E-selectin and the Natural Tetrasaccharide Ligand sialyl Lewis^x: The Importance of Pharmacophore Pre-Organization in Glyco-Mimetics

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Declaration

I declare, that I wrote this thesis *"E-selectin and the natural tetrasaccharide ligand sialyl Lewis*^{x}*: the importance of pharmacophore pre-organization in glyco-mimetics"* with the help indicated and only handed it in to the faculty of science of the University of Basel and to no other faculty and no other university.

Alexander Titz, Basel, 31st of January 2008

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Preface

The work described in this thesis was conducted from October 1st 2004 until January 31st 2008 at the Institute of Molecular Pharmacy at the University of Basel under the guidance of Prof. Dr. Beat Ernst.

Parts of this thesis have already been published in peer reviewed journals or will be submitted for publication. These manuscripts are included in the corresponding paragraphs the way they were published in or prepared for submission to the corresponding journals. Compound numbering and references in these sections are independent of the rest of this work. However, the compounds are included in the compound overview (*page 173*).

Manuscripts published in or submitted to peer-reviewed journals:

- Titz, A.; Radic, Z.; Schwardt, O.; Ernst, B. A safe and convenient method for the preparation of triflyl azide, and its use in diazo transfer reactions to primary amines *Tetrahedron Lett.* **2006**, *47*, 2383.

- Titz, A.; Ernst, B. Mimetics of Sialyl Lewis^x: The Pre-Organization of the Carboxylic Acid is Essential for Binding to Selectins. *CHIMIA* **2007**, *61*, 194.

- Titz, A.; Patton, J.; Alker, A.; Porro, M.; Schwardt, O.; Hennig, M.; Francotte, E.; Magnani, J.; Ernst, B. Is adamantane a suitable substituent to pre-organize the acid orientation in E-selectin antagonists? *Bioorg. Med. Chem.* ***2008***, *16*, 1046.

- Titz, A.; Papandreou, G.; Cutting, B.; Wagner, B.; Dondoni, A.; Marra, A.; Magnani, J.; Schwardt, O.; Ernst, B. Lipophilic interactions between the fucose α-face and galactose β-face: Nature stabilizes the internal conformation of the core of sialyl Lewis^x in the bioactive conformation *Angew. Chem., Int. Ed.* ***in preparation***.

- Titz, A.; Patton, J.; Radic, Z.; Schwardt, O.; Magnani, J.; Ernst, B. Probing the Carbohydrate Recognition Domain of E-Selectin by a

Click-Chemistry Approach: The Importance of the Acid Orientation in sLe^x Mimetics for Binding *Bioorg. Med. Chem.* ***in preparation***.

Parts of this work were presented at conferences:

Oral presentation

- Titz, A.; Wagner, B.; Ernst, B. The pre-organization of the trisaccharide core of sialyl Lewis^x is essential for binding to E-selectin. *Fall Meeting of the Swiss Chemical Society* September 12th **2007**, Lausanne, Switzerland.

- Titz, A.; Wagner, B.; Ernst, B. The pre-organization of the trisaccharide core of sialyl Lewis^x is essential for binding to E-selectin. OP35, *13th European Carbohydrate Symposium* August **2005**, Bratislava, Slovak Republic.

Posters

- Titz, A.; Alker, A.; Porro, M.; Schwardt, O.; Hennig, M.; Francotte, E.; Ernst, B. The pre-organization of the carboxylic acid of sialyl Lewis^x mimetics is essential for binding to E-selectin. *BioValley Science Day* October 23rd **2007**, Basel, Switzerland.

- Titz, A.; Patton, J.; Radic, Z.; Magnani, J.; Ernst, B. The pre-organization of the carboxylic acid of sialyl Lewis^x mimetics is essential for binding to E-selectin (Part II). *14th European Carbohydrate Symposium* September **2007** Lübeck, Germany.

- Titz, A.; Alker, A.; Porro, M.; Schwardt, O.; Hennig, M.; Francotte, E.; Ernst, B. Pre-organization of the carboxylic acid of sialyl Lewis^x mimetics is essential for binding to E-selectin. *Benzon Symposium No.54: Glycosylation-Opportunities in Drug Development* June **2007**, Copenhagen, Denmark.

- Titz, A.; Alker, A.; Hennig, M.; Francotte, E.; Ernst, B. The pre-organization of the carboxylic acid of sialyl Lewis^x mimetics is essential for binding to E-selectin. *Fall Meeting of the Swiss Chemical Society* October 13th **2006**, Zurich, Switzerland.

Abstract

The selectins play a key role in the inflammatory cascade. The initial tethering and rolling of the leukocytes on the vascular endothelial cells, mediated by the selectins, is an essential mechanism of the host immune defense. Excessive infiltration of leukocytes into inflamed tissue can, however, lead to severe pathological consequences as observed in various diseases (*e.g.* rheumatoid arthritis, stroke or reperfusion injury). Therefore, blocking of the selectins is a valuable pharmaceutical approach.

The tetrasaccharide sialyl Lewis^x (sLe^x) is the natural binding epitope common to all selectin ligands, and consequently served as a lead compound in selectin antagonist research. As observed for many carbohydrate-protein interactions, the affinity of sLe^x towards the selectins is in the low millimolar range. Its bio-active conformation has been determined by trNOE-NMR experiments and X-ray crystallography in complex with the selectins. Because binding of the selectins to their ligands occurs under shear stress conditions, the stabilization of the pharmacophores in sLe^x mimetics in the bio-active conformation is a pre-requisite for binding.

In this PhD thesis, the importance of pre-organization of the acid orientation in sLe^x mimetics, but also the correct orientation of the core were studied.

The stabilization of the acid orientation was studied by a non-covalent approach, using (*R*)- and (*S*)-adamantyllactic acid as replacements for the *N*-acetyl neuraminic acid in sLe^x (*chapter 2.1*). Then, a '*click chemistry*' library, directed towards the exploration of additional enthalpic contributions to binding, based on a pre-organized triazololactate was investigated (*chapter 2.2*). The results obtained from these studies finally led to the project where the acid orientation is covalently locked in the bio-active orientation (*chapter 2.3*).

The core conformation of sLe^x and mimetics thereof was studied and found to be stabilized *via* a lipophilic interresidue interaction between fucose and galactose (*chapter 2.4*).

Abbreviations

$[\alpha]_D^{20}$	optical rotary power
Ac	acetyl
Ad	adamantyl
AD	asymmetric dihydroxylation
AE	asymmetric epoxidation
AIBN	azo-*bis*-isobutyronitrile
All	allyl
AMBER	assisted model building and energy refinement
approx.	approximately
Ar	aryl
Arg	arginine
Asn	asparagine
Asp	aspartic acid
ax	axial
bb	backbone
Bn	benzyl
br s	broad singlet
Bt	benzotriazolyl
Bu	butyl
Bz	benzoyl
c	concentration
Cbz	carboxybenzyl
CCDC	Cambridge crystallographic data center
CD	cluster of differentiation
COSY	correlation spectroscopy
CR	complement regulatory-like
CRD	carbohydrate recognition domain
CSA	camphorsulfonic acid
Cy	cyclohexyl
d	doublet
d.e.	diastereomeric excess

d.r.	diastereomeric ratio
DBTO	dibutyltin oxide
DCM	dichloromethane
dd	doublet of a doublet
δ	chemical shift
DEPT	distortionless enhancement by polarization transfer
DET	diethyl tartarate
DIBAl-H	diisobutylaluminium hydride
DIPEA	diisopropylethylamine
DMAP	4-*N*,*N*-dimethylamino-pyridine
DME	1,2-dimethoxyethane
DMF	*N*,*N*-dimethylformamide
DMSO	dimethylsulfoxide
DMTST	dimethyl(methylthio)sulfonium triflate
EE	ethyl acetate
e.e. or ee	enantiomeric excess
EGF	epidermal growth factor
ELAM-1	endothelial leukocyte adhesion molecule-1
ELISA	enzyme-linked immunosorbent assay
eq	equatorial
equiv.	equivalents
ESI	electrospray-ionization
ESL-1	E-selectin ligand 1
Et	ethyl
Fuc	fucose
Gal	galactose
GB/SA	generalized Born model/hydrophobic solvent accessible surface area
GBSA	see GB/SA
GDP	guanosine-diphosphate
Glc*N*Ac	*N*-acetyl glucosamine
Glu	glutamic acid
GlyCAM-1	glycosylation dependent cell adhesion molecule-1

Gul	gulose
HIV	human immunodeficiency virus
HL-60	human promyelocytic leukemia cells-60
HMBC	heteronuclear multiple bond correlation
HPLC	high performance liquid chromatography
HR-MS	high resolution mass spectrometry
HSQC	heteronuclear single quantum coherence
HUMIRA	Adalimumab
HUVEC	human umbilical vein endothelial cells
IC_{50}	half maximal inhibitory concentration
ICAM-1	intercellular adhesion molecule-1
IgG	immunoglobulin G
IL-1	interleukin-1
IMP	Institute of Molecular Pharmacy
iPr	*iso*-propyl
IR	infrared
IUPAC	International Union of Pure and Applied Chemistry
JBW	jumping-between-wells
k.o.	knock out
k_{off}	rate constant of dissociation
k_{on}	rate constant of association
Lac	lactate
LAD-2	leukocyte adhesion deficiency type 2
LAM-1	lymphocyte adhesion molecule-1
Le^a	$Lewis^a$
LECAM-1	leukocyte endothelial cell adhesion molecule-1
LECAM-2	leukocyte endothelial cell adhesion molecule-2
Le^x	$Lewis^x$
LG	leaving group
LPS	lipopolysaccharide
Lys	lysine
m	multiplet (NMR)
m	medium (IR)

