m, 1H, MeCy); ^{13}C NMR (125.8 MHz, CD_3OD): δ 183.4 (*C*O), 102.9 (Gal-C1), 100.0 (Fuc-C1), 84.2 (MeCy-C2), 83.4 (Cy-C_q), 79.9 (MeCy-C1), 78.3 (Gal-C3), 76.0 (Gal-C5), 73.9 (Fuc-C4), 71.4 (Fuc-C3), 70.9, 70.4 (Fuc-C2, Gal-C2), 69.5 (Gal-C4), 67.5 (Fuc-C5), 63.1 (Gal-C6), 40.3, 35.4, 35.0, 34.4, 31.9, 26.9, 24.2, 23.1, 22.9 (9C, 5C Cy, 4C MeCy), 19.7 (MeCy-*C*H$_3$), 16.8 (Fuc-C6); $[\alpha]_D$ - 67.7 (*c* 0.92, MeOH); HR-MS (ESI) *m*/*z*: calcd for$C_{26}H_{44}NaO_{13}^+$ $[M+H]^+$: 587.2674; found: 587.2670; HPLC-purity > 99.5 % (B).

3-*O*-(Sodium carboxyprop-2-yl)-β-D-galactopyranosyl-(1→1)-[α-L-fucopyranosyl-(1→2)]-(1*R*,2*R*,3*S*)-3-methylcyclohexane-1,2-diol 4.

A dispersion of **16** (6.0 mg, 0.012 mmol) in aqueous NaOH (0.03 N, 0.45 ml) was stirred at r.t. for 1 h. Purification *via* RP chromatography (H_2O/MeOH) and lyophilization from water afforded **4** as white fluffy solid (4.5 mg, 0.008 mmol, 70%).

^{1}H NMR (500.1 MHz, CD_3OD): δ 5.01 (d, ^3J = 4.0Hz, 1H, Fuc-H1), 4.88 (m, 1H, Fuc-H5), 4.32 (d, ^3J = 7.4Hz, 1H, Gal-H1), 3.88-3.83 (m, 2H, Fuc-H3, Gal-H4), 3.76-3.59 (m, 6H, Fuc-H2, Fuc-H4, Gal-H2, Gal-H6a,b, MeCy-H1), 3.57 (dd, ^3J = 2.9, 9.6Hz, 1H, Gal-H3), 3.42 (m, 1H, Gal-H5), 3.23 (t, ^3J = 9.3Hz, MeCy-H2), 2.11 (m, 1H, MeCy), 1.71-1.57 (m, 3H, MeCy), 1.46, 1.43 (2s, 6H, C(CH_3)$_2$CO$_2$), 1.41-1.22 (m, 2H, MeCy), 1.20 (d, ^3J = 6.6Hz, 3H, MeCy-CH_3), 1.12 (d, ^3J = 6.4Hz, 3H, Fuc-H6), 1.08 (m, 1H, MeCy); ^{13}C NMR (125.8 MHz, CD_3OD): δ 183.3 (C(CH$_3$)$_2$*C*O$_2$), 102.8 (Gal-C1), 100.0 (Fuc-C1), 84.2 (MeCy-C2), 81.5 (*C*(CH$_3$)$_2$CO$_2$), 79.8 (MeCy-C1), 78.7 (Gal-C3), 76.0 (Gal-C5), 73.9 (Fuc-C4), 71.4 (Fuc-C3), 70.8, 70.4, 70.1 (Fuc-C2, Gal-C2, Gal-C4), 67.5 (Fuc-C5), 63.1 (Gal-C6), 40.4 (MeCy-C3), 35.0 (MeCy-C4), 31.8 (MeCy-C6), 28.1, 26.0 (2C, C(*C*H$_3$)$_2$CO$_2$), 24.2 (MeCy-

C5), 19.7 (MeCy-*C*H_3), 16.7 (Fuc-C6); $[\alpha]_D$ - 60.9 (*c* 0.62, MeOH); HR-MS (ESI) *m*/*z*: calcd for $C_{23}H_{40}NaO_{13}^+$ $[M+H]^+$: 547.2361; found: 547.2365; HPLC-purity > 99.5 % (B).

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2.5.2 Approaches towards the synthesis of 2,2-dialkyl-2-*O*-glycosyl glycolic acid derivatives.

The synthetic route presented in section 2.5.1 was the result of extensive screening and optimization efforts, which are described in section 2.5.2.1. Section 2.5.2.2 comprises alternative routes, which were discontinued in favor of the spiroepoxide route.

2.5.2.1 Spiroepoxide opening with carbohydrate building blocks

When opening an epoxide, especially with a carbohydrate nucleophile, several aspects should be considered. Since positive charge is better stabilized on tertiary carbon atoms than on secondary or primary ones, epoxides can be opened regioselectively at the higher substituted position under acidic conditions (Figure 2.5.1). A typical side reaction under acidic conditions is a 1,2-hydride shift, resulting in aldehyde **5.3**, which can form hemiacetal **5.4** with alcohols.

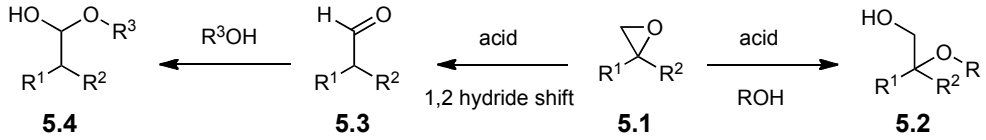
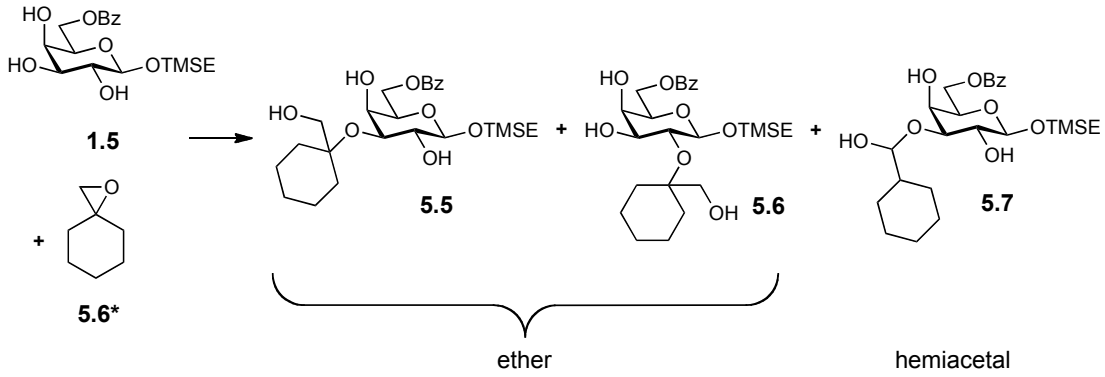


Figure 2.5.1 Reaction of an asymmetric epoxide and an alcohol under acidic conditions.

To prevent this side reaction, a highly reactive nucleophile is required. The attacking galactoside should consequently offer a reactive 3-OH group and little steric bulk. Protecting groups should therefore not reduce the reactivity neither by electronic nor by steric factors and must be compatible with the reaction conditions. Lewis or Brønstedt acids can be used for the activation of the epoxide. The solvent must allow high substrate concentrations, but should not be too polar, as this would decrease the nucleophilicity of the hydroxyl group. Low temperatures usually increase selectivity, but might also favor hemiacetal formation *via* hydride shift due to the decreased reactivity of the galactoside. To evaluate the protecting group pattern and suitable reaction conditions, several D-Gal derivatives were synthesized and reacted with spiroepoxide **5.6*** under different reaction conditions. The reactions were monitored by TLC and mass spectrometry, which did not allow to distinguish regioisomers. In case product isolation was possible, NMR was used for further characterization. The results are summarized in Tables 2.5.1 and 2.5.2.

Table 2.5.1 Reaction of spiroepoxide **5.6*** with galactoside **1.5**.

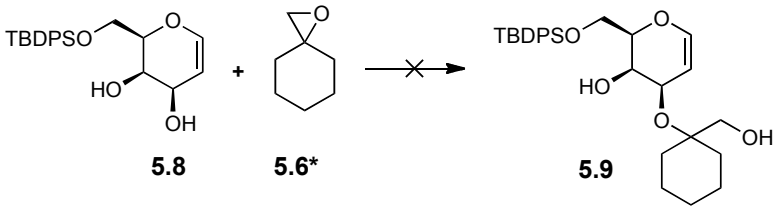


Entry	$c_{1.5}$[M]	Ratio **1.5** /**5.6***	Catalyst (eq.)	Solvent	T	t [h]	Observation
A)	0.5	1:1	Hydrazine sulfate (0.1)	THF	60°C	17	no reaction
B)	0.5	1:2	(±)CSA (0.1)	THF	r.t.	17	hemiacetal
C)	0.5	1:2	$SnCl_4$ (0.1)	THF	r.t.	17	ether, hemiacetal
D)	1.0	10:1	DDQ (1.0)	CH_2Cl_2/ THF	r.t.	48	no reaction
E)	2.5	2:1	Hydrazine sulfate (1.0)	CH_2Cl_2	50°C	17	Ether (NMR) hemiacetal
F)	2.5	1:2	(±)CSA (0.1)	CH_2Cl_2	r.t.	17	Ether hemiacetal
G)	0.9	1:10	(±)CSA (1.0)	-	r.t.	16	Oligomerisation of **5.6*** multiple alkylation
H)	4.2	2.6:1	i. $SnCl_4$ ii. (±)CSA	DMF	r.t.	68	no reaction
I)	0.1	1:1	i. $ZnBr_2$ (0.1) ii. TMSOTf (0.3)	CH_2Cl_2	-78°C to r.t.	12	hemiacetal
J)	0.3	1:1	TBSOTf (0.4)	CH_2Cl_2	-15°C to r.t.	0.2	no product red solid

The initial attempts presented in Table 2.5.1 allowed to identify **5.5**, **5.6**, and **5.7**, which were formed with approximately 10%, 5%, and 4%, respectively (entry E). Starting material **1.5** could be recovered to 41%. Unfortunately, these reaction conditions did not reproducibly yield the three products. All reactions (entry A to J) suffered from poor reaction rates, which was partially attributed to the electron pulling, and thereby reactivity decreasing effect of the benzoyl protecting group. Using **5.6*** as solvent to increase the reaction rate led to oligomerisation reactions (entry G). Reactions with silyltriflates (entries I and J), thought to quench the newly formed primary alcohol, were hard to control and often led to polymerization reactions.

To increase the reaction rate of the alkylation, galactal **5.8** was synthesized (Table 2.5.2). It combines a highly reactive allylic hydroxy group, little steric bulk, and good selectivity, since only two hydroxyl groups are available. However, the acid lability of **5.8** strongly limited the range of catalysts and no product could be isolated.

Table 2.5.2 Reaction of galactal **5.8** with spiroepoxide **5.6***.

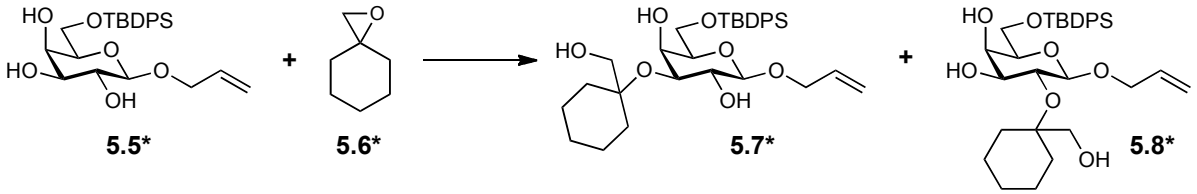


5.8 **5.6*** **5.9**

Entry	c_{gal} [M]	Ratio **5.8** /**5.6***	Catalyst (eq.)	Solvent	T	t [h]	Observation
A)	0.1	1:20	CSA (0.1)	THF	mw 40°C 150W	24 r.t. 2 mw	Ether + multiple alkylation (MS) Heating destroyed ether
B)	0.1	1:6	TMSOTf	THF	0°C	0.02	Strong reaction No starting material No ether
C)	0.03	1:6	TMSOTf	THF	-78°C to r.t.	2 3	No starting material Ether (MS)

Finally, **5.6*** was reacted with **5.5*** (Scheme 2.5.1, see also section 2.5.1). The anomeric allyl protecting group allowed to use $BF_3{\cdot}Et_2O$, which was not possible with TMSE protected D-Gal derivative **1.5**. We assumed that $BF_3{\cdot}Et_2O$ would lead to reduced hemiacetal formation

compared to Brønstedt acid (±)-camphorsulfonic acid ((±)-CSA). The silyl protecting group in 6-*O* position should increase the reactivity compared to *e.g.* a benzoate. The identified reaction conditions were successfully transferred to the synthesis of more complex tetrasaccharide mimetics (section 2.5.1).

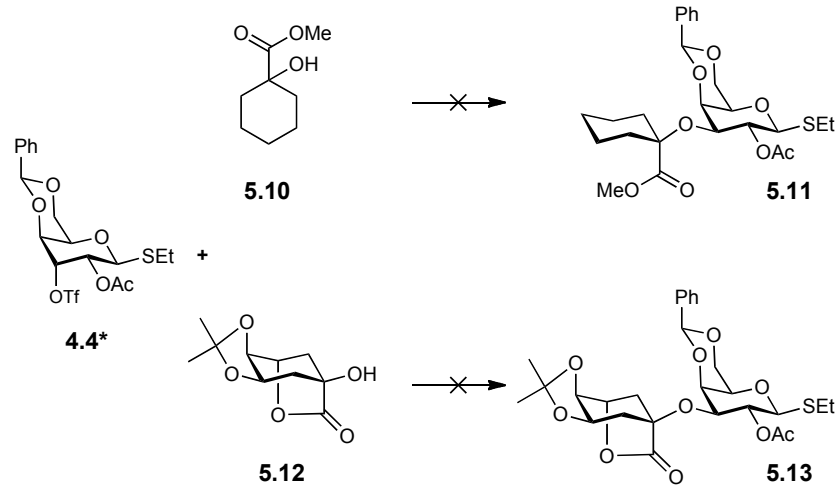


Scheme 2.5.1 $BF_3{\cdot}Et_2O$, CH_2Cl_2, -18°C, 135 min, (**5.7***: 3 %, **5.8***: 5%, **5.5***: 72%).