MAdCAM-1	mucosal addressin cell adhesion molecule-1
MBP-A	mannose binding protein A
MC	Monte Carlo
mCPBA	*meta*-chloroperbenzoic acid
MD	molecular dynamics
Me	methyl
Met	methionine
mol. sieves	molecular sieves
MS	mass spectrometry
Neu*N*Ac	*N*-acetyl neuraminic acid
NIS	*N*-iodo succinimide
NMR	nuclear magnetic resonance
NOE	nuclear Overhauser effect
NSAID	non-steroidal anti-inflammatory drugs
ORTEP	Oak ridge thermal ellipsoid plot
PDC	pyridinium dichromate
PE	petrol ether
PG	protecting group
Ph	phenyl
Pr	propyl
Pro	proline
PSGL-1	P-selectin glycoprotein ligand 1
py	pyridine
r.t.	room temperature
R_f	retention factor
rIC_{50}	relative IC_{50}
ROE	rotating frame Overhauser enhancement
ROESY	ROE spectroscopy
rt	room temperature
s	singlet (NMR)
s	strong (IR)
sc	side chain
Sia	sialic acid

sLe^a	sialyl $Lewis^a$
sLe^x	sialyl $Lewis^x$
STD	saturation transfer difference
SUMM	systematic unbounded multiple minimum
t	triplet
TBAF	tetrabutylammonium fluoride
TBAI	tetrabutylammonium iodide
^{t}Bu	*tert.* butyl
TCP	tetrachlorophthal-
TEA	triethylamine
TEMPO	2,2,6,6-tetramethylpiperidine-1-oxyl
tert.	*tertiary*
Tf	triflyl
THF	tetrahydrofuran
TLC	thin layer chromatography
TMS	trimethylsilyl
TNF-α	tumor necrosis factor α
TOCSY	total correlation spectroscopy
TOF	time-of-flight
t_R	retention time
trNOE	transferred NOE
Tyr	tyrosine
UV	ultraviolet
µW	microwave
w	weak
X-ray	Röntgen ray

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1. Introduction

Acute or chronic inflammatory diseases have a strong negative impact on the patient's quality of life but also on the budget of the public health system. Among those diseases are allergies, stroke, organ transplant rejection, psoriasis, rheumatoid arthritis and others of large socio-economical impact.

Many diseases have a significant inflammatory component and non-steroidal anti-inflammatory drugs (NSAID, *e.g.* cyclooxygenase inhibitors) as well as steroid-based drugs (*e.g.* cortisol) are widely used therapeutics. Usually, the treatment is not curing the patient but has a palliative character. The side effects of those drugs are well documented. Consequently, biologics for the treatment of inflammatory diseases have entered the market. Major pharmaceutical companies, *e.g.* Abbott Labs (HUMIRA) or Wyeth (Enbrel or etanercept), offer antibody treatment against chronic inflammatory diseases. However, the high costs of the treatment of patients with these novel biologics is only covered by the public health system when severe side effects with traditional medicine is observed.

Therefore, the research at the Institute of Molecular Pharmacy (IMP) at the University of Basel is focusing on the discovery of small molecules that interfere with the inflammatory cascade at an early stage without having the side effects and cost burden of the established treatment of inflammation. The selectins are a class of cell adhesion molecules involved in the early stage of the inflammatory cascade. The initial tethering and rolling step of the leukocytes mediated by the selectins is an essential mechanism of the host immune defense. Therefore, the blocking of the selectin – leukocyte interaction in case of excessive influx of leukocytes into adjacent tissue is the goal of our research and the topic of this PhD thesis.

1.1 Structure and prevalence of the selectins

The selectins form a family of calcium binding C-type lectins. Lectins are carbohydrate recognizing proteins, involved in cellular recognition.[1] The family of E-, P- and L-selectin share common structural motifs (*figure 1*). They possess an *N*-terminal carbohydrate recognition domain (CRD), followed by an epidermal growth factor like domain (EGF), a varying number of short consensus repeats or complement regulatory-like (CR) domains, one transmembrane region and a *C*-terminal cytosolic tail.[2] The major difference between the selectins is the number of the CR domains. The overall homology of the selectins is approx. 50%. The highest level of homology is found in their *N*-terminal CRD and EGF domains. The CRD domain hosts the carbohydrate ligand in the recognition process *via* a structural Ca^{2+} ion required for conformational stabilization. The EGF domain is required for binding, although its role is not fully understood. It may directly interact with the ligands or serve as chaperone for the correct fold of the CRD domain.[3,4]

The CR domains are thought to act as spacers between the membranes of the two interacting cells.[5] With the transmembrane domain the selectins are incorporated into the cell membrane, whereas the short *C*-terminal tail is believed to be involved in signal transduction.[6]

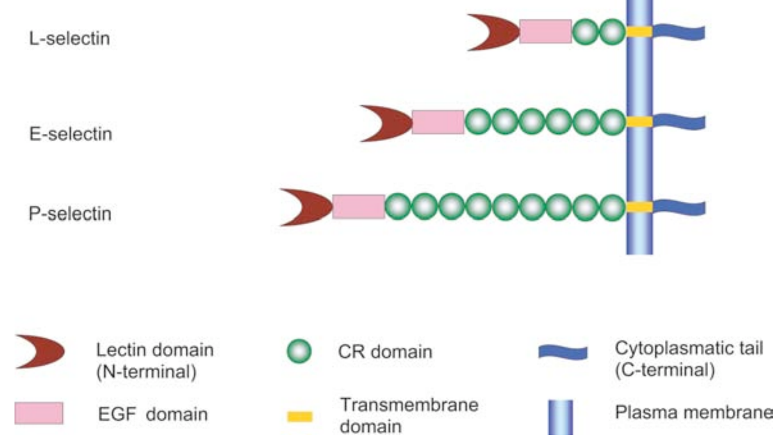


Figure 1: The domain organization of the selectins (adapted from [7])

The names of the selectins were given according to the site of their first identification.

- **E-selectin** (CD62E, ELAM-1 or LECAM-2) was discovered in the late 1980's by Bevilacqua and co-workers[8,9] and has first been identified on endothelial cells. Its sequence consists of 589 amino acids and it has a calculated weight of 64 kDa. Because of its 5 potential *N*-glycosylation sites, the protein is highly glycosylated and the observed molecular weight is increased to 115 kDa.[10,11]

- **L-selectin** (CD62L, LAM-1 or LECAM-1) was first discovered on lymphocytes, and was described as lymphocyte homing receptor.[12] It was also found on neutrophil granulocytes and monocytes, and its role in leukocyte migration into inflamed tissue was reported.[13,14] Its sequence consists of 372 amino acids and has a calculated weight of 42 kDa. L-selectin is highly glycosylated and the observed molecular weight is increased to 90 kDa.[15,16]

- **P-selectin** (GMP-140) was discovered on platelets by McEver[17] and Furie[18] in 1984. The sequence consists of 789 amino acids and has a calculated weight of 86 kDa. P-selectin is also highly glycosylated and the observed molecular weight is increased to 140 kDa.[19,20]

1.2 Ligands to the selectins

The natural ligands to the selectins are glycoproteins which usually bear sialylated and fucosylated terminal glycan epitopes.[21-23] The isomeric trisaccharides Lewis^x (**1**, Le^x, *figure 2*) and Lewis^a (**2**, Le^a), as well as their sialylated tetrasaccharide analogs sialyl Lewis^x (**3**, sLe^x) and sialyl Lewis^a (**4**, sLe^a) are generally considered to be the common terminal epitopes. Derivatives of sialyl Lewis^x with sulfation of the 6-positions of *N*-acetyl glucosamine or galactose were also shown to bind to the selectins.

E-selectin ligand 1 (ESL-1) was identified as a non-sulfated 150 kDa *N*-linked glycoprotein ligand to E-selectin.[24,25] Although it bears sLe^x motifs, it does not bind to P-selectin because of the lack of sulfation. The sequence of ESL-1 contains 5 possible *N*-glycosylation sites.[26]

GlyCAM-1,[27] MAdCAM-1,[28,29] CD34,[30] podocalyxin-like protein,[31] endo-mucin,[32] endoglycan[33] and PSGL-1[34] have been identified as ligands to L-selectin. GlyCAM-1, a sialo-mucin, is the best characterized L-selectin ligand. It is stored in the cytoplasmic granula of human endothelial venules in lymph node tissue.[27,35-39] The secretory glycoprotein GlyCAM-1 is believed to be a regulatory protein for the recruitment of lymphocytes into peripheral lymphnodes.[40,41]

Figure 2: Carbohydrate epitopes in selectin ligands

The best characterized and major natural P-selectin ligand is the P-selectin glycoprotein ligand 1 (PSGL-1) which has been discovered in 1993.[42] The 125 kDa protein forms dimers *via* a cysteine disulfide bridge. PSGL-1 possesses terminal sLe^x structures in its sialomucin type glycans. This glycoprotein is also a ligand to E- and L-selectin, but for P-selectin binding, additional sulfation at two of the three *N*-terminal tyrosine residues (Tyr46, Tyr48 and Tyr51) is required.[43-45]

An overview on the selectins and their ligands was published by Vestweber and Blanks[40] and is shown in *figure 3*.