2.5.2.2 Alternative strategies

2.5.2.2.1 Substitution

Regioselective alkylations of galactosides are typically performed by reacting a tin acetal activated galactoside with the corresponding alkyl triflates in presence of cesium fluoride. These S_N2-type reactions proceed nicely with secondary triflates. Tertiary triflates, especially with an adjacent carboxy group, are very prone to elimination reactions and consequently not suited for substitutions. An alternative approach is the substitution of the triflyl guloside **4.4*** with the corresponding alcoholate (Scheme 2.5.2). **4.4*** has already been successfully converted to the corresponding 3-azido galactoside (chapter 2.4.1). Although azides are by far better nucleophiles than tertiary alcoholates, we tried to react guloside **4.4*** with alcoholates **5.10** and **5.12** (Scheme 2.5.2). However, no product formation was observed by TLC and mass spectrometry.



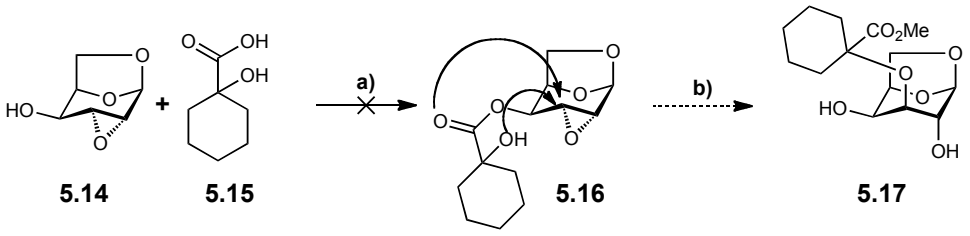
Scheme 2.5.2 NaH, DMF, 0°C to r.t.

2.5.2.2.2 Nucleophilic attack of anhydro carbohydrates

In analogy to nucleophilic substitutions, epoxide opening can be achieved by two different strategies; either by opening a spiroepoxide with a galactoside (sections 2.5.1 and 2.5.2.1) or a sugar epoxide with an alcohol. Latter strategy will be discussed in the following section.

According to the Fürst-Plattner rule,[1] epoxide opening on carbohydrates favors the formation of two axial hydroxyl groups. Consequently, epoxide 1,6:2,3-dianhydro-β-D-gulopyranoside **5.14** should be used to ensure the correct stereochemical outcome.

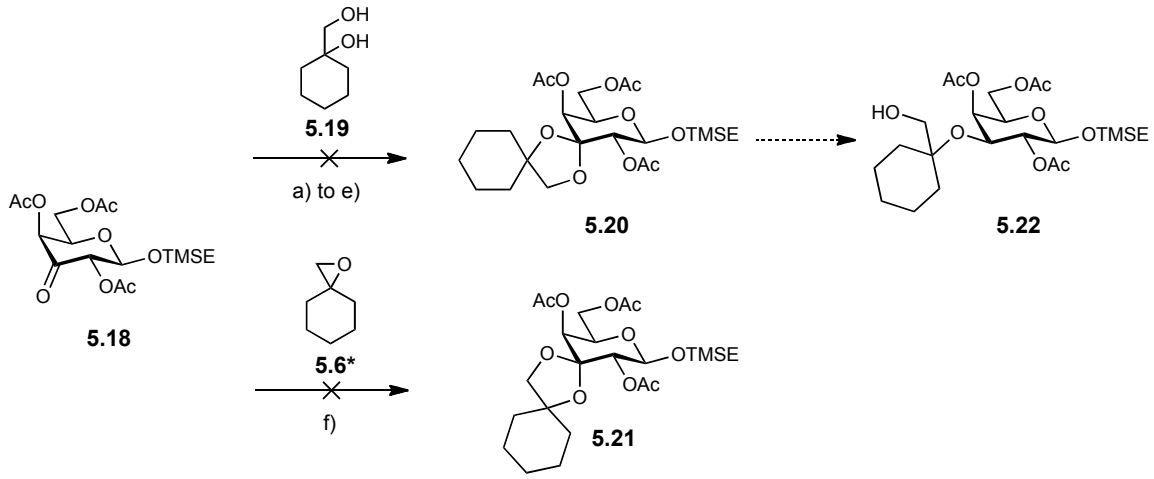
Since nucleophilic epoxide opening of the 4-*O*-benzylated derivative of **5.14** was successful with NaN_3[2] and Et_2AlCN[3], modified conditions with an alcohol as nucleophile might be as well. To compensate for the low reactivity of the tertiary alcohol **5.15**, an intramolecular reaction with **5.19** was planned (Scheme 2.5.3). This would favor epoxide opening entropically and kinetically and would reduce the need for protecting groups. Although ester formation on the 4-OH of **5.14** is known[2] and **5.15** can be transformed into esters without protection of the α-hydroxy group,[4] no product **5.16** could be isolated. As the sterically demanding CH_2 group in position 6 as well as epoxide opening *via* the carbonyl group in **5.16** might also hamper the formation of **5.17**, this strategy was not further pursued.



Scheme 2.5.3 a) DIC, DMAP, CH_2Cl_2, DMF, r.t., 12 d; b) i. NaH, THF, 0°C to r.t. ii. MeOH.

2.5.2.2.3 Acetal formation

Reaction of **5.18** with **5.19** was planned to yield acetal **5.20**, which could then be opened regioselectively under reducing conditions, *e.g.* with Et_3SiH and $TiCl_4$, to give **5.22** (Scheme 2.5.4). Acetyl groups were chosen as protecting groups to reduce the electron density of the ring and thereby activate the keto group for acid catalyzed acetal formation. The reaction was followed by TLC and mass spectrometry. Various conditions a) to e) did not yield the desired acetal, although hemiacetal formation was observed by mass spectrometry in case of a) and d). These results indicated that condensation might be the rate-limiting factor, and **5.18** was reacted with spiroepoxide **5.6***. Mass spectrometry indicated adduct formation, however it did not allow to distinguish between hemiacetal and acetal. Due to the extremely slow reaction rate and uncertain stereochemical outcome, this route was discontinued.

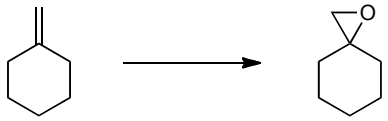


Scheme 2.5.4 a) (±)CSA, CH_2Cl_2, r.t., 2 d; b) (±)CSA, toluene 70°C to 110°C, 10 h; c) amberlyst 15, $CaSO_4$, DCE, 75°C to r.t., 10 h; d) p-TsOH·H_2O, $CaSO_4$, DCE, 75°C 2 h, r.t. 8 h; e) amberlyst 15, DCE, r.t., 7.5 h; f) amberlyst 15, CH_2Cl_2, r.t., 2 d.

2.5.2.3 Synthesis of building blocks

2.5.2.3.1 Synthesis of building block 5.6*

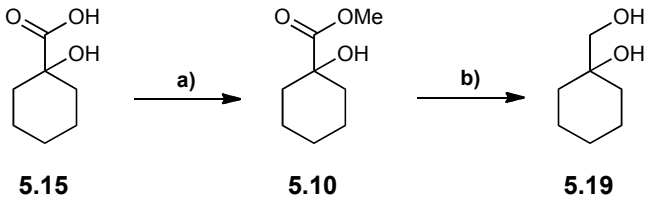
Peroxidation of alkene **5.23** with 3-chloroperbenzoic acid afforded spiroepoxide **5.6*** in 47% yield.



Scheme 2.5.5 MCPBA, CH_2Cl_2, 0°C to r.t., 47%.

2.5.2.3.2 Synthesis of building blocks 5.10 and 5.19

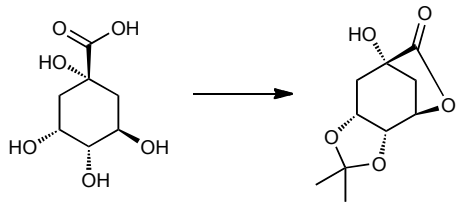
5.15 was reduced to diol **5.19** *via* methyl ester **5.10** using $LiAlH_4$.



Scheme 2.5.6 a) $(CH_3)_3SiCHN_2$, Et_2O, MeOH, 10 min, r.t., quant.; b) $LiAlH_4$, Et_2O, r.t., 54%.[5]

2.5.2.3.3 Synthesis of building block 5.12

Lactone **5.12** was readily available from (-)-quinic acid **5.24** *via* acid catalyzed acetal formation/lactonisation.[6]

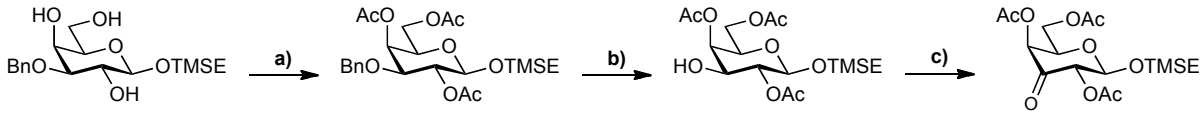


5.24 **5.12**

Scheme 2.5.7 2,2-DMP, (±) CSA, acetone, 70°C, 3.5 h, 65%.

2.5.2.3.4 Synthesis of building block 5.18

5.18 was readily available in three steps from intermediate **3.23**. Acetylation of **3.23** and hydrogenolytic debenzylation afforded **5.26**, which was oxidized with pyridinium chlorochromate to **5.18** in 48% overall yield.

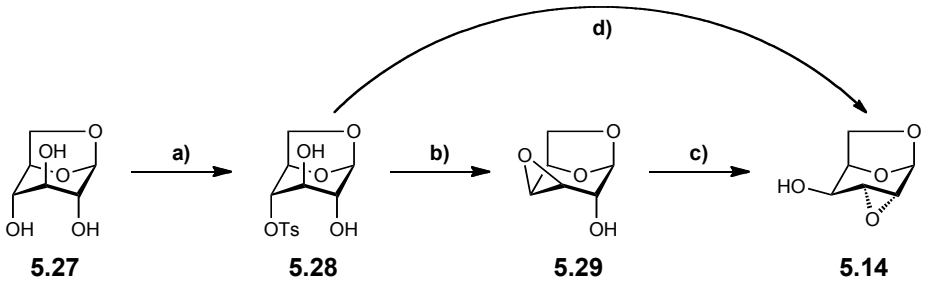


3.23 **5.25** **5.26** **5.18**

Scheme 2.5.8 a) Ac_2O, pyridine, DMAP, r.t., quant.; b) H_2, $Pd(OH)_2/C$, dioxane, H_2O r.t., 81%; c) PCC, MS 4 Å, CH_2Cl_2, r.t., 60%.

2.5.2.3.5 Synthesis of building block 5.17

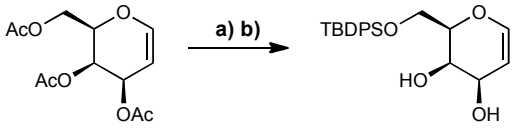
Following a procedure of Grindley and Thangarasa, monotosylation of levoglucosan **5.27** was achieved *via* the stannylene intermediate.[7] Tosylate **5.28** was then converted to epoxide **5.29** using Amberlite IRA 400, a strongly basic ion exchange resin.[8] Epoxide migration (**5.29 → 5.14**) does not take place under these conditions. A method developed by Mubarak and Fraser was applied, since a higher yield of **5.14** is expected when using sodium hydride in THF instead of sodium hydroxide in MeOH.[3] Direct conversion of tosylate **5.28** to epoxide **5.14** under basic reflux conditions[9] mainly led to the hydrolysis of the epoxide. However, epoxide formation and Payne rearrangement can be combined in one step using sodium hydride in DMF. This procedure allows the synthesis of **5.14** from inexpensive **5.27** in two steps (steps a & d) and 42% yield. The developed route might further be explored for the efficient synthesis of 3-azido-3-deoxy galactosides.



Scheme 2.5.9 a) i. Bu_2SnO, toluene, MeOH, 70°C; ii. TsCl, Et_3N, DME, MS 4 Å, r.t., 51%; b) Amberlite IRA400-OH, MeOH, r.t., 3 min, 79%; c) NaH, THF, 0°C to r.t., 63%; d) NaOH, EtOH, H_2O, reflux (epoxide hydrolysation) or NaH, DMF, 82%.

2.5.2.3.6 Synthesis of building block 5.8

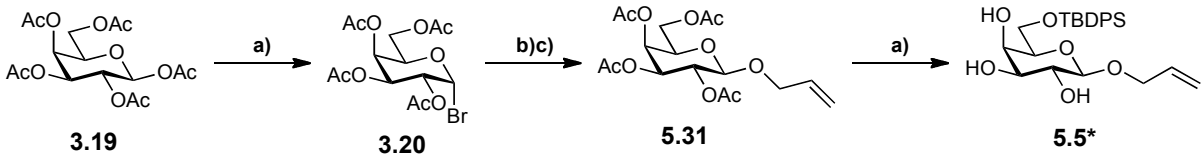
Tert-butyldiphenylsilyl protected galactal **5.8** was synthesized from **5.30** in 65% over two steps.[10]



Scheme 2.5.10 a) NaOMe, MeOH, r.t., 13 h; b) TBDPSCl, Et_3N, DMAP, DMF, r.t., 6 d, 65%.

2.5.2.3.7 Synthesis of building block 5.5*

5.5* was synthesized in four steps from peracetylated D-galactose **3.19**. The anomeric allyl protecting group was introduced *via* the 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide **3.20** using Koenigs-Knorr conditions. Deacetylation under Zemplén conditions provided allyl galactoside **5.31**. Selective protection of the 6 position of D-galactose was achieved with the sterically demanding *tert* butyldiphenylsilyl group, which was installed in 77% yield.