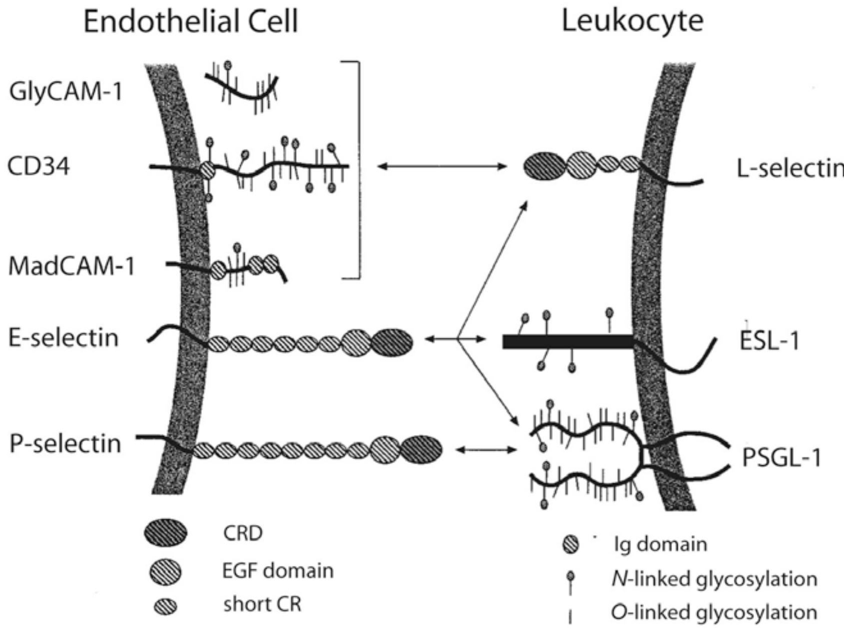


Figure 3: The selectins and their natural glycoprotein ligands.[40]

1.2.1 The role of the selectins in the inflammatory cascade

The inflammatory cascade is an important step of the defense mechanism of the immune system. In healthy humans, an invasion of pathogens or an injury triggers this complex cascade, ultimately leading to neutralization of the pathogen and healing of the injury. This process can be described by six directly linked steps (see *figure 4*).

First, an inflammatory stimulus causes an immune response in the host. Inflammatory mediators, such as cytokines (*e.g.* TNF-α or IL-1) or LPS, stimulate endothelial cells to display E- and P-selectin on their cell surface. P-selectin is transiently expressed and usually stored in α-granulocytes in platelets and in Weibel-Palade bodies in endothelial cells.[46,47] Upon histamine or thrombin stimulation P-selectin is rapidly translocated to the cell surface within minutes. In contrast to the rapid exposure of P-selectin, E-selectin is expressed *de novo* by TNF-α, IL-1 or LPS mediated stimulation of transcription.[48,49] The peak level of expression is observed 3-4 h after stimulation.[50]

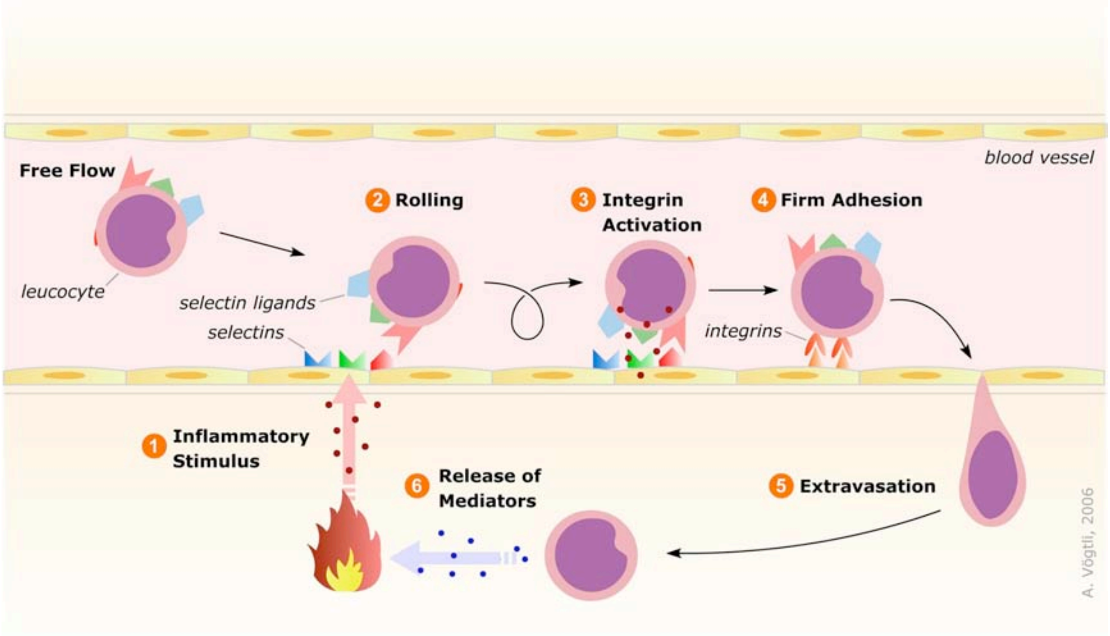
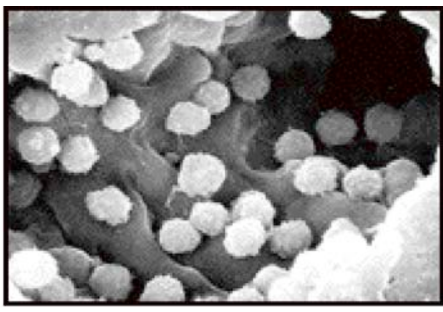


Figure 4: The inflammatory cascade[51]

As a second step, the initially freely flowing leukocytes interact *via* their surface glycoproteins (*e.g.* PSGL-1 and ESL-1) with the exposed E- and P-selectins on the endothelial cell surface. This fast association, fast dissociation contact leads to the observed tethering and rolling process of the leukocytes along the blood vessel (see also *figure 5*).[52,53] In fact, observations of this process and the following extravasation of leukocytes have been reported already at the end of the 19th century.[54-56]



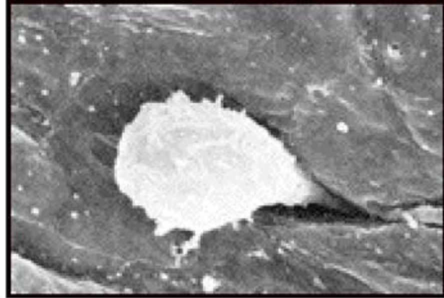


Figure 5: Leukocyte rolling (left) on endothelial cells and migration into tissue (right)[57]

L-selectin is constitutively expressed at the surfaces of leukocytes and can interact *via* its own glycan structures with E-selectin.[58] The pool of leukocytes attracted to the site of inflammation is further expanded *via* leukocyte-

leukocyte interactions of PSGL-1 on already tethered leukocytes with L-selectin on new leukocytes.[59,60]

In a third step, chemokines from the endothelial cells are responsible for the secondary activation of the leukocytes. As a result, integrins, a second class of cell adhesion molecules located on leukocytes are activated,[61,62] and can then interact with their ligands on endothelial cells initiating the firm adhesion in the fourth step of the inflammatory cascade. This firm adhesion mediated by the integrin interaction with their ligands, *e.g.* VCAM-1, ICAM-1 and also MAdCAM-1[61,62] is necessary for the subsequent step of extravasation of leukocytes from the blood stream into the inflamed tissue (see also *figure 5*). Finally, the leukocytes are attracted to the site of inflammation where they fight the inflammatory stimulus with cytokines, oxidative agents and proteases.

1.2.2. Physiological and pathophysiological importance of the selectins

In many acute or chronic inflammatory diseases excessive recruitment of leukocytes into tissue is a principal element. The disruption of the selectin-leukocyte interaction was shown to have a severe effect on the course of these diseases.[63-65]

Selectin-deficient mice showed impaired inflammatory responses.[66,67] But also the application of selectin directed antibodies led to a severe decrease in the host's response.[68-70]

Further evidence for the importance of the selectins was reported in 1992. In a rare disease called leukocyte adhesion deficiency type-2 (LAD-2), the patient's inflammatory response is impaired.[71,72] In those patients, a mutation in a GDP-fucose transporter gene leads to a dramatic effect on the glycans expressed.[73,74] These patients lack fucosylation in their glycoconjugates and as a result, rolling of leukocytes is reduced.

Selectins play major roles in a variety of inflammatory diseases like ischemia and reperfusion injury,[75,76] asthma,[77-80] and rheumatoid arthritis.[81-84] An

overview on these pathological functions would exceed the frame of this thesis and it is therefore recommended to the interested reader to go into the references cited.