Scheme 2.5.11 a) HBr, AcOH, CH_2Cl_2, 0°C to r.t., 2.5 h, 92%; b) allyl alcohol, Ag_2CO_3, CH_2Cl_2, MS 3 Å, r.t., 3 h; c) NaOMe, MeOH, r.t., 4 h, 86% from 3.20; d) TBDPSCl, Et_3N, DMAP, DMF, r.t., 12 h, 77%.

Experimental

General experimental conditions are given in section 2.5.1.

1-Oxaspiro[2.5]octane 5.6*.

To a stirred solution of 3-chloroperbenzoic acid (9.00 g, 77%) in anhydrous CH_2Cl_2 (100 mL) under argon was slowly added methylene cyclohexene (5.00 g, 52 mmol) at 0°C. The solution was stirred at r.t. for 12 h, cooled to 0°C and filtered. The filtrate was washed with satd. aqueous $Na_2S_2O_3$ (100 mL) and satd. aqueous $NaHCO_3$ (100 mL) and checked for peroxides. The organic layer was dried over Na_2SO_4, filtered, and concentrated. Distillation afforded **5.6*** as clear oil (2.75 g, 24.5 mmol, 47%). Analytical data were in accordance with literature.[11]

Methyl 1-hydroxycyclohexanecarboxylate 5.10.

To a stirred solution of 1-hydroxycyclohexanecarboxylic acid (500 mg, 3.47 mmol) in MeOH (10 mL) and Et_2O (10 mL) was slowly added a solution of (trimethylsilyl)diazomethane in hexanes (2.0 M, 1.80 mL, 3.60 mmol) at r.t. The reaction was monitored by mass spectrometry and upon completion volatiles were removed under reduced pressure (40°C, 60 mbar) to give a slightly yellow oil, which was directly used in the next step (550 mg, 3.48 mmol).

^{1}H NMR (500.1 MHz, $CDCl_3$): δ 3.77 (s, 3H, CO_2CH_3), 1.83-1.73 (m, 2H), 1.72-1.54 (m, 7H), 1.28 (m, 1H); MS (ESI) *m/z*: calcd for $C_8H_{14}NaO_3^+$ $[M+Na^+]^+$: 181.08; found: 180.81.

1-Hydroxycyclohexylmethanol 5.19.

To a stirred solution of **5.10** (550 mg, 3.48 mmol) in anhydrous Et_2O (3.0 mL) under argon was slowly added a suspension of $LiAlH_4$ (79 mg, 2.09 mmol) in anhydrous Et_2O (2.0 mL). The suspension was heated to reflux for 1 h and stirred at r.t. for 12 h. The reaction mixture was diluted with Et_2O (50 mL) and washed with satd. aqueous $NaHCO_3$ (2 · 50 mL). The aqueous layer was extracted with Et_2O (2 · 50 mL) and the combined organic layers were dried (Na_2SO_4), filtered, and concentrated under reduced pressure to give **5.19** as white solid (245 mg, 1.88 mmol, 54%). Analytical data were in accordance with literature.[5]

3,4-*O*-Isopropylidene-1,5-quinic lactone 5.12.

A solution of (-)-quinic acid (10.0 g, 52 mmol), (±)-camphor sulfonic acid (100 mg, 0.43 mmol), and 2,2-dimethoxypropane (23.0 mL, 188 mmol) in acetone (50.0 mL) was stirred at

70 °C. After 3.5 h, the reaction was quenched with Et_3N (1.5 mL), and volatiles were evaporated under reduced pressure. Column chromatography on silica afforded **5.12** as white solid (7.30 g, 34 mmol, 65%). Analytical data were in accordance with literature.[12]

2-(Trimethylsilyl)ethyl 2,4,6-tri-*O*-acetyl-3-*O*-benzyl-β-D-galactopyranoside 5.25.

To a stirred solution of **3.23** (2.59 g, 6.99 mmol) and DMAP (cat) in pyridine (20 mL) was added acetic anhydride (4.0 mL, 42.6 mmol). After stirring at r.t. for 15 h, volatiles were evaporated under reduced pressure. Column chromatography on silica (PE/EtOAc) afforded **5.25** as white solid (3.44 g, 6.93 mmol, 99%). Analytical data were in accordance with literature.[13]

2-(Trimethylsilyl)ethyl 2,4,6-tri-*O*-acetyl-β-D-galactopyranoside 5.26.

5.25 (3.19 g, 6.42 mmol) was dissolved in dioxane/water (4/1, 35 mL) under argon. $Pd(OH)_2/C$ (100 mg, 10% $Pd(OH)_2$) was added and the resulting mixture was hydrogenated (3 bar H_2) at r.t. After 20 h, the mixture was filtered and the solvent removed under reduced pressure to give **5.26** as clear crystals (2.11 g, 5.19 mmol, 81%).

^{1}H NMR (500.1 MHz, $CDCl_3$): δ 5.31 (d, ^3J = 3.1Hz, 1H, H-4), 4.94 (dd, ^3J = 7.9, 10.0Hz, H-2), 4.43 (d, ^3J = 7.9Hz, 1H, H-1), 4.14 (d, ^2J = 6.6Hz, 2H, H-6a,b), 3.98 (m, 1H, $CH_2CH_2Si(CH_3)_3$), 3.81 (m, 2H, H-3, H-5), 3.56 (m, 1H, $CH_2CH_2Si(CH_3)_3$), 2.16, 2.11, 2.05 (3s, 9H, $COCH_3$), 1.03-0.86 (m, 2H, $CH_2CH_2Si(CH_3)_3$), 0.00 (s, 9H, $CH_2CH_2Si(CH_3)_3$); ^{13}C NMR (125.8 MHz, $CDCl_3$): 171.4, 171.1, 170.7 (3C, $COCH_3$), 100.5 (C-1), 73.0 (C-2), 71.8, 71.0 (C-3, C-5), 69.9 ($CH_2CH_2Si(CH_3)_3$), 67.6 (C-4), 62.0 ($CH_2CH_2Si(CH_3)_3$), 21.1, 20.9 20.8 (3C, $COCH_3$), 18.0 ($CH_2CH_2Si(CH_3)_3$), -1.3 (3C, $CH_2CH_2Si(CH_3)_3$); $[\alpha]_D$ -16.0 (*c* 0.88, $CHCl_3$); MS (ESI) *m*/*z*: calcd for $C_{17}H_{30}NaO_9Si$ $[M+Na]^+$: 429.16; found: 429.08.

2-(Trimethylsilyl)ethyl 2,4,6-tri-*O*-acetyl-β-D-*xylo*-hex-3-ulopyranoside 5.18.

To a stirred suspension of PCC (1.326 g, 6.15 mmol) and powdered activated molecular sieves 4 Å (2.5 g) in anhydrous CH_2Cl_2 (20 mL) was slowly added a solution of **5.26** (1.0 g, 2.46 mmol) in CH_2Cl_2 (5.0 mL) at r.t. After stirring for 19 h, the reaction mixture was filtered (Celite) and run through a short silica column (EtOAc) to give ketone **5.18** as slightly yellow oil (600 mg, 1.48 mmol, 60%), which was directly used in the next step.

1,6-Anhydro-4-*O*-*p*-tolylsulfonyl-β-D-glucopyranose 5.28.

A solution of 1,6-anhydro-β-D-glucopyranose (1.00 g, 6.16 mmol) and dibutyltin oxide (2.30

g, 9.24 mmol) in anhydrous toluene (50 mL) and anhydrous methanol (10 mL) was stirred at 60°C under argon. After 5 h, volatiles were removed under reduced pressure and the resulting white residue was dried under high vacuum for 12 h. The tin acetal was dissolved in anhydrous 1,2-dimethoxyethane (50 mL) and powdered activated molecular sieves 4 Å (3.0 g), and a solution of *p*-toluenesulfonyl chloride (1.29 g, 6.77 mmol) in anhydrous 1,2-dimethoxyethane (20 mL) was added. After stirring vigorously at r.t. for 14 h, the reaction mixture was filtered, the filtrate was diluted with CH_2Cl_2 (50 mL) and washed with brine (2 · 50 mL). The aqueous layers were extracted with CH_2Cl_2 (2 · 50 mL) and the combined organic layers were dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Column chromatography on silica (PE/EtOAc) afforded **5.28** as white solid (993 mg, 3.14 mmol, 54%). Analytical data were in accordance with literature.[7]

1,6;3,4-Dianhydro-*β*-D-galactopyranose 5.29.

To a solution of **5.28** (100 mg, 0.32 mmol) in anhydrous MeOH (2.0 mL) was added Amberlite IRA 400 OH^- ® (1.4 mL) and the resulting mixture was shaken at r.t. for 15 min. The mixture was filtered, the filter was washed with MeOH, and the combined filtrates were concentrated under reduced pressure to give **5.29** as clear oil (36 mg, 0.25 mmol, 79%). Analytical data were in accordance with literature.[14]

1,6;2,3-Dianhydro-*β*-D-gulopyranose 5.14.

From 1,6;3,4-Dianhydro-β-D-galactopyranose **5.29**: A suspension of NaH (56 mg, 60% NaH) was washed several times with anhydrous PE under argon. Subsequently, anhydrous THF (5.0 mL) was added and the dispersion was cooled to 0°C. A solution of **5.29** (100 mg, 0.69 mmol) in anhydrous THF (4.0 mL) was added and the reaction mixture was stirred slowly warmed to r.t. After stirring for 22 h, the mixture was poured on an ice-water mixture (30 mL) and the aqueous layer was extracted with EtOAc (30 mL). The organic layer was washed with brine (30 mL) and the aqueous layers were extracted with EtOAc (3 · 30 mL). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Column chromatography on silica (PE/EtOAc) afforded **5.14** as white solid (63 mg, 0.44 mmol, 63%). Analytical data were in accordance with literature.[2]

From 1,6-Anhydro-4-*O*-*p*-tolylsulfonyl-β-D-glucopyranose **5.27**: Following the previous procedure, **5.28** (40 mg, 0.13 mmol) was reacted with NaH suspension (10 mg) in anhydrous THF (2.0 mL) for 14 h to give **5.14** (15 mg, 0.10 mmol, 82%) after workup and column chromatography (PE/EtOAc). Analytical data were in accordance with literature.[2]

1,5-Anhydro-6-*O*-(*tert*-butyl)diphenylsilyl-2-deoxy-D-*lyxo*-hex-1-enitol 5.8.

To a freshly prepared solution of NaOMe in MeOH (0.03 M, 50 mL) under argon at r.t. was added 3,4,5-tri-*O*-acetyl-D-galactal **5.30** (4.09 g, 15.0 mmol). The mixture was stirred for 13 h and subsequently concentrated under reduced pressure to afford the D-galactal as white solid (2.20 g, 15.0 mmol, quant.), which was directly used in the next step. The residue (1.0 g, 6.8 mmol) was dissolved in anhydrous DMF (7.0 mL) under argon at r.t. and DMAP (cat.), Et_3N (1.90 mL), and *tert*-butyldiphenylsilyl chloride (1.90 mL, 7.4 mmol) were added. After stirring at r.t. for 6 d, the solution was diluted with EtOAc (100 mL) and washed with water (4 x 100 mL). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Column chromatography on silica (CH_2Cl_2/EtOAc) afforded **5.9** as clear oil (1.712 g, 4.45 mmol, 65%). Analytical data were in accordance with literature.[10]

2-Propen-1-yl β-D-galactopyranoside 5.31.

Allyl alcohol (10.0 mL, 146 mmol) and **3.20** (10.0 g, 24.3 mmol) in anhydrous CH_2Cl_2 (60 mL) were stirred with powdered activated molecular sieves 3 Å (6.0 g) at r.t. under argon for 3 h. Ag_2CO_3 (8.0 g, 29.2 mmol) was added slowly and the mixture was stirred under argon for 13 h. The mixture was filtered (celite) and volatiles were evaporated to yield a clear sticky solid (9.32 g, 24.0 mmol), which was used in the next step without further purification.

The crude product was dissolved in anhydrous MeOH (50 mL) under argon and a freshly prepared solution of NaOMe in MeOH (1 M, 1.0 mL) was slowly added. After 4 h, the solution was neutralized with HCl in MeOH (0.1 M) and concentrated. Column chromatography on silica (CH_2Cl_2/MeOH 9/1 to 8/2) afforded **5.31** as white solid (4.63 g, 21.0 mmol, 86%). Analytical data were in accordance with literature.[15]

2-Propen-1-yl 6-*O*-(*tert*-butyldiphenylsilyl)-β-D-galactopyranoside 5.5*.

tert-Butyldiphenylsilyl chloride (4.40 mL, 17.2 mmol) was slowly added to a stirred solution of **5.31** (3.44 g, 15.5* mmol), Et_3N (4.35 mL, 31.0 mmol) and DMAP (cat.) in anhydrous DMF (15 mL) under argon. After 12 h, the solution was diluted with EtOAc (100 mL) and washed with water (3 ·100 mL). The organic layer was dried (Na_2SO_4) and concentrated. Column chromatography on silica (PE/EtOAc 1/1 to 0/1) yielded **5.5*** as white foam (1.50 g, 12.0 mmol, 77%). Analytical data were in accordance with literature.[16]

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2.6 Sialyl Lewis^X: A "Pre-organized Water Oligomer"?

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Author contributions: F.P.C. Binder: synthesis of selectin antagonists **3**, **4**, and **5**, manuscript; K. Lemme: ITC measurements, bioassay, protein expression & purification, manuscript; R.C. Preston: generation of the 7A9 antibody column used for the functional purification of E-selectin.


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VIP Glycomimetics

Sialyl Lewis^x: A "Pre-Organized Water Oligomer"?**

*Florian P. C. Binder, Katrin Lemme, Roland C. Preston, and Beat Ernst**

In memory of Daniel Bellûs

In recent years, lectins, such as selectins,[1] galectins,[2] or siglecs[3] have received increasing attention as drug targets. Among them, selectins are the most extensively studied, since they are key players in the early stages of inflammation and therefore promising targets for the treatment of diseases with an inflammatory component, such as stroke, asthma, psoriasis, or rheumatoid arthritis.[4] The key role of selectins is to promote the initial step of the inflammatory cascade, by allowing leukocytes to roll along the vascular endothelial surface. This step is followed by the integrin-mediated firm adhesion and the final extravasation to the site of the inflammatory stimulus.[5]

The specific interaction between E-selectin and its physiological ligand ESL-1 (E-selectin ligand-1) is mediated by the tetrasaccharide sialyl Lewis^x (sLe^x, **1**).[6] Consequently, sLe^x (**1**) became the lead structure for the search of drug-like, high-affinity selectin antagonists.[1,7] Elucidation of the structure activity relationship (SAR),[8] mutation studies,[9] transferred nuclear overhauser enhancement NMR spectroscopy (trNOE-NMR),[10] saturation transfer difference NMR spectroscopy (STD-NMR),[11] molecular modeling,[12] and finally X-ray crystallography[13] yielded a precise picture of the interactions of sLe^x and E-selectin on an atomic level (Figure 1). Because docking studies[7] and STD-NMR experiments[11] revealed that the *N*-acetyl-D-glucosamine (D-Glc*N*Ac) and *N*-acetyl-D-neuraminic acid (D-Neu5Ac) moieties have only weak interactions with the protein,[12] they were replaced with structurally simplified mimics, resulting in E-selectin antagonists with significantly improved binding affinities. However, the affinity of these antagonists,

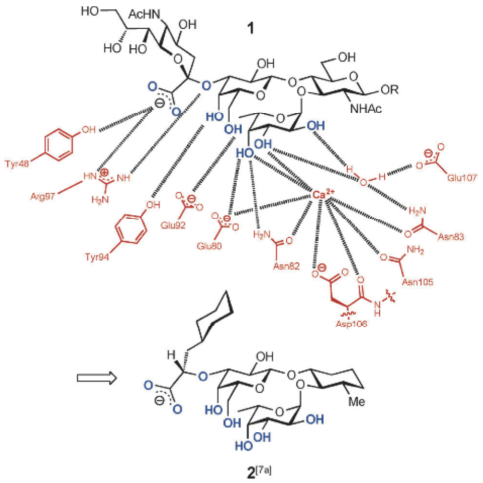

Figure 1. Top: Detailed representation of the interactions between sLe^x (**1**) and E-selectin as observed in the crystal structure;[13] the pharmacophores of **1** are highlighed in blue. Bottom: The glycomimetic **2** exhibits a 13 μM affinity in a cell-free ligand-based competitive binding assay.[7a]

for example, **2** in Figure 1,[7a] is still only in the low micromolar range.

Despite the progress made, the driving force of the interaction of E-selectin with its ligands has not been fully characterized to date, neither for sLe^x (**1**), nor for any low molecular weight selectin antagonist. However, Wild et al. estimated the enthaplic contribution of the E-selectin/ESL-1 interaction by van't Hoff analysis, which involves the correlation of the binding affinity measured at different temperatures. The results indicated that enthalpic changes contribute only 10 to 25% of the binding free energy ΔG and that the interaction is primarily driven by favorable entropy changes.[14]

Recently, the thermodynamic aspects of protein–ligand interactions have gained increasing interest in drug discovery.[15] Particularly enthalpy and entropy changes provide valuable information for lead optimization. Having access to these individual components of binding affinity rather than the overall value facilitates the successful design of high-affinity ligands. Herein, we report a comprehensive study on the thermodynamic fingerprint of a series of E-selectin antagonists.

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Supporting information for this article (details of the synthesis of antagonist **4**, the expression and purification of E-selectin/IgG, the competitive binding assay, and the isothermal calorimetry experiments) is available on the WWW under http://dx.doi.org/10.1002/anie.201202555.

The binding free energy (ΔG) associated with a protein–ligand interaction is composed of enthalpic (ΔH) and entropic ($-T\Delta S$) contributions ($\Delta G = \Delta H - T\Delta S$). The binding energy under standard conditions ($\Delta G°$), where all reactants and products are at a concentration of 1 mol L^{-1}, is calculated from the dissociation constant K_D using the equation $\Delta G = RT\ln K_D$. With isothermal titration calorimetry (ITC),[16] K_D and the enthalpy ΔH are measured directly if no changes in the protonation states occur during the interaction. The enthalpic term (ΔH) represents the contribution of non-covalent interactions upon binding,[15b] that is, hydrogen bonds, electrostatic, and dipole–dipole interactions between ligand and receptor.[17] The entropy term can be dissected into translational and rigid-body rotational entropy,[18] solvation entropy,[19] and conformational entropy.[20]

For our study, an E-selectin/IgG construct consisting of the lectin domain, the EGF-like domain, and six short consensus repeats fused to the Fc part of human IgG1 was used.[21] The 148 kDa protein was expressed in Chinese Hamster Ovarian cells and purified from the conditioned culture medium by affinity chromatography, first with protein A-Sepharose, followed by a second functional purification with the monoclonal anti-hE-selectin antibody 7A9 (see the Supporting Information). The high degree of purity and functionality of the protein is reflected by the stoichiometry (N) of the calorimetric experiments (Table 1). Batches of up to 50 mg E-selectin/IgG were necessary for ITC measurement to reach c values close to 1.

Our calorimetric investigation had two goals: first, the determination of K_D values of a series of E-selectin ligands and their comparison with data collected by a competitive binding assay (Table 1)[22] and second, the elucidation of the thermodynamic fingerprints of these ligands. The K_D value for sLe^x (**1**) binding to E-selectin determined by ITC is (878 ± 93) μM and is thereby in good agreement with previously reported data (e.g. 1.1 to 2.0 mM,[23] (0.7 ± 0.4) mM[10b]). In addition, the relative K_D values (rK_D) for the antagonists **2** to **6** also nicely correlate with their relative IC_{50} values (rIC_{50}). In analogy to earlier findings,[7a] replacement of D-Glc*N*Ac with carbocyclic mimics enhanced binding affinity up to 25-fold (**1**→**4**), whereas the replacement of D-Neu5Ac by (*S*)-cyclohexyllactic acid improved binding 2- to 5-fold (**1**→**5**; **3**→**6**; **4**→**2**).

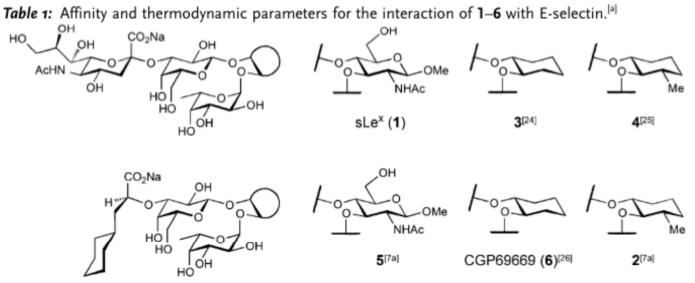
Except for some isolated cases,[14,27] lectin–oligosaccharide interactions are typically enthalpy driven with mostly unfavorable entropies.[28] In contrast, the binding of sLe^x to E-selectin is driven by a large entropy term ($-T\Delta S = -23$ kJ mol^{-1}). Clearly, the entropy costs caused by the loss of translational and rotational degrees of freedom and conformational changes of ligand and protein upon binding are overcompensated by the beneficial entropy arising from the release of bound water molecules.[29] This argumentation is supported by two experimental observations. First, the bound conformation was identified as one of two low-energy solution conformations of sLe^x,[30] demanding only minor conformational adjustments upon binding. Second, the comparison of the crystal structure of apo-E-selectin and E-selectin bound to sLe^x revealed only minor conformational differences.[13]

The beneficial entropy term, however, is partially compensated by an unfavorable change in enthalpy. To enable the pharmacophoric groups of sLe^x (**1**) to interact with their target, a predominantly polar surface area of approximately 275 Å^2[13a] on both interacting moieties has to be desolvated. Because the newly formed polar interactions between the pharmacophores of sLe^x and E-selectin do not fully compensate for the desolvation penalty of the polar binding interface (Figure 2),[31] a net loss of enthalpy ($\Delta H° = +5.4$ kJ mol^{-1}) is observed. Thus, the directed polar interactions of the pharmacophores contribute to specificity rather than affinity.

Thus, sLe^x (**1**) represents a surrogate of clustered water molecules attached to a scaffold. As a "pre-organized water oligomer" it offers an array of directed hydrogen bonds for the highly specific binding to E-selectin. The clear entropic benefit of the release of water molecules from the large binding interface to bulk water and the high degree of pre-organization of sLe^x result in the observed large entropy gain which provides the impetus for the binding process.

The concept of conformational pre-organization was also exploited for the development of selectin antagonists (Figure 3). More precisely, in sLe^x (**1**), D-Glc*N*Ac acts as

Table 1: Affinity and thermodynamic parameters for the interaction of **1**–**6** with E-selectin.[a]



Ligand	rIC_{50}	rK_D	K_D [μM]	ΔG [kJ mol^{-1}]	ΔH [kJ mol^{-1}]	$-T\Delta S$ [kJ mol^{-1}]	N
1	1	1	878 ± 93	−17.5 ± 0.2	+5.4 ± 0.7	−23 ± 1	1
3	0.3	0.36	317	−20.0	−0.5	−19.5	1
4	0.05	0.04	38	−25.3	+0.9	−26.2	0.94
5	0.27	0.30	260	−20.5	−2.2	−18.3	1
6	0.08	0.07	59 ± 4	−24.2 ± 0.2	−5.3 ± 0.4	−18.9 ± 0.6	0.93 ± 0.08
2	0.014	0.02	19 ± 2	−27.1 ± 0.2	−5.8 ± 0.1	−21.3 ± 0.4	0.97 ± 0.01

[a] Relative IC_{50} values (rIC_{50}) and relative K_D values (rK_D) are reported relative to the reference compound sLe^x (**1**). IC_{50} values were determined in a competitive binding assay.[22] K_D and ΔH were measured in ITC experiments, ΔG, and $T\Delta S$ were calculated according the equations $\Delta G = \Delta H - T\Delta S$ and $\Delta G = RT\ln K_D$. N = stoichiometric ratio of ligand and protein.

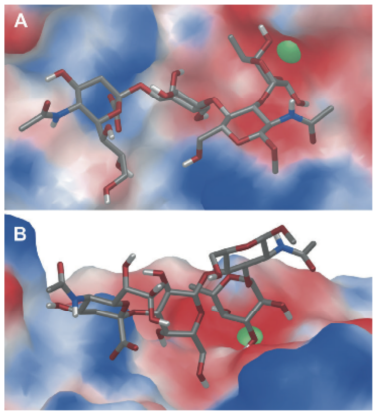


Figure 2. sLe^x (**1**) bound to E-selectin as observed in the crystal structure (protein data bank (PDB) code: 1G1T).[13a] A) top view: the binding epitope on E-selectin is dominated by polar residues [polar residues in red (positively charged) and blue (negatively charged), nonpolar in white]. The contact area is 275 Å^2. B) side view: Only a small part of sLe^x directly contributes to binding (graphics generated by Maestro[32]).

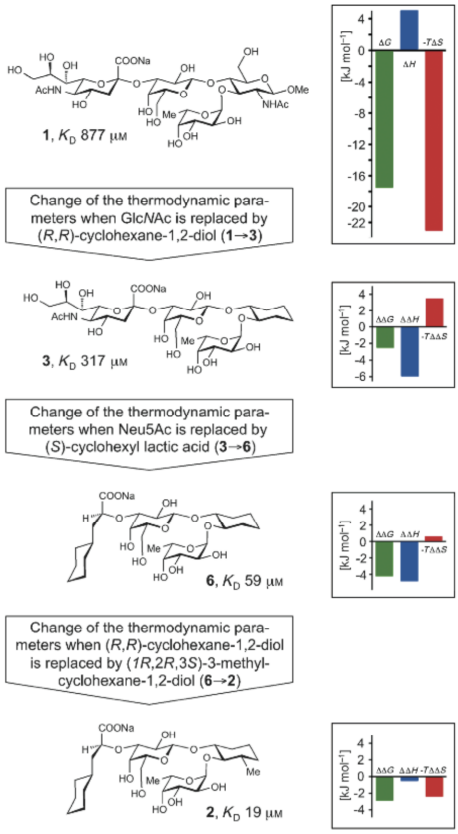


Figure 3. Thermodynamic signature (ΔG, ΔH, $-T\Delta S$) of sLe^x (**1**) (the corresponding data for the antagonists **2**–**6** are summarized in Table 1) and the changes of the thermodynamic parameters ($\Delta\Delta G$, $\Delta\Delta H$, and $-T\Delta\Delta S$) when D-GlcNAc in **1** is replaced by (*R*,*R*)-cyclohexane-1,2-diol (**1**→**3**), D-Neu5Ac in antagonist **3** by (*S*)-cyclohexyl lactic acid (**3**→**6**), and (*R*,*R*)-cyclohexane-1,2-diol in antagonist **6** by (1*R*,2*R*,3*S*)-3-methylcyclohexane-1,2-diol (**6**→**2**).

a scaffold to ensure the correct spatial orientation of L-Fuc and D-Gal in the bioactive conformation, that is, its role is to pre-organize the Le^x core. D-GlcNAc itself has only weak contacts with the target protein (Figure 2B).[13] A comparable role is attributed to D-Neu5Ac, which only contributes to binding through a salt bridge involving its carboxylate group.[13] Consequently, mimics of D-GlcNAc and D-Neu5Ac were designed to stabilize the bioactive conformation and to keep the entropic costs for binding low or virtually the same as for the highly pre-organized sLe^x (**1**). With (*R*,*R*)-cyclohexane-1,2-diol (**1**→**3**) which was demonstrated to be a moderate mimic of D-GlcNAc,[7a] substantial entropy costs arose ($-T\Delta\Delta S$: 3.5 kJ mol^{-1}, Figure 3). When D-Neu5Ac in **3** was replaced by (*S*)-cyclohexyl lactic acid (**3**→**6**, $-T\Delta\Delta S$: 0.6 kJ mol^{-1}), only a small entropy penalty emerged. Finally, (1*R*,2*R*,3*S*)-3-methylcyclohexane-1,2-diol (**6**→**2**) proved to be an optimal replacement of D-GlcNAc (Figure 3),[7a] resulting in an entropy term similar to that of sLe^x (**1**; Table 1). Compared to antagonist **6**, the improved pre-organization of the core conformation in **2** led to a substantial reduction of the entropy costs ($-T\Delta\Delta S$: -2.4 kJ mol^{-1}). Because the investigated mimics are significantly less polar than D-GlcNAc and D-Neu5Ac, an additional effect needs to be taken into account, namely a substantial alteration of the solvation properties. Although only partial desolvation is necessary, the desolvation of the carbocyclic mimics is enthalpically less unfavorable compared to that of the more polar D-GlcNAc or D-Neu5Ac moieties.[31]

Strikingly, the introduction of (*R*,*R*)-cyclohexane-1,2-diol (**1**→**3**) has the same relative effect on enthalpy and entropy as the exchange of D-Neu5Ac for (*S*)-cyclohexyllactic acid (**1**→**5**), which is reflected in the same slope in the entropy–enthalpy plot in Figure 4. In both cases, a significant gain in enthalpy is partially compensated by a loss in entropy. Furthermore, when both mimics are combined in one molecule (→**6**), the effect is not additive, that is, less entropy is lost but also less enthalpy is gained than expected (**6** vs $\mathbf{6}_{expected}$, Figure 4). Clearly, the exchange of the carbohydrate moieties does not only change local conformational and solvation properties, but rather the properties of the entire ligand.