Carbohydrate epitopes which bind to the selectins were also reported as tumor-associated antigens.[85] Carcinoma cell metastasis is believed to progress *via* the interaction of their sialylated and fucosylated mucin surface proteins with the selectins.[86] Recently, Varki and Varki reported on the association between cancer metastasis and P-selectin.[87]

1.3 Sialyl Lewis^x as lead

Sialyl Lewis^x is the minimal epitope recognized by all three selectins[21,22,88] and served therefore as a lead structure for selectin antagonist research.[89-92] Sialyl Lewis^x was chosen for this purpose although it only has, similar to most carbohydrate - receptor interactions, a modest affinity in the millimolar range. Soluble sLe^x was shown to have beneficial effects in *in vitro* and *in vivo* models.[93-96]

1.3.1 The assay format

The binding kinetics of the selectins to their *in vivo* ligands show fast association - fast dissociation kinetics with k_{on} values typically in the range of 10^4 $M^{-1}s^{-1}$ and k_{off} values in the range of 1 s^{-1}.[97-99] Sialyl Lewis^x shows also similar binding kinetics to a sLe^x specific antibody GSLA-2.[100,101] This behavior of the ligand is essential to overcome the shear forces under flow conditions. Interestingly, the overall affinity of sLe^x is rather low and results from only a few, mostly electrostatic interactions.[99] To have a significant read-out of a cell-free assay, the reference ligand is therefore displayed in an oligo-valent fashion.[102,103]

sLe^x has been reported with various affinities to the selectins as a result of the wide variety of target-based assays applied in this field. This is a significant drawback when comparing different compounds that have been evaluated by

different groups, *i.e.* with different assays. Therefore, the affinities reported in this work will always be relative to sLe^x or to one of its well studied mimetics (CGP69669A,[69] *vide infra*). In addition, false positives were reported due to the presence of trace amounts of polyanionic ion exchange resins especially for P-selectin which requires additional sulfation for binding.[104]

Under static cell-free conditions, various ELISA-based assays are reported in the literature. Either sLe^x is grafted on microtiter plates *via* sLe^x-glycolipids and an antibody directed against the glycan with a secondary read-out motif is displaced by the testing compounds[105-108] or the inverse process is used where selectin is immobilized and a polymeric sLe^a with a secondary signal is displaced by the compound of interest.[102,109,110] Also HL-60 cells displaying sLe^x on their surface have been used to determine affinities of compounds of interest using selectin-IgG constructs and myeloperoxidase activity after cell lysis as read-out.[111,112]

Since *in vivo* the binding process occurs under shear stress conditions, more sophisticated dynamic assay formats were developed. Because of their low throughput, however, they are only used as secondary assays. Rolling of neutrophils on a stimulated HUVEC cell monolayer can be observed in the flow-assay reported by the Novartis team.[113] Intravital microscopy was developed as a convenient *in vivo* characterization method.[69,114-119] The tethering, rolling and extravasation process in a blood vessel can directly be observed by this format.

1.3.2 Essential pharmacophores

Systematic studies towards the replacement of all functional groups in sLe^x gave a good picture of the essential pharmacophores. These are:

a, the 3- and 4-hydroxyl groups of fucose[120,121]

b, the 4- and 6-hydroxyl groups of galactose[122,123]

c, the carboxylic acid of neuraminic acid (and additionally, 4-OH and hydrophobic contributions for P-selectin)[120,124-12]

From several studies, it became evident that Glc*N*Ac is not directly involved in the binding process: it does not bear essential pharmacophores for selectin binding.[127-129]

1.3.3 Conformation of the tetrasaccharide motif

Oligosaccharides were regarded for a long time as conformationally flexible when compared to other classes of biopolymers, *e.g.* polypeptides. However, intrinsic properties of saccharides lead to a stabilization, respectively a conformational preference across glycosidic linkages. The exo-anomeric effect[130,131] is a main reason for this preference, but also inter-residue hydrogen bonds and other effects, *e.g.* steric constraints, contribute to a preferred population of the possible conformations of saccharides.

Sialyl Lewis^x has attracted the attention of many researchers in the field of medicinal chemistry. Consequently, there is a large number of reports on conformational issues of sLe^x in solution but also bound to the selectins. An overview on this topic was given by Porro.[132]

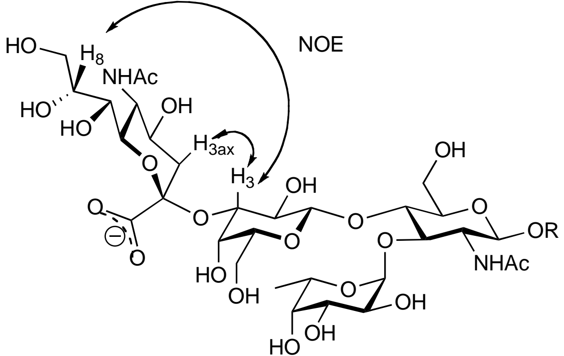


Figure 6: Simultaneous NOEs between Gal-H3 and Neu*N*Ac-H3$_{ax}$ and H8 prove the existence of at least two conformations of sLe^x in solution

In solution, sLe^x populates three conformational clusters resulting from different interglycosidic dihedral angles. However, the degree of population of the individual clusters is still debated. Results have been obtained from computational studies and NMR experiments of the ligand alone.[133-140] There is a general agreement on the conformational stability of the Lewis^x core of

sLe^x. However, a certain flexibility around the glycosidic linkage of sialic acid with galactose is topic of discussion. Especially the presence of NOEs between sialic acid H3[134,137,140] and H8[138,139] with the galactose H3 proton supports the co-existence of two conformations in solution (*figure 6*).

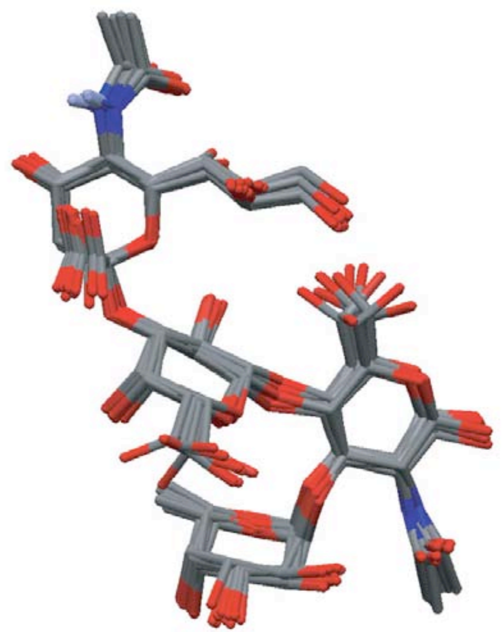


Figure 7: The bioactive conformation of sLe^x from trNOE NMR experiments[141,142]

Finally, structural analysis in presence of the protein revealed the bioactive conformation with the help of transferred-NOE experiments (*figure 7*).[141,142] Saturation transfer difference (STD) spectroscopy could show the epitopes of sLe^x which are in close contact with the protein in the bound state.[143]

Later, in the year 2000, this bioactive conformation was confirmed by X-ray crystallography of sLe^x in complex with all three selectins as reported by Camphausen and co-workers,[126] based on the crystal structure of the apo-protein published in 1994.[144]

Figure 8: Contacts of the pharmacophores in sLe^x with E-selectin (adapted from [126])

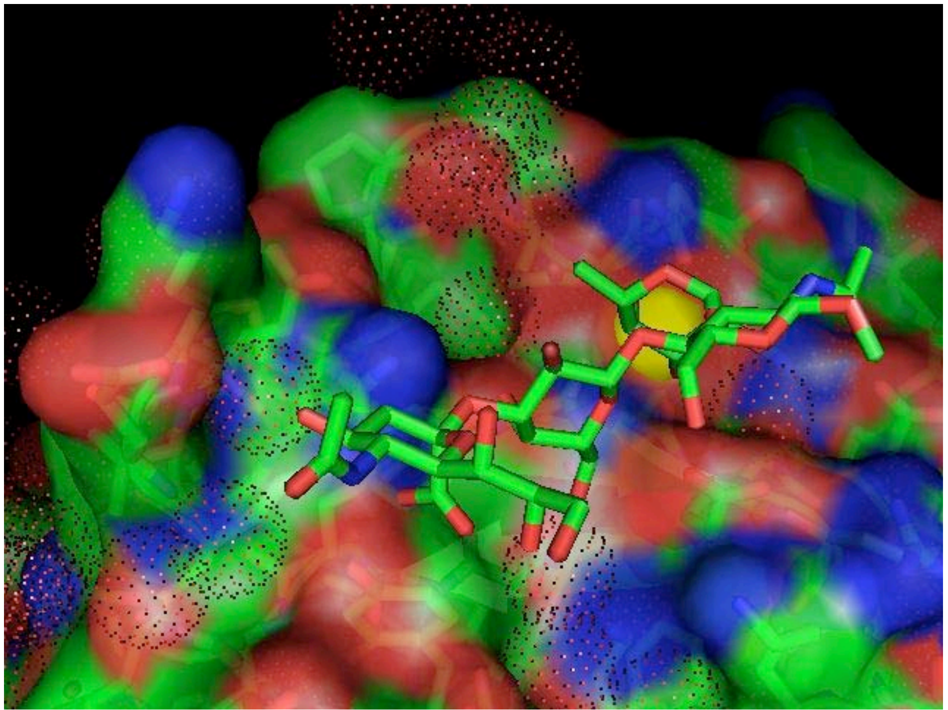


Figure 9: Top-view on sLe^x bound to E-selectin as determined by X-ray[126]: The essential pharmacophores are oriented in a plane complementary to the receptor, whereas Glc*N*Ac and Neu*N*Ac, with exception of its carboxylic acid, are not in contact with the protein (yellow sphere: Ca^{2+} ion, dotted spheres: H_2O molecules)

An information already obtained from the X-ray structure of the highly homologous mannose binding protein (MBP-A)[145] in complex with its glycan ligand proved true also in the case of the complex of sLe^x with the selectins: the fucose (mannose in MBP-A) residue is involved in inner-sphere complexation of the protein bound Ca^{2+} ion. The interactions of the remaining pharmacophores of sLe^x with E-selectin are depicted in *figure 8*. The essential carboxylate of neuraminic acid is involved in a tyrosine supported salt-bridge with Arg97, whereas the remaining functional groups of this residue are not in contact with the protein. From this structure, it also becomes evident that the Glc*N*Ac moiety just serves as a spacer to keep the fucose and the galactose residues in their appropriate positions, as Glc*N*Ac itself has no contacts with the protein surface (*figure 9*).