In summary, thermodynamic binding parameters for the interaction of E-selectin with sLe^x (**1**) and the glycomimetics **2**–**6** were investigated by ITC. The interaction of sLe^x with E-selectin is driven by a large favorable entropy term which is partially compensated by an unfavorable enthalpy contribution. The exchange of residues acting as scaffolds with less

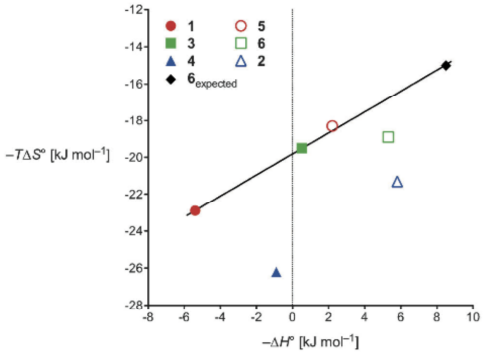


Figure 4. Entropy–enthalpy plot for ligands **1** to **6** and the values expected for **6** ($\mathbf{6}_{expected}$) in case the effects caused by the mimetic replacements of D-Glc*N*Ac by cyclohexane-1,2-diol and D-Neu5Ac by (*S*)-cyclohexyllactic acid were additive.

polar mimics, that is, D-Neu5Ac for (*S*)-cyclohexyllactic acid and D-Glc*N*Ac for (*R*,*R*)-cyclohexane-1,2-diol, resulted in improved binding enthalpy, however accompanied by a loss of binding entropy. Only for mimetic structures that maintain the pre-organization of sLe^x (**1**) in its bioactive conformation, as it is the case for the replacement of D-Glc*N*Ac by (1*R*,2*R*,3*S*)-3-methylcyclohexane-1,2-diol (**1**→**4** or **5**→**2**), a similar entropy term was found. Overall, the almost 50-fold improved affinity of **2** compared to **1** results from a gain in binding enthalpy, whereas the binding entropy is not significantly changed.

The development of glycomimetics with improved binding properties is intrinsically difficult because of the similarity of the ligand (carbohydrate) and the solvent (water). The results of this thermodynamic study suggest, that for a successful development of glycomimetics, carbohydrate moieties with predominantly structural tasks and no or only weak contacts with the target protein should be replaced by hydrophobic mimics, resulting in reduced desolvation penalties and therefore improved enthalpic contributions to binding. In addition, the mimetic replacement should contribute to an improved pre-organization of the binding conformation to optimize the entropy term as well. When the carbohydrate ligand is already almost optimally pre-organized in solution, as it is the case for sLe^x (**1**), the identification of such mimics is a most challenging task.[7a]

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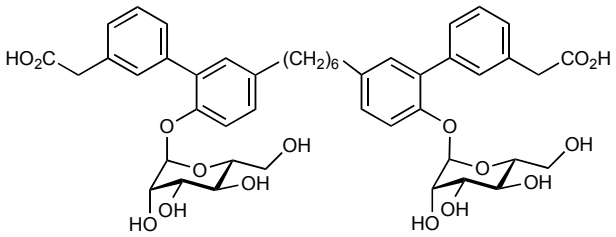
2.7 Synthesis of literature known small molecule selectin antagonists

Numerous selectin antagonists have been developed over the past 15 years (see chapter 1.3.). To evaluate the potency of some of these small molecule antagonists in our own assays, three representative compounds were synthesized (Figure 2.7.1).

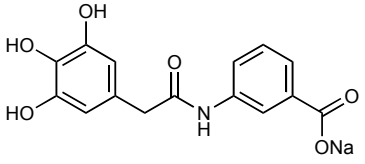
Revotar's TBC1269 (**7.1**)[1] is one of the most promising selectin antagonists so far and is currently in Phase II clinical trials for the treatment of asthma and psoriasis. Nevertheless, it should be noted, that the mechanism of action of TBC1269 (**7.1**) is controversially discussed. Beauharnois *et al.* for example found Ca^{2+} independent binding of TBC1269 (**7.1**) to P-selectin in a Biacore experiment,[2] and Hicks *et al.*, showed that TBC1269 (**7.1**) does not influence rolling in an intravital microscopy experiment.[3]

Based on the pharmacophore of TBC1269 (**7.1**), researchers at Revotar also developed a series of polyhydroxyphenols.[4] From this series, **7.2** was selected as it was reported to exhibit remarkable IC_{50} values in a static binding assay (PAA-sLe^x-TYS) for all three selectins (IC_{50}= 0.8 μM E-selectin, IC_{50} = 1.2 μM P-selectin, and IC_{50}= 1.4 μM L-selectin).[4] However, the authors point out that the compound has to be freshly prepared before testing, because rapid oxidation by air occurs.

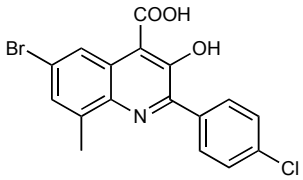
Wyeth (now Pfizer) developed a series of quinoline salicylic acid derivatives,[5] which led to the identification of a clinical candidate which is currently in Phase I clinical trials.[6] Compound **7.3** was chosen as representative example of this series.



7.1 (TBC1269)



7.2

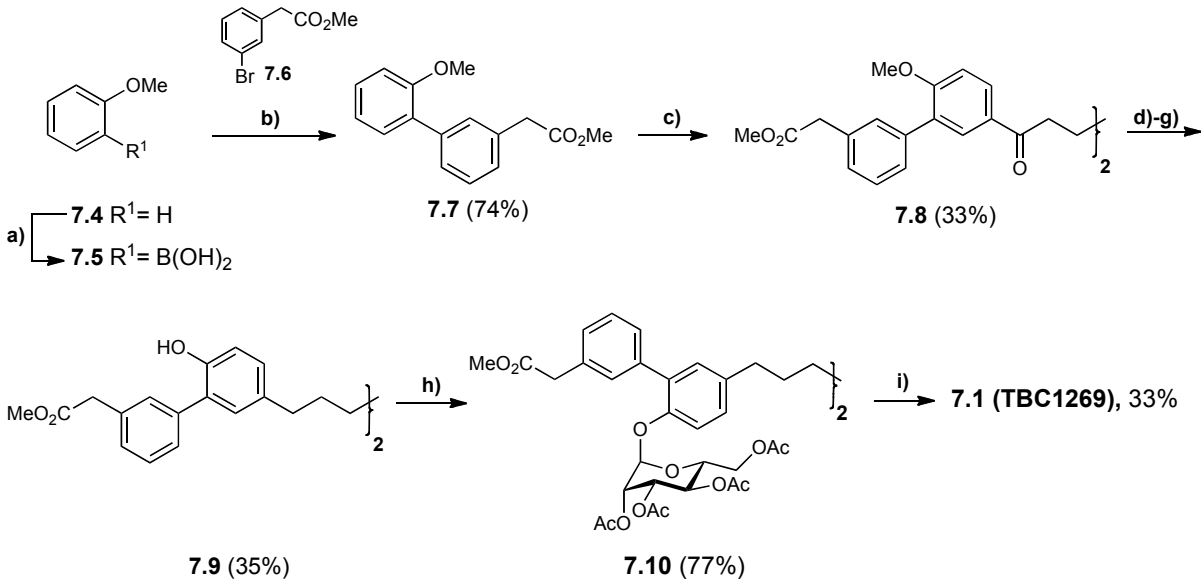


7.3

Figure 2.7.1 Small molecule selectin antagonists developed by Revotar and Pfizer.

2.7.1 Synthesis of TBC1269 (7.1)

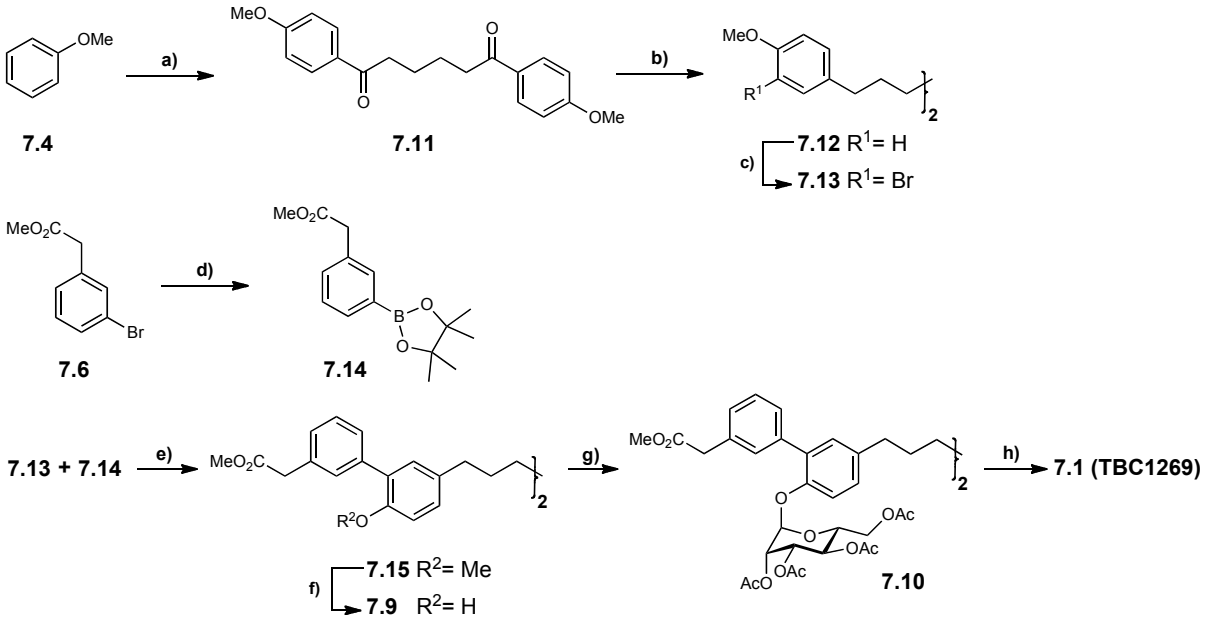
The published synthesis of TBC1269 (**7.1**) suffers from an extremely poor overall yield of 3% starting from **7.7** (Scheme 2.7.1).[1] Compound **7.7** is not commercially available and is prepared in two steps in 74% yield. Since the expected overall yield of 2% was considered unreasonably low, the synthesis was discontinued after step b) and an alternative route was explored (Scheme 2.7.2).



Scheme 2.7.1 a) i. TMEDA, n-BuLi, Et_2O, -78°C to r.t., ii. $B(OMe)_3$, -10°C to r.t., iii. aq. HCl, r.t.; b) CsF, $Pd(Ph_3)_4$, DME, mw 100°C; c) adipoyl dichloride, $AlCl_3$, DCE, 0°C to r.t.; d) aq. LiOH, MeCN, r.t., then aq. 2N HCl; e) i. N_2H_4, DMSO, 80°C; ii. KO*t*Bu, DMSO, 80°C, then aq. 2 N HCl; f) BBr_3, CH_2Cl_2, -78°C to r.t.; g) H_2SO_4, MeOH, reflux; h) α-D Man pentaacetate, DCE, $BF_3{\cdot}Et_2O$; i) aq. LiOH, MeCN, r.t.

In contrast to the published route (Scheme 2.7.1), the second route is convergent and allows to shift the Suzuki coupling from the beginning of the synthesis to the fourth step (Scheme 2.7.2). Friedel Crafts acylation of anisole **7.4** (→**7.11**)[1] followed by reduction (→**7.12**)[1] proceeded in excellent yield. Regiospecific bromination of **7.12** with NBS in acetonitrile[7] gave **7.13** ready for Suzuki coupling in 67% yield. Pinacol arylboronate **7.14** was obtained from ester **7.6** and bis(pinacolato)diboron in excellent yield using optimized microwave conditions[8] of the cross-coupling procedure developed by Miyaura.[9] Subsequent Suzuki coupling of **7.13** and **7.14** proceeded quantitatively. Cleavage of the methoxy groups with boron tribromide gave diol **7.9**. Since dimannosylation with 1,2,3,4,6-penta-*O*-acetyl-α-D-mannopyranose and $BF_3{\cdot}Et_2O$ proceeded in poor yields, the resulting mixture was reacted again with 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl trichloroacetimidat to provide **7.10**.

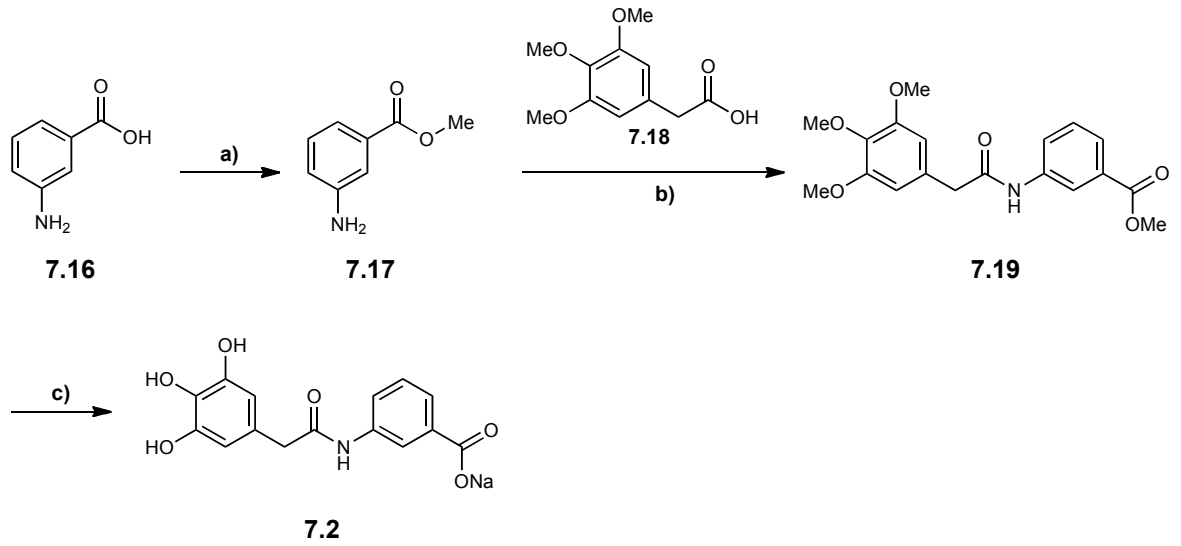
Final hydrolysation of the ester groups of **7.10** and purification *via* HPLC afforded **7.1** in 9 % overall yield.



Scheme 2.7.2 a) adipoyl dichloride, $AlCl_3$, CH_2Cl_2, -18°C to r.t., 25 min, 98%; b) TFA, TES, $BF_3{\cdot}Et_2O$, CH_2Cl_2, r.t., 2.5 h, quant.; c) NBS, MeCN, r.t., 3 h, 68%; d) bis(pinacolato)diborane, KOAc, $PdCl_2$(dppf), dppf, dioxane, mw: 300 W, 120°C, 2 h, 94%; e) $Pd(Pph_3)_4$, CsF, dioxane, mw, 120°C, 3.5 h, quant. ; f) BBr_3, CH_2Cl_2, -78°C to r.t., 2 h, 63%; g) i. 1,2,3,4,6-penta-*O*-acetyl-α-D-mannopyranose, $BF_3{\cdot}Et_2O$, CH_2Cl_2, 0°C to r.t., 12 h; ii. 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl trichloroacetimidat, TMSOTf, toluene, r.t., 5 h, 68%; h) aq. LiOH, MeCN, r.t., 12 h, 32%.

2.7.2 Synthesis of compound 7.2

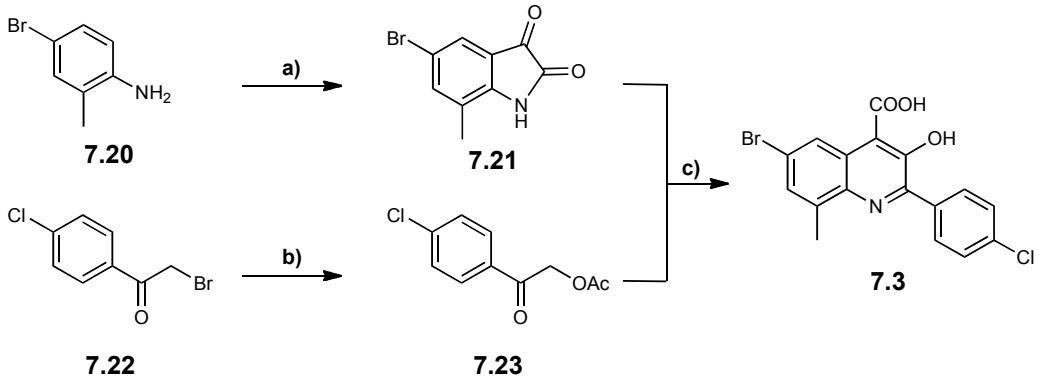
Compound **7.2** was synthesized in analogy to reference [4] (Scheme 2.7.3). Acid catalyzed ester formation of nicotinic acid (**7.16**) gave **7.17**, which was coupled to **7.18** under standard coupling conditions. Amide **7.19** was deprotected and transferred to the sodium salt to give the title compound **7.2**.



Scheme 2.7.3 a) H_2SO_4 (conc.), MeOH, reflux, 2 d, 99%; b) EDC·HCl, Et_3N, DMAP, anhyd. CH_2Cl_2, r.t., 24 h, 62%; c) i. BBr_3, anhyd. CH_2Cl_2, -78°C to r.t., ii. H_2O, r.t.; iii. ion exchange resin, H_2O, 27% from **7.19**.

2.7.3 Synthesis of compound 7.3

Compound **7.3** was synthesized following literature procedures (Scheme 2.7.4).[5] Sandmeyer-isatin synthesis with 4-bromo-2-methylaniline **7.20** gave isatin **7.21**, which was reacted with **7.23** in a Pfitzinger reaction.



Scheme 2.7.4 a) i. chloral hydrate, NH_2OH·HCl, Na_2SO_4, 2M HCl (aq), H_2O, 55°C, 24 h, ii. H_2SO_4, 55°C to 80°C, 81%; b) $NaOAc·3H_2O$, AcOH, H_2O, reflux, 2.5 h, 75%; c) 6M KOH (aq), EtOH, 100°C to reflux, 4 h, 55%.

Biological evaluation

Up to date no biological data is available.

Experimental

NMR spectra were recorded on a Bruker Avance DMX-500 (500 MHz) spectrometer. Assignment of ^{1}H and ^{13}C NMR spectra was achieved using 2D methods (COSY, HSQC, HMQC, HMBC). Reactions were monitored by TLC using glass plates coated with silica gel 60 F_{254} (Merck) and visualized by using UV light and/or by charring with a molybdate solution (a 0.02 M solution of ammonium cerium sulfate dihydrate and ammonium molybdate tetrahydrate in aqueous 10% H_2SO_4. Column chromatography was performed on automated systems (RediSep Companion) from Teledyne Isco with normal phase RediSep columns from the same. LC-MS separations were carried out using Sunfire C_{18} columns (19 x 150 mm, 5.0 µm) on a Waters 2525 LC, equipped with Waters 2996 photodiode array and Waters micromass ZQ MS for detection. Size exclusion chromatography was performed with Bio-Gel® P-2 Gel (45-90 mm) from Bio-Rad. Solvents were purchased from Sigma-Aldrich or Acros. Solvents were dried prior to use where indicated. Dichloromethane (CH_2Cl_2) and Dichloroethane (DCE) were dried by filtration over Al_2O_3 (Fluka, type 5016 A basic). Methanol was dried by distillation from sodium methoxide, Electron spray ionization mass spectra (ESI-MS) were obtained on a Waters micromass ZQ. HRMS analysis were carried out using a Agilent 1100 LC equipped with a photodiode array detector and a Micromass QTOF I equipped with a 4 GHz digital-time converter. Microanalysis was performed at the Institute of Organic Chemistry at the University of Basel, Switzerland.

1,6-Bis(4-methoxyphenyl)hexane-1,6-dione 7.11.

Adipoyl dichloride (1.46 mL, 10.0 mmol) and anisole (2.63 mL, 24.0 mmol) were dissolved in anhydrous DCE (30 mL) under argon and the resulting solution was cooled to -18°C. Aluminium chloride (6.67 g, 50 mmol) was added in small portions. After stirring for 25 min at -18°C, the reaction was quenched with ice water and extracted with CH_2Cl_2. The combined organic layers were dried over Na_2SO_4, filtered, and concentrated. Column chromatography on silica (CH_2Cl_2/MeOH) gave **7.11** as white solid (3.19 g, 9.77 mmol, 98%). Analytical data were in accordance with literature.[1]

1,6-Bis(4-methoxyphenyl)hexane 7.12.

To a stirred solution of diketone **7.11** (3.19 g, 9.77 mmol) in CH_2Cl_2 (70 mL) was slowly added TFA (6.0 mL, 78 mmol), $BF_3{\cdot}Et_2O$ (9.8 mL, 79 mmol) and Et_3SiH (6.3 mL, 39 mmol). The mixture was stirred at r.t. for 165 min, cooled to 0°C and mixed with water. The mixture was extracted with CH_2Cl_2 and the combined organic layers were dried over Na_2SO_4, filtered,

and concentrated under reduced pressure. Column chromatography on silica (PE/EtOAc) gave **7.12** as white crystals (3.10 g, 10.4 mmol). Analytical data were in accordance with literature.[1]

1,6-Bis(3-bromo-4-methoxyphenyl)hexane 7.13.

A solution of **7.12** (60 mg, 0.20 mmol) and NBS (80 mg, 0.45 mmol) in MeCN (3.0 mL) was stirred at r.t. for 4 h. Volatiles were evaporated under reduced pressure and the resulting white crystalline residue was washed with CCl_4. Column chromatography on silica (PE/EtOAc) gave **7.13** as white crystals (62 mg, 0.14 mmol, 68%). Analytical data were in accordance with literature.[1]

Methyl 2-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)acetate 7.14.

A microwave tube was charged with methyl 2-(3-bromophenyl)acetate **7.6** (60 mg, 0.26 mmol), potassium acetate (77 mg, 0.78 mmol), bis(pinacolato)diborane (80 mg, 0.32 mmol), $PdCl_2$(dppf) (6.4 mg, 7.8 μmol), and dppf (4.4 mg, 7.9 μmol) and evacuated at high vacuum. After 10 min, the tube was flushed with argon and anhydrous dioxane (1.5 mL) was added under vigorous stirring. The solution was degassed in an ultrasonic bath for 15 min and subsequently flushed with argon for another 10 min. Microwave irradiation at 120°C (300 W) for 2 h gave the crude product as red solution. Dioxane was evaporated under reduced pressure and the residue was dissolved in CH_2Cl_2 (50 mL) and washed with brine (2 x 25 mL). The organic layer was dried over Na_2SO_4, filtered and concentrated under reduced pressure. Column chromatography on silica (CH_2Cl_2, short column) gave **7.14** as clear oil (68 mg, 0.25 mmol, 94%). Analytical data were in accordance with literature.[4]

Dimethyl 2,2'-(hexane-1,6-diylbis(2'-methoxy-[1,1'-biphenyl]-5',3-diyl))diacetate 7.15.

A microwave tube was charged with **7.13** (36 mg, 0.08 mmol), **7.14** (72 mg, 0.26 mmol), $Pd(PPh_3)_4$ (4 mg, 3.5 μmol), and CsF (790 mg, 5.2 mmol) and evacuated at high vacuum. After 10 min, the tube was flushed with argon and anhydrous dioxane (5.0 mL) was added under vigorous stirring. The solution was degassed in an ultrasonic bath for 15 min and subsequently flushed with argon for another 10 min. Microwave irradiation at 120°C (300 W) for 5 h gave the crude product. Dioxane was evaporated under reduced pressure and the residue was dissolved in CH_2Cl_2 (50 mL) and washed with brine (2 x 25 mL). The organic layer was dried over Na_2SO_4, filtered and concentrated under reduced pressure. Column

chromatography on silica gave **7.15** as orange oil, which was directly used in the next step (47 mg, 0.08 mmol, quant.).

1,6-Bis[3-(3-carbomethoxymethylphenyl)-4-hydroxyphenyl]hexane 7.9.

To a solution of **7.15** (62 mg, 0.10 mmol) in anhydrous CH_2Cl_2 (4.0 mL) under argon at -78°C was slowly added BBr_3 (80 μL, 0.8 μmol). The solution was slowly warmed to -20°C and stirred at -20°C for 1h. The reaction was quenched with ice-water and extracted with CH_2Cl_2. The organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Column chromatography on silica gave **7.9** (37 mg, 0.07 mmol, 63%) as slightly yellow oil. Analytical data were in accordance with literature.[1]

1,6-Bis[3-(3-carbomethoxymethylphenyl)-4-(2,3,4,6-tetra-*O*-acetyl-α-D-manno pyranosyloxy)phenyl]hexane 7.10.

To a stirred solution of **7.9** (25 mg, 0.04 mmol) and 1,2,3,4,6-penta-*O*-acetyl-α-D-mannopyranose (52 mg, 0.13 mmol) in anhydrous CH_2Cl_2 (2.0 mL) under argon at 0°C was slowly added $BF_3{\cdot}Et_2O$ (65 μL, 0.51 mmol). The solution was stirred at 0°C for 20 min and at r.t. overnight. The reaction mixture was diluted with CH_2Cl_2 and washed with water, satd. aq. $NaHCO_3$, and brine. The organic layer was dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Column chromatography on silica gave three still impure fractions. The product **7.10** (25 mg, 0.02 mmol), monoglycosylated **7.9**, and **7.9**. The three fractions were combined and reacted again, this time with trichloroacetimidat activated α-D-Man tetraacetat.

To a stirred solution of the three fractions, additional **7.9** (21 mg, 0.04 mmol) and 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl trichloroacetimidat (87 mg, 0.13 mmol) in anhydrous toluene (3.0 mL) under argon at r.t. was added TMSOTf (2.6 μL, 0.014 mmol). The solution was stirred for 5 h, diluted with toluene (5 mL) and quenched with aq. satd $NaHCO_3$ (5 mL). The aq. layer was extracted with toluene and the combined organic layers were dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Column chromatography on silica gave **7.10** as slightly yellow foam (68 mg, 0.06 mmol, 68%). Analytical data were in accordance with literature.[1]

TBC1269 7.1.

To a stirred solution of **7.10** (18 mg, 15 μmol) in acetonitrile (0.5 mL) was slowly added a solution of LiOH (10 mg, 0.42 mmol) in water (0.5 mL). The mixture was stirred at r.t. for 24

h, diluted with water (10 mL), and acidified to pH 3 with conc. HCl. Evaporation of volatiles, subsequent purification *via* HPLC, and lyophilization gave **TBC1269** as white powder (4 mg, 4.6 μmol, 32%). Analytical data were in accordance with literature.[1]

Methyl 3-aminobenzoate 7.17.