1.4 Selectin antagonists

One major drawback in the development of sLe^x antagonists were the testing conditions. Because of this wide variety of assays, it is difficult to compare the non-standardized affinities measured by different groups. Furthermore, false-positives as a result of ion-exchange resin contamination should always be taken into account when comparing reported data (*vide supra*).[104]

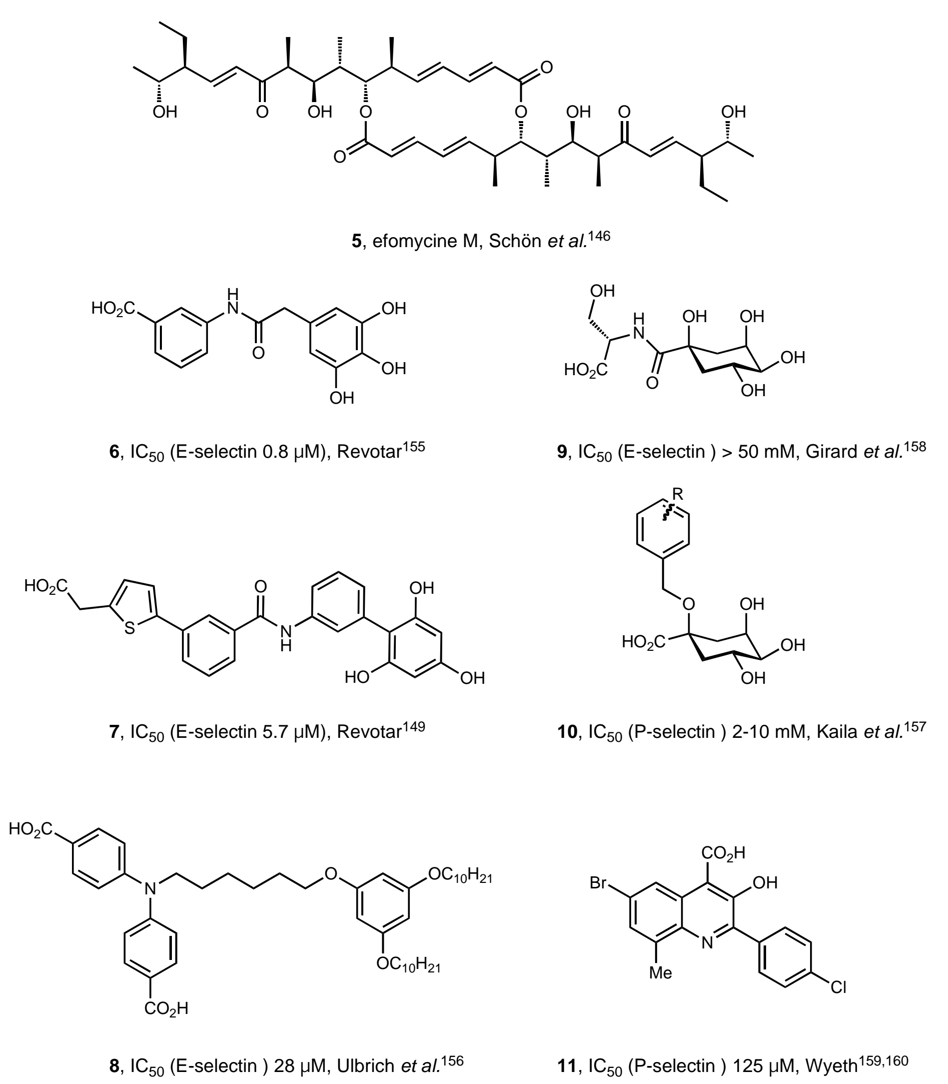


Figure 10: Recently reported selectin binding molecules

The numerous small molecule mimetics of sialyl Lewis^x based on replacements of single saccharide subunits, but also *de novo* design of E-selectin antagonists were summarized by Wong and co-workers[92] in 1998 and

in a more recent review by Ernst, Kolb and Schwardt.[57] Here, I want to mention the latest research results which are not included in those two review articles.

Schön further analyzed efomycine M (**5**, *figure 10*), a natural product which is supposed to bind to the selectins.[146] This molecule has been mentioned before in a patent by Bayer AG, Germany, as a potential treatment for psoriasis.[147] However, when tested by an independent group at Schering AG, Germany, these results could not be confirmed.[148]

A team led by Aydt at Revotar, Germany, published a series of patents[149-154] and one paper[155] on various *de novo* designed pan-selectin antagonists (*e.g.* **6** and **7**, *figure 10*). These molecules do not resemble sLe^x because of the total loss of any carbohydrate motif. In a competitive binding assay, IC_{50} values were reported to be in the micromolar range.

Ulbrich and co-workers[156] synthesized and tested selectin antagonists based on long C_{10}-alky chains and carboxylic acids (**8**, *figure 10*), that resemble detergents. The IC_{50}s reported are thus not expected to result from functional selectin inhibition.

Kaila[157] and co-workers at Wyeth reported on quinic acid derivatives as selectin binding molecules (**10**, *figure 10*). Quinic acid was chosen since it has the same relative orientation of the hydroxyl groups as in fucose. The affinities towards P-selectin were in the millimolar range, and crystals of selectin could be soaked with solutions of these small molecules to give insight in their binding properties. Independently, Girard *et al.* published the synthesis of quinic acid derivatives and their evaluation in an HL-60 cell based assay (**9**, *figure 10*).[158] The IC_{50}s were reported to be greater than 50 mM.

In 2007, Kaila and co-workers published two papers on the optimization of quinolone derivatives as P-selectin antagonists (*figure 10*).[159] These molecules moved on to clinical trials this year at Wyeth, although they display IC_{50}s in the micromolar range.[160]

1.4.1 Locked Conformations

It is a well known concept in medicinal chemistry, that a conformational pre-organization of the pharmacophores in their bioactive spatial arrangement ultimately leads to a higher binding affinity. This results from decreased entropy costs upon binding when compared to a flexible analog. This phenomenon has been shown in various fields of supramolecular assembly[161] and medicinal chemistry (*vide infra*). In 1993, Carver was discussing the idea of reducing conformational freedom of molecules in solution to obtain oligosaccharides with higher binding affinity towards their receptors.[162]

A beautiful example of this approach, where the affinity benefits largely from the locked conformation, was the introduction of cyclic ureas as HIV protease inhibitors in 1994.[163] Bundle recently reported on the stabilization of the bioactive conformation of a trisaccharide, mimicking the LPS of *Shigella flexneri* for antibody binding.[164,165] They could reduce the entropic cost of binding (TΔS) by 1.2 kcal mol^{-1} *via* a locked bioactive conformation.

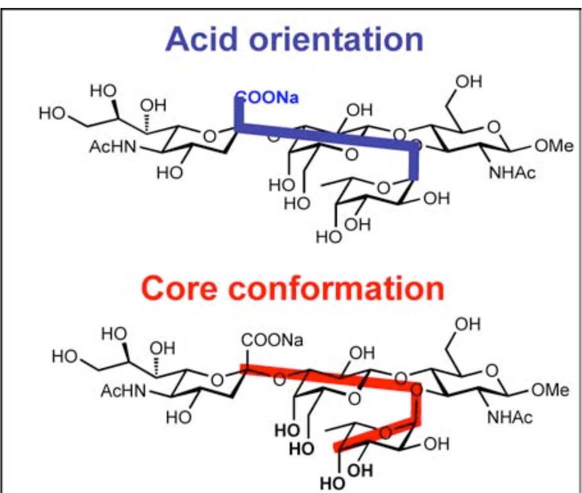


Figure 11: Internal coordinates of sLe^x: the core conformation indicates the relative spatial arrangement of the fucose and galactose residues, the acid orientation defines the tilting angle of the C1-C2 bond vector of sialic acid relative to the core

Entropic contributions are much more important for binding under shear stress conditions, as they are present in the selectin-mediated inflammatory cascade, than in cases where binding occurs under static conditions. It is therefore the philosophy of our research group that only small molecules with pre-organized pharmacophores will be functional selectin blockers.[166]

This working hypothesis has evolved from two reports by Kolb and Ernst in 1997, where biological affinities of selectin ligands were predicted as function of their conformational preference *in silico*.[167,168] For better visualization of the internal conformation of sLe^x in the bound conformation, two internal coordinates were defined. These are

- the core conformation, *i.e.* the relative spatial arrangement of fucose and galactose and
- the acid orientation which defines the tilting angle of the C1-C2 bond vector of sialic acid with the core (*figure 11*).

The bioactive conformation of sLe^x[142] and the prediction of the conformational preference of sLe^x in solution as a function of these two coordinates are shown in f*igure 12* as 2-dimensional plot.

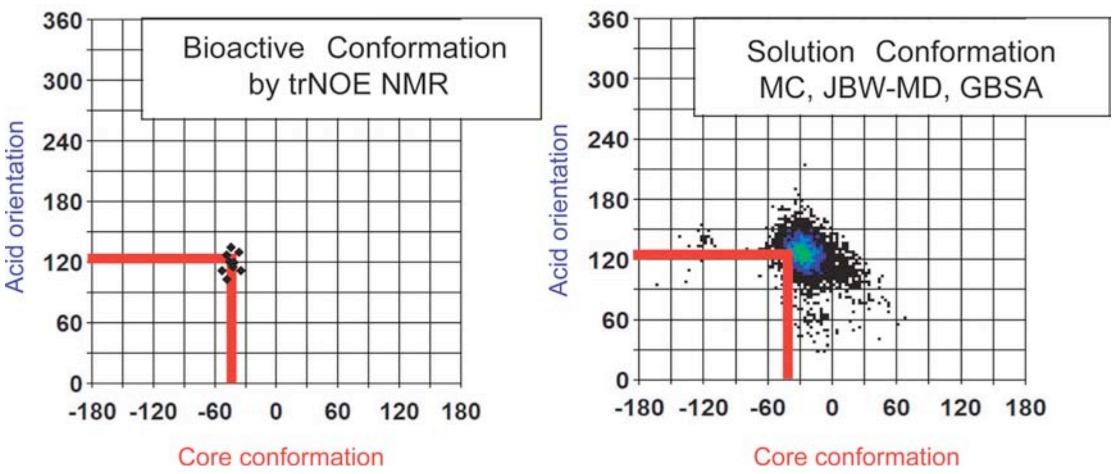


Figure 12: The bioactive conformation of sLe^x as a function of acid/core dihedrals (left) and the computationally predicted behavior in solution (right)[167,168]

This concept of conformational restriction is the basis of the present thesis:

- The pre-organization of the core as a result of lipophilic inter-residual stabilization was studied (*chapter 2.4*).
- The major part of this thesis however, deals with the flexibility of the neuraminic acid, the impact of this flexibility on binding and how the conformation can be pre-stabilized in the correct orientation (*chapter 2.1, 2.2, 2.3*).

1.4.2 Replacements for Neu*N*Ac in sLe^{x} mimetics: importance of pre-organization

The literature regarding replacements of Neu*N*Ac as well as the importance of the above mentioned pre-organization was summarized in a review for CHIMIA.

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Mimetics of Sialyl Lewis^{x}: The Pre-Organization of the Carboxylic Acid is Essential for Binding to Selectins

Alexander Titz§* and Beat Ernst
§SCS Poster Prize Winner

Abstract: Selectins play a key role in leukocyte trafficking during the inflammatory response of the organism, *i.e.* the recruitment and extravasation of leukocytes from the blood stream into inflamed tissue. Antagonizing the interaction of selectins with their physiological ligands was shown to be a validated approach for the treatment of inflammatory disorders like rheumatoid arthritis, stroke or reperfusion injuries. Although numerous research efforts to identify small molecule selectin antagonists have been reported, no successful drug has been identified so far. This mini-review describes selectin antagonists, where the N-acetylneuraminic acid moiety of the natural ligand sialyl Lewis^{x} is replaced by mimetics containing the essential carboxylic acid function. The prerequisite of a pre-organization of the carboxylate in the bioactive conformation is discussed.

Keywords: Antagonist · Pre-organization · Selectin · Sialyl Lewis^{x} · Sialyl mimetics

1. Introduction

Excessive extravasation of leukocytes from blood vessels into the adjacent inflamed tissue can cause acute or chronic reactions, as observed in reperfusion injuries, stroke or rheumatoid arthritis.[1,2] In the early stage of the inflammatory process, E-, P- and L-selectin, a family of closely related cell adhesion molecules, play a key role. They are responsible for the rolling of leukocytes on the endothelial cell surface, which results in firm adhesion and finally extravasation of leukocytes into the inflamed tissue (Fig. 1).[3,4] With selectin knock-out mice, it has been demonstrated that the rolling stage is a prerequisite for the inflammatory cascade to occur.[5,6] Therefore, antagonism of selectins is a validated approach for the treatment of inflammatory diseases.

Fig. 1. The inflammatory cascade: Upon an inflammatory stimulus (1), the selectins are expressed on endothelial cells. As a result, the formerly free-flowing leukocytes tether to the selectins and start to roll on the endothelial surface (2). This rolling initiates the activation of integrins which interact with members of the IgG superfamily (3), leading to firm adhesion of the leukocytes to the endothelial surface (4). Finally, leukocytes extravasate (5) into the inflamed tissue where they fight the inflammatory stimulus (6) (figure courtesy of Alexander Vögtli)

2. The Natural Ligand Sialyl Lewis^{x}

The minimal common epitope present in all physiological selectin ligands, is sialyl Lewis^{x} (**1**, sLe^{x}, Fig. 2a).[7,8] Although the affinity of the tetrasaccharide sLe^{x} is only in the millimolar range,[9–11] beneficial effects in *in vitro* assays as well as in disease models have been shown.[12]

sLe^{x} was therefore selected as the lead compound in selectin antagonist research.[13] Numerous academic and industrial research groups have been involved in the identification of the essential pharmacophores of sLe^{x}, which are as follows:

i) the hydroxyl groups in position 3 and 4 of the fucose moiety,

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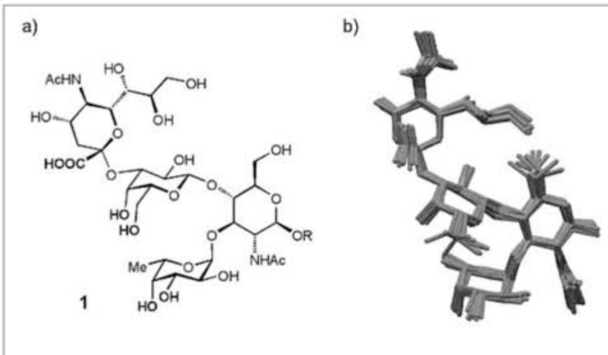

Fig. 2. a) The natural epitope sialyl Lewis^x (**1**, sLe^x) consists of N-acetyl-D-neuraminic acid (D-NeuNAc), D-galactose (D-Gal), N-acetyl-D-glucosamine (D-GlcNAc) and L-fucose (L-Fuc). The pharmacophores are highlighted in bold type. b) The bioactive conformation of sLe^x was determined by trNOE NMR measurements.[15]

ii) the 4- and 6-hydroxyl groups of galactose and
iii) the carboxylic acid of N-acetyl neuraminic acid.[10,14]

In addition, the bioactive conformation of sLe^x (Fig. 2b), has been determined by trNOE NMR analysis[15] and subsequently confirmed by X-ray crystallography.[16] In this bound conformation, L-Fuc and D-Gal are stacked upon each other and D-GlcNAc merely acts as a spacer to guarantee the appropriate spatial orientation of the carbohydrate moieties. The binding face of sLe^x is formed by the carboxylate of D-NeuNAc together with the pharmacophoric hydroxyls of fucose and galactose (Fig. 2b).

This mini-review summarizes the research of the past 15 years directed towards the replacement of the NeuNAc moiety of sLe^x. Along with aspired improved affinity for selectins, these mimetics are synthetically more accessible than the parent structure. The numerous contributions focusing on modifications of the trisaccharide core are not discussed, as detailed information is available from recent reviews.[10,17]

3. sLe^x Derivatives with N-Acetyl Neuraminic Acid Replacements

Since the carboxylic acid function is the dominant pharmacophore of the NeuNAc moiety of sLe^x (**1**), numerous antagonists substituted with acidic fragments have been synthesized. The 3'-sulfo Lewis^x derivative **2** (Fig. 3)[18–20] and 3'-sulfo Lewis^a **3** (Fig. 3)[20] were reported to exhibit significant E-selectin binding affinity. Pure 3'-sulfo-Lewis^x derivatives showed E-selectin binding with affinity comparable to sLe^x as reported by Hasegawa and co-workers in 1993.[21] Later, an IC_{50} of 3 mM was determined by Kiessling *et al.*[22,23] In 1996, Hasegawa and co-workers reported sLe^x analogs, where neuraminic acid has been replaced by a sulfate (→**4**), a phosphate (→**5**) or glycolic acid (→**6**). In addition, the reducing end was substituted by a C_{30}-tail (→**4, 5**) or a ceramide (→**6**).[24] At least part of the reported affinities is probably resulting from micelle formation, leading to multivalency.[25] Sulfate and phosphate derivatives (→**7, 8**) containing a modified Lewis^x core also exhibit affinities in the millimolar range.[26]

The replacement of NeuNAc by glycolic acid was originally introduced by Musser *et al.*[14] Both derivatives, **9** and **10**,[27,28] showed affinity towards E-selectin comparable to that of sLe^x. An extensive study of 3'-O-carboxymethyl substituted Lewis^x derivatives (*e.g.* **10**, Fig. 3) was published by Glaxo as a result of their search for suitable GlcNAc replacements.[27–29]

In 1997, Hasegawa's group[30] reported on a C-linked carboxymethyl group that places the pharmacophore closer to the galactose 3-position, *i.e.* the 3'-C-carboxymethyl Lewis^x derivative (**11**, Fig. 3). Its affinity is more than 50-fold better when compared to a sLe^x derivative with the same substitution at the reducing end. However, the molecules contain a different central carbohydrate. In **11** D-glucose was used instead of D-GlcNAc.

Another interesting approach to replace NeuNAc (see **12**, Fig. 3) was reported by Borbás *et al.*[31,32] The authors argued that the sulfonated fructose derivative **12** should show improved binding affinity, mainly due to the increased acidity of the sulfonate when compared to a carboxylate. Unfortunately, no biological data are available for this sLe^x mimetic.