To a stirred solution of 3-aminobenzoic acid **7.16** (296 mg, 2.16 mmol) in MeOH (10.0 mL) was added sulfuric acid (0.12 mL) and the reaction mixture was refluxed for 2 d. After cooling to r.t., volatiles were removed under reduced pressure, the residue was dissolved in EtOAc (50 mL) and washed with satd. aq. $NaHCO_3$ (50 mL). The aq. layer was extracted with EtOAc (3 x 50 mL) and the combined organic layers were washed with brine and dried over Na_2SO_4. Solvent was evaporated under reduced pressure to give **7.17** as red oil, which was directly used in the next step (320 mg, 2.11 mmol, 98%).

Methyl 3-(2-(3,4,5-trimethoxyphenyl)acetamido)benzoate 7.19.

To a solution of EDC hydrochloride (604 mg, 3.15 mmol) and Et_3N in anhydrous CH_2Cl_2 (12.0 mL) was added 3,4,5-trimethoxyphenylacetic acid **7.18** (716 mg, 3.16 mmol) and DMAP (cat.). After stirring for 10 min at r.t. under argon, **7.17** (320 mg, 2.11 mmol) was added and the reaction mixture was stirred at r.t. overnight. The reaction was quenched by addition of a satd. aq. NH_4Cl solution (20 mL), which was extracted with CH_2Cl_2 (3x 20 mL). The combined organic layers were washed with brine (50 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure to afford a brown oil. Column chromatography on silica (PE/EtOAc) gave **7.19** as orange sticky solid (476 mg, 1.32 mmol, 62%). Analytical data were in accordance with literature.[4]

Sodium 3-[2-(3,4,5-trihydroxyphenyl)-acetamido]-benzoate 7.2.

To a stirred solution of **7.19** (160 mg, 0.45 mmol) in anhydrous CH_2Cl_2 (2.0 mL) under argon at -78°C, was added BBr_3 (0.56 mL, 5.8 mmol) within 30 min. The resulting green solution was stirred at r.t. for 4 h before it was cooled to 0°C and water (2 mL) was added slowly under vigorous stirring. Methanol and CH_2Cl_2 were added and the mixture was extracted with EtOAc (3x). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Purification *via* HPLC, conversion to the sodium salt *via* ion exchange resin (DOWEX 50X8) and purification *via* SEC gave the final compound **7.2** (40 mg, 0.12 mmol, 27%). Analytical data were in accordance with literature.[4] Elemental

analysis calcd (%) for $C_{15}H_{13}NO_6$ + 1.1 H_2O: C 52.09, H 4.16, N 4.05; found: C 52.02, H 4.11, N 4.26.

5-Bromo-7-methylindoline-2,3-dione 7.21.

Chloral hydrate (1.19 g, 7.2 mmol) was added to a stirred suspension of hydroxylamine hydrochloride (1.50 g, 21.5 mmol), sodium sulfate (6.80 g, 47.9 mmol), 4-bromo-2-methylaniline **7.20** (1.12 g, 6.0 mmol) in water (40 mL) and aq. HCl (2N, 2.0 mL). The mixture was stirred at 55°C o.n. After cooling to 0°C, the hydroxyiminoacetanilide was isolated by filtration, washed with cold water, and dried under high vacuum o.n. The cyclisation was carried out by adding the hydroxyiminoacetanilide in small portions to a flask containing sulfuric acid (4.0 mL, which had been heated to 55°C. The temperature was maintained below 70°C during the addition. After complete addition, the dark solution was heated to 80°C for 10 min, cooled to r.t., poured onto crushed ice (20 mL), and allowed to stand on the ice for 30 min. The resulting brown precipitate was collected by filtration, washed with water (3x 30 mL) and dried under high vacuum over night to give isatin **7.21** as brown powder (1.17 g, 4.9 mmol, 81%). Analytical data were in accordance with literature.[5]

2-(4-Chlorophenyl)-2-oxoethyl acetate 7.23.

To a solution of 2-bromo-4'-chloroacetophenone **7.22** (1.40 g, 6.0 mmol) in ethanol (5.0 mL) was added a solution of sodium acetate trihydrate (0.93 g, 6.8 mmol) in water (3.1 mL) and acetic acid (0.31 mL). The solution was refluxed for 2.5 h, cooled to r.t. and stored in a freezer at -18°C o.n. The white crystalline product was collected by filtration (0.96 g, 4.5 mmol, 75%). Analytical data were in accordance with literature.[5]

6-Bromo-2-(4-chlorophenyl)-3-hydroxy-8-methylquinoline-4-carboxylic acid 7.3.

A suspension of isatin **7.21** (600 mg, 2.50 mmol) in aq. potassium hydroxide (6.0 mL) was heated to 100°C. A solution of **7.23** (532 mg, 2.50 mmol) in warm ethanol (4.0 mL) was added over the course of 1 h, and upon completion of the addition, the mixture was refluxed for 2.5 h. After cooling to r.t., ethanol was removed under reduced pressure, the residue was diluted with water (15 mL), treated with charcoal, filtered and the clear solution was acidified to pH 1 with aq. HCl (1N). The resulting yellow precipitate was collected by filtration, washed with water, and dried under high vacuum. The crude product was purified by column chromatography on silica gel (70:2.5 EtOAc/MeCN + 0.5% Et_3N) and converted back to the free acid by precipitation from an acidic solution of MeCN/H_2O (20% MeCN, acidified with

HCl) to give the title compound **7.3** as pale yellow solid (537 mg, 1.37 mmol, 55%). Analytical data were in accordance with literature.[5] Compound purity was confirmed by elemental analysis: calcd (%) for $C_{17}H_{11}BrClNO_3$: C 52.00, H 2.82, N 3.57; found: C 52.13, H 2.77, N 3.42.

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3 Outlook

Introduction of different amide and ester substituents in position 2 of D-galactose has proven beneficial for the design of E-selectin antagonists (see introduction). Since an acetyl ester increased binding affinity to P-selectin as well, bulkier substituents like benzoates might be used to further enhance the affinity of P-selectin antagonists.

Lactones between the carboxy group of a sialic acid mimic and the 2-*O* or 4-*O* position of D-galactose could be useful for the development of prodrugs with improved pharmacokinetic properties (in analogy to 5.15*, 5.16*).

Given the unexpectedly poor binding affinity of E-selectin antagonists **5.3*** and **5.4***, as well as the conflicting data of STD-NMR studies and the crystal structure of sLe^x in complex with E-selectin, crystal structures of E-selectin antagonists in complex with E-selectin are urgently needed to overcome roadblocks and promote our understanding in selectin glycomimetic interactions.

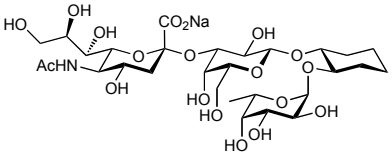
The binding of PSGL-1 to P-selectin is associated with conformational changes in both the lectin and the EGF domain, shifting P-selectin from a bent to an extended conformation. Stabilization of the extended conformation *via* a glycan wedge between lectin and EGF domain was shown to increase the binding affinity towards PSGL-1 (see introduction). These information might be used to develop a new class of allosteric selectin antagonists, suited to overcome the unfavorable properties of sLe^x mimetics.

P-selectin bears a second binding site close to the sLe^x binding epitope, which ensures the high binding affinity of its natural ligand PSGL-1 (see introduction). This second site offers promising preconditions for a fragment based drug discovery approach and might be explored with small molecules mimicking non-carbohydrate binding epitopes of PSGL-1, *e.g.* tyrosine sulfate.

Finally, it is well known that the potency of selectin antagonists strongly depends on the assay setup. As the natural binding process between selectins and their ligands takes place under flow-conditions, a flow assay might prove useful for the characterization of future selectin antagonists.

4 Formula Index

Chapter 2.1



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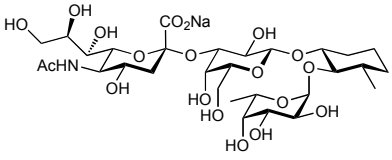

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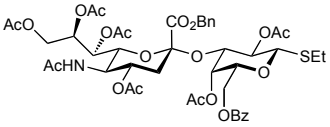

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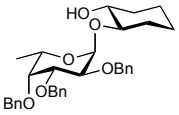

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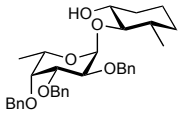

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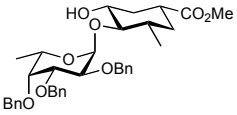

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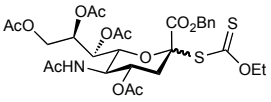

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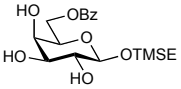

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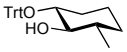

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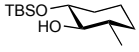

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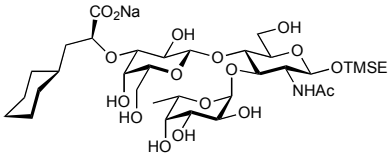

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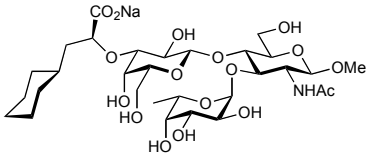

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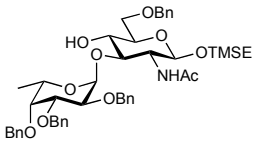

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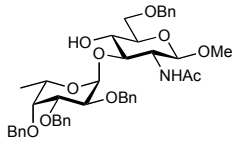

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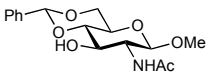

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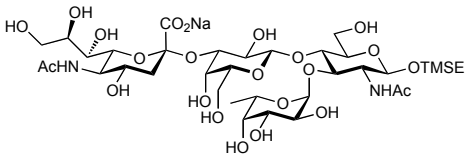

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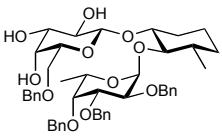

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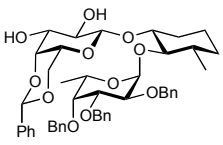

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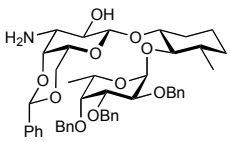

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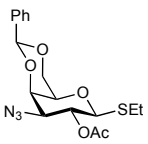

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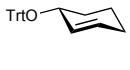
Chapter 2.2

2.2
FB45

2.3
FB46

2.4
FB48



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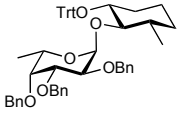

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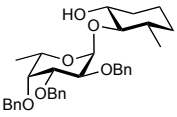

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FB304

2.6*
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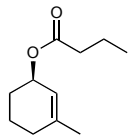

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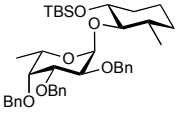

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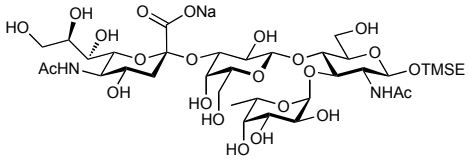
Chapter 2.3.1



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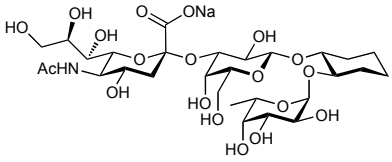

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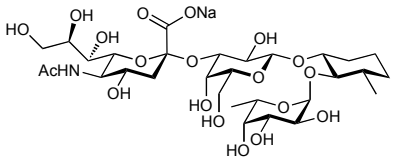

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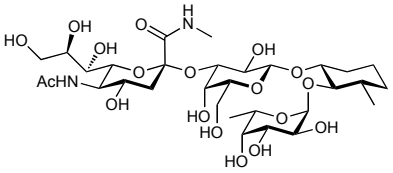

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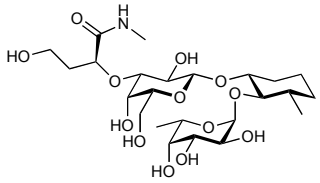

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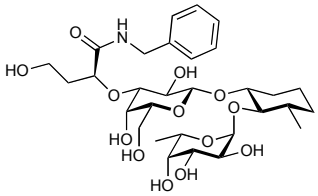

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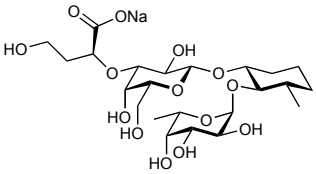

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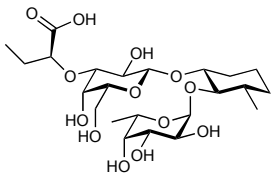

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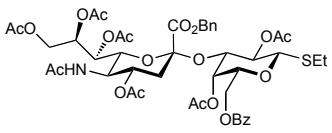

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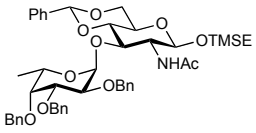

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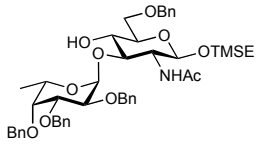

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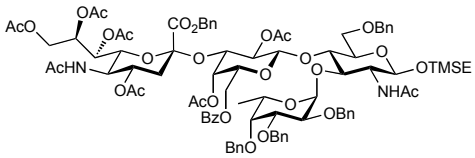

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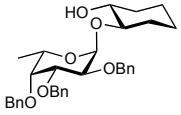

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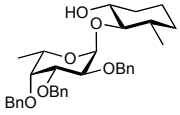

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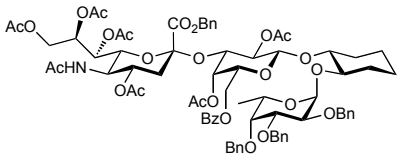

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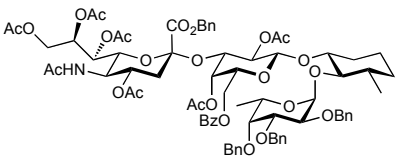

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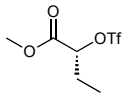

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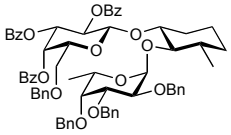

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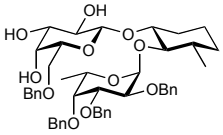

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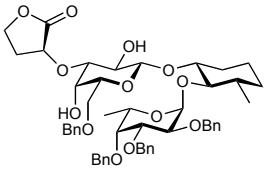

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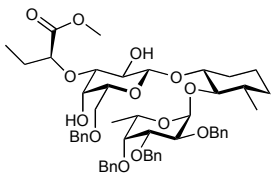

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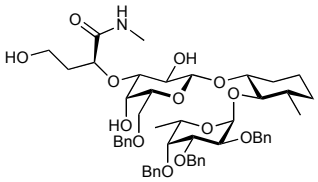

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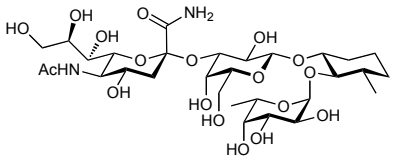
Chapter 2.3.2



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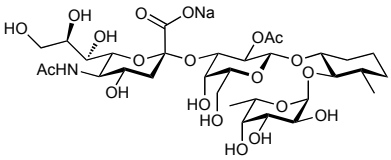

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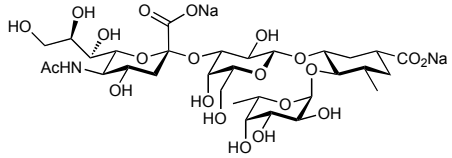

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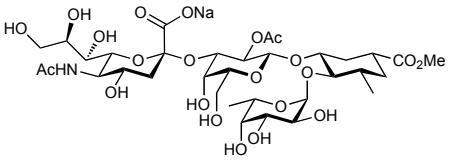

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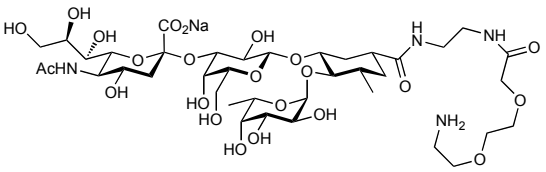

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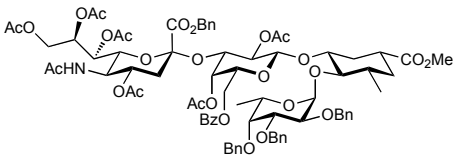

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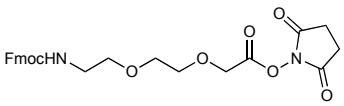

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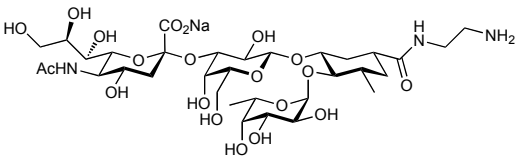

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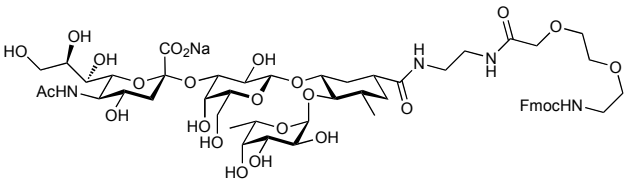

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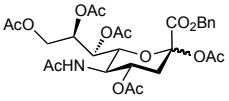

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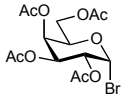

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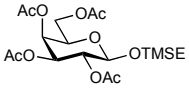

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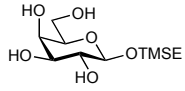

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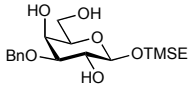

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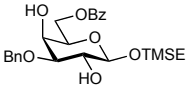

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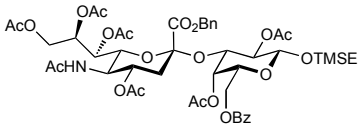

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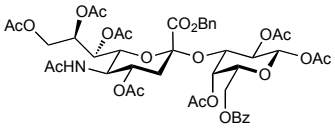

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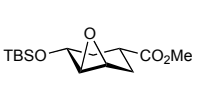

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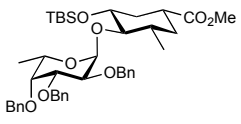

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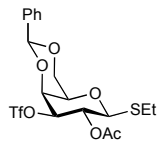

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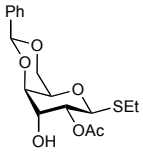

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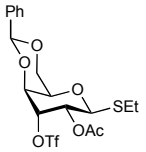

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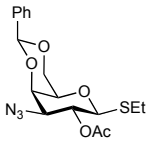

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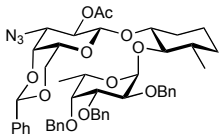

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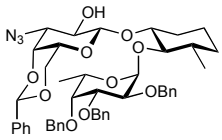

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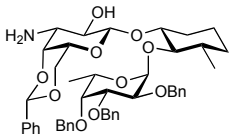

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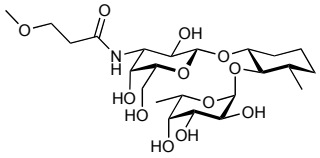

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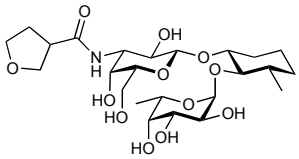

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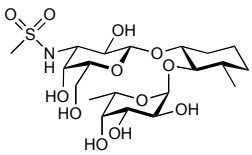

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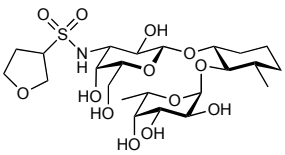

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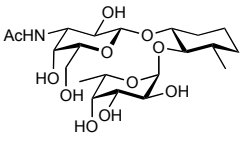

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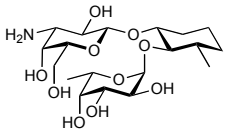

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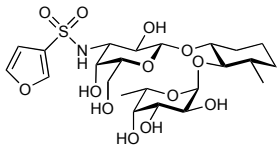

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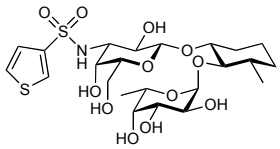

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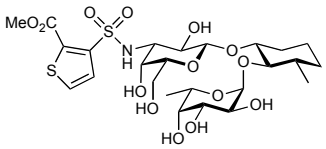

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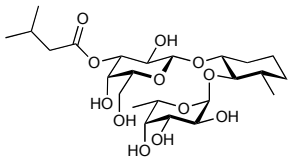

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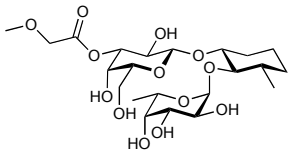

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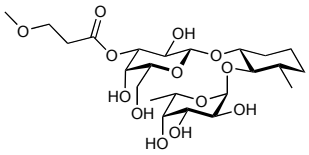

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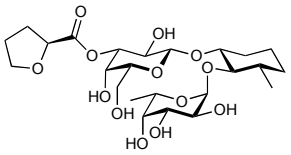

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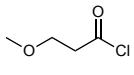

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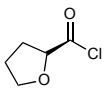

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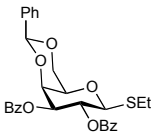

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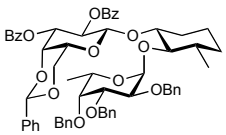

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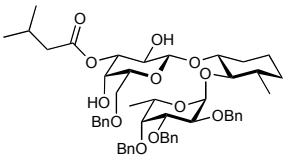

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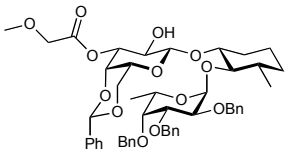

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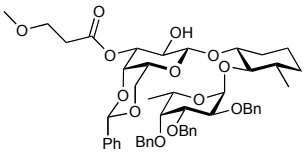

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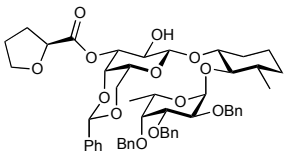

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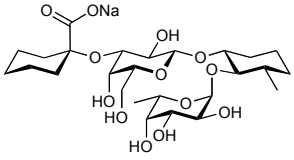
Chapter 2.5.1



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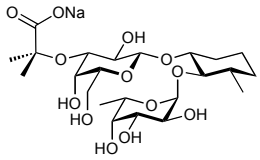

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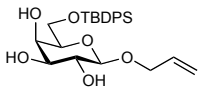

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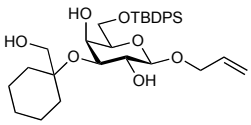

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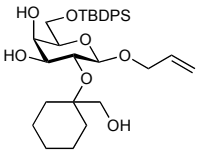

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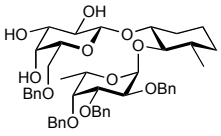

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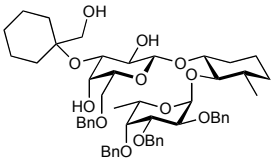

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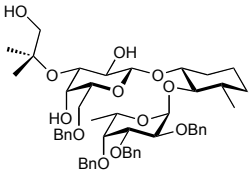

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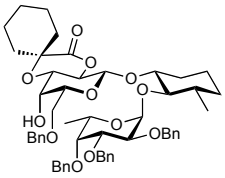

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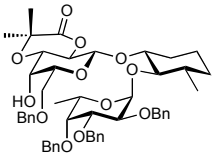

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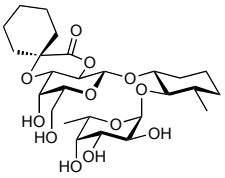

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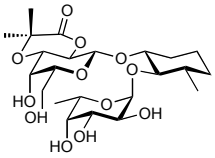

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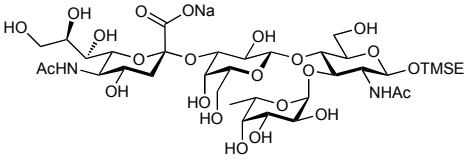

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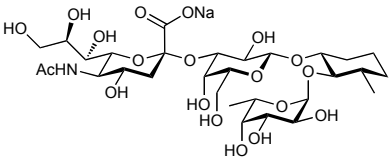

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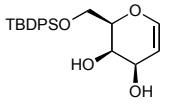

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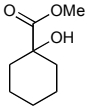

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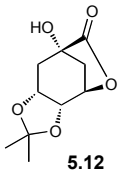

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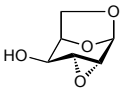

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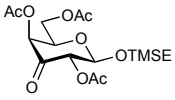

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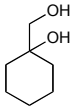

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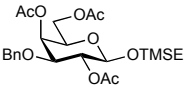

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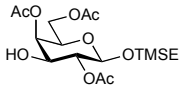

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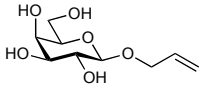

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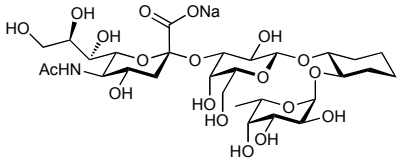

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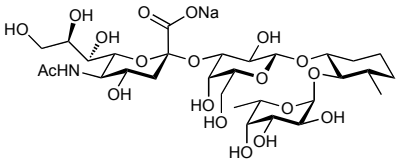

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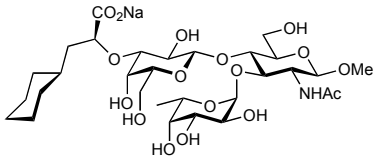
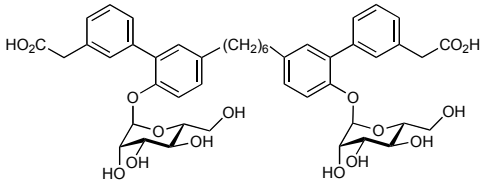


6.5*
1.8
FB329

Chapter 2.7



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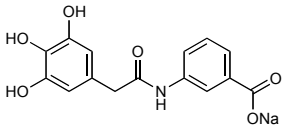

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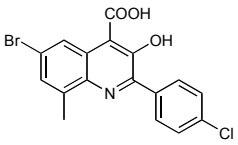
FB3



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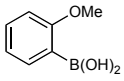

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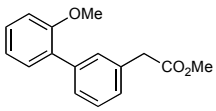

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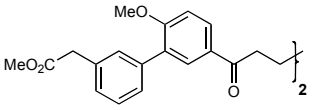

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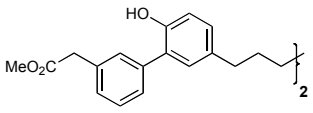
FB16
FB19



7.9
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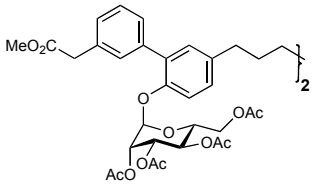

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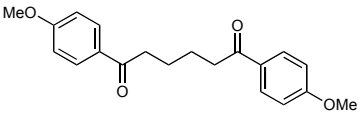

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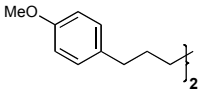
FB9



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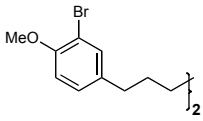

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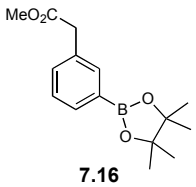

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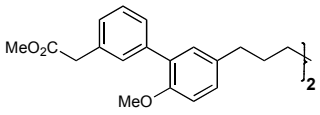

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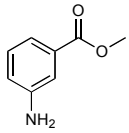
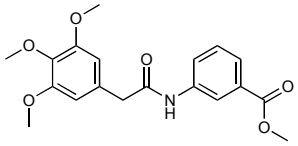
FB12
FB13



7.19
FB1



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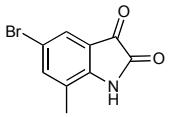

7.23
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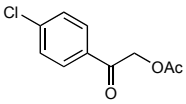

7.25
FB5