4. Pre-Organization of the Acid Orientation in the Bioactive Conformation

The exo-anomeric effect[33] contributes to the conformational preference of the glycosidic linkages in sLe^x. In addition to this stereoelectronic effect, the Lewis^x core is further stabilized by steric compression[34] and lipophilic interactions between the α-face of L-fucose and the β-face of D-galactose.[35] The terminal Sia-Gal linkage, however, does not benefit from additional effects and is therefore more flexible. A general approach to minimize the entropic cost of a ligand binding to a receptor, resulting from conformational flexibility, is the pre-organization of the ligand in its bioactive orientation.

The bioactive conformation of the natural selectin ligand sLe^x exhibits a high similarity to one of its low energy conformations in solution. As a consequence, only minimal conformational changes upon binding are

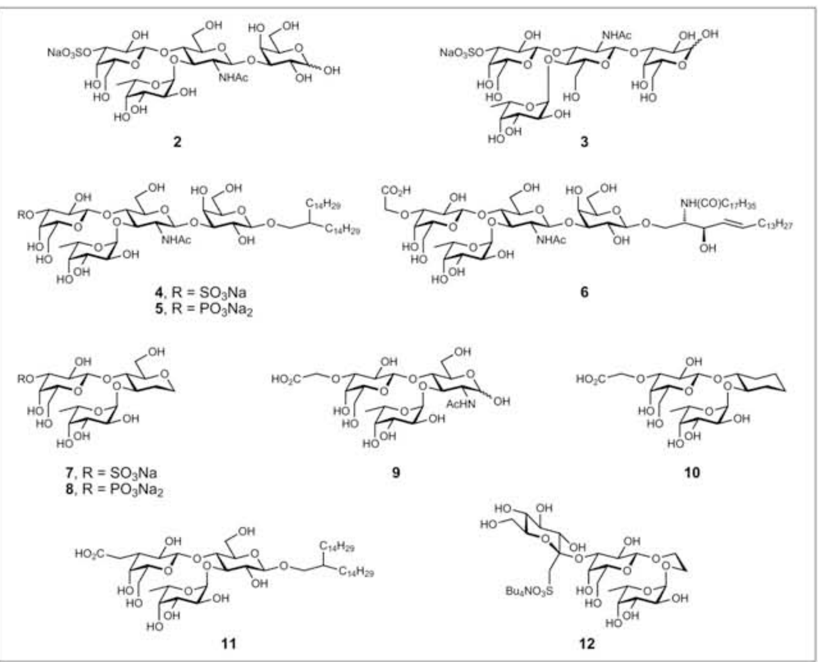

Fig. 3. Anionic replacements for N-acetylneuraminic acid in sLe^x (**1**)

(*S*)-13, R = H; rIC_{50} = 4
(*S*)-14, R = Ph; rIC_{50} = 2.6

(*R*)-13, R = H; rIC_{50} > 10
(*R*)-14, R = Ph; rIC_{50} > 10

(*S*)-15, R = Ph; rIC_{50} = 0.3
(*S*)-16, R = C_6H_{11}; rIC_{50} = 0.1
(*S*)-17, R = 1-adamantyl; rIC_{50} = 0.21

(*R*)-15, R = Ph; rIC_{50} > 10
(*R*)-16, R = C_6H_{11}; rIC_{50} > 10
(*R*)-17, R = 1-adamantyl; rIC_{50} > 10

Fig. 4. Lactic acid derived selectin antagonists. The IC_{50} values are reported relative to sLe^x (**1**). This results in relative IC_{50}s (rIC_{50}) below 1.0 for derivatives binding better than **1** and rIC_{50}s above 1.0 for compounds with a lower affinity than that of **1**.

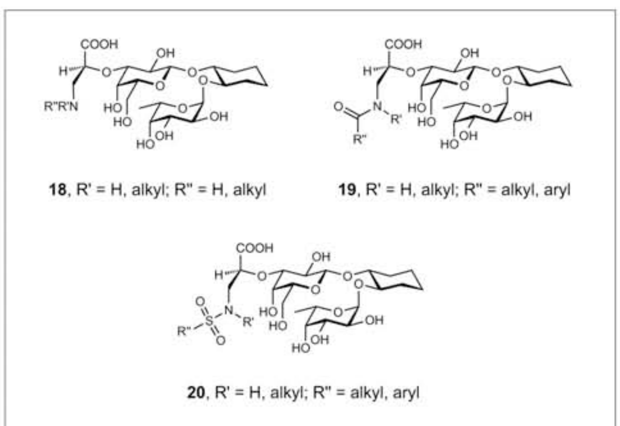

Fig. 5. A lactic acid derived library

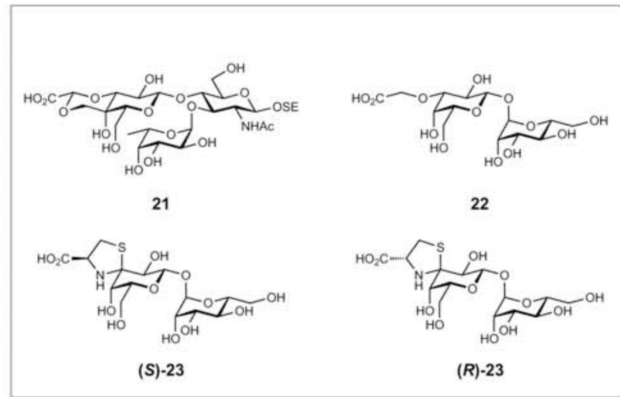

Fig. 6. Conformationally locked carboxylic acid moieties and the flexible reference compound **22**

necessary, leading to low entropic cost. For the replacement of NeuNAc only conformationally biased substituents, where the carboxylic acid is already pre-organized in the bioactive orientation in solution, should therefore be considered.

In 1997, Kolb and Ernst[36,37] developed a computational method for the prediction of the affinity of selectin antagonists as a function of their pre-organization with respect to the acid and the core orientation. With (*S*)-configurated lactic acid as present in (*S*)-**13** (Fig. 4), the acid orientation is similar to the one in the physiological ligand, whereas in the (*R*)-configurated derivative (*R*)-**13** the acid adopts a conformation different to the bioactive one. As a consequence, (*R*)-**13** has to undergo a large conformational change upon binding leading to substantial entropic costs.

Kolb and Ernst further demonstrated a beneficial effect of steric bulk on the binding affinity through pre-organization by using various substituted lactic acids to mimic NeuNAc.[36,37] A series of (*R*)- and (*S*)-configured lactate derivatives (**14–16**, Fig. 4) were computationally analyzed for their conformational preference in solution. The predicted binding behavior as a function of the population of the bioactive conformation could be confirmed in the biological assay. All lactic acid derivatives with (*S*)-configuration at the C-2 of the lactate moiety ((*S*)-**13–16**) bound to E-selectin, whereas the corresponding (*R*)-isomers ((*R*)-**13–16**) were all inactive due to an acid orientation outside of the bioactive window. In the (*S*)-series, the affinity could be improved by increasing the steric bulk at the 2-position of the lactic acid. Thus, (*S*)-**13** shows a rIC_{50} of 4.0 when compared to sLe^x, whereas the bulkier phenyllactic acid derivative (*S*)-**14** was a better ligand to E-selectin with a rIC_{50} of 2.6. In the series where GlcNAc is replaced by cyclohexanediol, the bulkier cyclohexyllactate (*S*)-**16** was the more potent ligand (rIC_{50} = 0.1) when compared to the phenyllactate derivative (*S*)-**15** (rIC_{50} = 0.3). In contrast, the glycolate **10** (Fig. 3) shows only a moderate affinity for E-selectin, since in the absence of a steric bias the glycolate side chain can freely rotate and the acid does not preferably populate the bioactive conformation.

Recently, as a continuation of the preceding studies, we synthesized selectin antagonists bearing the bulky adamantly group, (*S*)-**17** and (*R*)-**17** (Fig. 4).[38] Again, the importance of the correct absolute configuration at the α-carbon of the adamantyllactate was confirmed by the test results. Whereas (*R*)-**17** is inactive, the (*S*)-adamantyl derivative (*S*)-**17** displays a rIC_{50} of 0.21. Preliminary data suggests that the α-substituent of (*S*)-lactate derivatives is solvent exposed upon binding to the protein.[39] Therefore, the slightly lower affinity of (*S*)-**17** compared to (*S*)-**16** might be a result of disfavored solvent interactions of the more hydrophobic adamantyl moiety.

Further selectin antagonists substituted with a wide variety of lactic acid derivatives have been patented by Novartis.[40] For this purpose, a library of antagonists not only aiming at an improved pre-organization, but also at additional enthalpic contribution was designed. Various amines (**18**), amides (**19**) and sulfonamides (**20**) (Fig. 5) were synthesized and tested. Most of the reported compounds antagonized E-selectin better than the lead structure sLe^x. However, none had an IC_{50} comparable to that of (*S*)-**16** (Fig. 4).

Another possibility to pre-organize the carboxylic acid in the bioactive conformation can be achieved by incorporating the pharmacophore in a ring system. However, when the locked conformation differs from the bioactive conformation, a substantial loss of binding affinity is inevitable. An interesting example, which demonstrates the drawback of this approach, was published by Thoma and co-workers.[41] In mimetic **21** (Fig. 6), the carboxylic acid was incorporated in a cyclic acetal leading to a presentation of the pharmacophore outside of the bioactive conformation. As a consequence, a salt bridge with an arginine of the receptor can no longer be established. Since this inter-

action is crucial for binding, a complete loss of activity was observed.

Another approach to lock the carboxylate within a cyclic motif was published by Wong *et al.* The starting point was the disaccharide mimetic **22**[42,43] (Fig. 6), which contains a carboxymethyl group at the 3-position of galactose. By incorporating the carboxylate in a thiazine ring system, (*S*)- and (*R*)-**23** derived from L- and D-cysteine were obtained.[44] Compared to the parent compound **22**, both diastereomers **23** turned out to exhibit a higher, but almost equipotent affinity towards P-selectin. The authors hypothesized that the improved affinity for both diastereomers is not linked to the locked conformation of the carboxylic acid, but rather the result of a new lipophilic contact enabled by the thiazine ring system.

Conclusion

A vital pharmacophore of sLe^x is the carboxylic acid function on NeuNAc, which forms an essential salt bridge in all three sLe^x-selectin complexes.[16] Consequently, a large number of bioisosters of the acid function have been synthesized. This covers sulfates and phosphates but also organic replacements (Fig. 3).[18–32]

The importance of the pre-organization of the carboxylate was demonstrated by detailed computational and experimental studies.[36,37] It could be clearly shown that the pre-organization of the acid leads to a substantial improvement of affinity, whereas locking of the carboxylate outside of the bioactive conformation causes a severe loss of affinity.

The most successful substitution of NeuNAc was found to be lactic acid and derivatives thereof. The degree of pre-organization could be linked to the configuration at the α-position of the lactic acid. In addition, the influence of the size of α-substituents of lactic acid on the acid-orientation (*e.g.* (*S*)-**13**–(*S*)-**17**) was investigated. Finally, the possibility to establish supplemental hydrophilic or lipophilic interactions (*e.g.* **18–20**) with the binding site of the receptor was studied.

Today, after substantial research efforts, small molecule pan-selectin antagonists with affinities in the nanomolar range are still not available. To demonstrate that pre-organization significantly influences the affinity and could therefore lead to a new generation of selectin antagonists, was the aim of this mini-review.

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1.5 Status of low molecular weight compounds in clinical and pre-clinical development

Various low molecular weight selectin antagonists have entered preclinical and clinical trials. In 2003, Schön[169] and Ulbrich[170] summarized the compounds in clinical development. Here, an updated version is given (*figure 13*).

Several molecules entered clinical trials, which however were stopped due to inefficacy. Bristol-Meyers-Squibb was active in the field with BMS-190394, a sulfatide analog (**12**, *figure 13*).[171] Cytel Corp. launched its P-selectin sulfatide sLe^x analogous antagonist CY-1503[172] and went into clinical trials.[173] Nippon Organon was studying OJ-R9188 (**13**, *figure 13*) in 2001, a pan-selectin inhibitor in allergic dermatitis without any further reporting until now.[174] Another selectin-inhibitor, OC-229648 (**14**, *figure 13*) from Ontogen Corporation, was developed as a non-carbohydrate derived selectin antagonist but was not further pursued.[175,176] As mentioned above, Wyeth is now entering preclinical development with its quinolinone inhibitors (**11**, *figure 10*).[160]

Bimosiamose (**15**, TBC1269, *figure 13*), a selectin directed dimeric sLe^x mimicking compound based on mannose as fucose replacement has been developed by Revotar (Berlin, Germany) in asthma and psoriasis. Its current status for both indications is clinical phase II.[177] However, in 2005 Norman could show that bimosiamose does not inhibit rolling of leukocytes *in vivo* and is thus not a functional selectin antagonist.[178]

12, BMS-190394

13, OJ-R9188

14, OC229648

15, bimosiamose, TBC-1269

16, CGP69669A

Figure 13: Selectin antagonizing low molecular weight compounds in clinical and pre-clinical development

In collaboration together with the Institute of Molecular Pharmacy at the University of Basel, GlycoMimetics Inc., Maryland, is continuing to develop the Ciba-Geigy compound CGP69669A[69] (**16**, *figure 13*). Various doctoral theses, including the present one, were dedicated to gain further insight into the blocking of the selectins with molecules derived from sLe^x.[7,132,179-181]

2. Results and Discussion

2.1 Pre-organization of the acid as a result of non-covalent restrictions

The concept of pre-organiztion of the carboxylic acid in sLe^x mimetics of Kolb and Ernst[167,168] was further studied by substitution of Neu*N*Ac with adamantyllactic acids of both absolute configurations (*R*) and (*S*).

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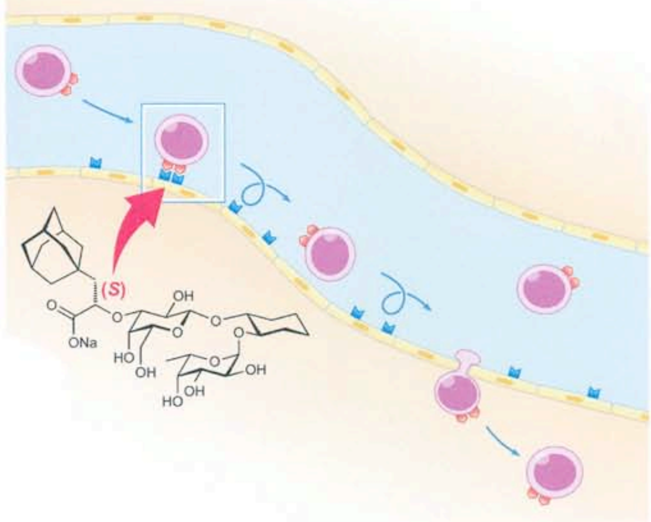

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Is adamantane a suitable substituent to pre-organize the acid orientation in E-selectin antagonists?

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Abstract—The selectins play a key role in the inflammatory process, that is, the recruitment of leukocytes from blood vessels into inflamed tissue. Because excessive infiltration of leukocytes can induce acute or chronic reactions, the control of leukocyte extravasation is of great pharmaceutical interest. All physiological ligands of the selectins contain the tetrasaccharide epitope sialyl Lewis^x, which therefore became the lead structure in selectin antagonist research. Previous studies indicated that an important factor for the affinity of sLe^x is the fact that in solution its pharmacophores are already conformationally pre-organized in the bioactive orientation. In mimics where the Glc*N*Ac- and the Neu*N*Ac-moieties of sLe^x were replaced by (*R*,*R*)-cyclohexane-1,2-diol and (*S*)-cyclohexyllactic acid, respectively, an optimized pre-organization of the pharmacophores could be realized, leading to antagonists with improved affinities. To further optimize the pre-organization of the carboxylic acid, a pharmacophore essential for binding, the replacement of Neu*N*Ac by bulky (*R*)- and (*S*)-adamantyl-lactic acid was studied. Although antagonist (***S***)-**7** showed a slightly reduced affinity, the expected beneficial effect of the (*S*)-configuration at C-2 of the lactate could be confirmed.
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1. Introduction

The selectins play a key role in the body's defense mechanism against inflammation.[1] They form a class of three cell adhesion molecules (E-, P-, and L-selectin), which, in case of an inflammatory stimulus, are responsible for the initial steps of the inflammatory response, that is, the tethering and rolling of leukocytes on endothelial cells. As shown with anti-selectin antibodies[2–4] and E-, P-, and L-selectin k.o. mice,[5,6] these early steps are a prerequisite for the inflammatory cascade to take place. The following steps, that is, firm adhesion and finally extravasation of leukocytes into the adjacent inflamed tissue, do not take place when the initial rolling is prevented. On the other hand, excessive infiltration of leukocytes into the adjacent tissue can lead to acute or chronic reactions, as observed in reperfusion injuries, stroke or rheumatoid arthritis.[7] Therefore, the antagonism of selectins is regarded as a valuable pharmaceutical goal.

Since all physiological ligands of the selectins contain the sialyl Lewis^x motif (sLe^x, **1**, Fig. 1),[8] this tetrasaccharide, although it exhibits only a moderate affinity (IC_{50} = 1 mM), was chosen as lead structure in the search for E-selectin antagonists. The solution[9] and the bioactive[10,11] conformation of sLe^x are known, and its pharmacophores have been identified.[12–15]

We have shown that the pre-organization of the pharmacophores in the bioactive conformation contributes substantially to the affinity of E-selectin antagonists.[16,17] To describe the degree of pre-organization of E-selectin antagonists, two internal dihedral angles have been defined (Fig. 2): (i) the core conformation depicting the relative orientation of L-fucose and D-galactose and (ii) the acid orientation indicating the tilting angle of the carboxylic acid toward the D-Gal(β1–4)[L-Fuc(α1–3)]D-Glc*N*Ac core. By a Monte-Carlo (jumping between

Keywords: E-selectin; Sialyl Lewis^x; Pre-organization; Adamantane.
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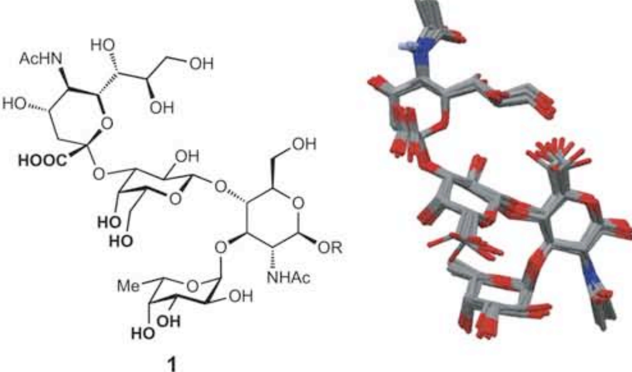


Figure 1. The natural tetrasaccharide epitope sialyl Lewis^x (**1**, sLe^x, essential pharmacophores for binding to E-selectin are highlighted in boldface) and its bioactive conformation as determined by trNOE NMR.[10,11]

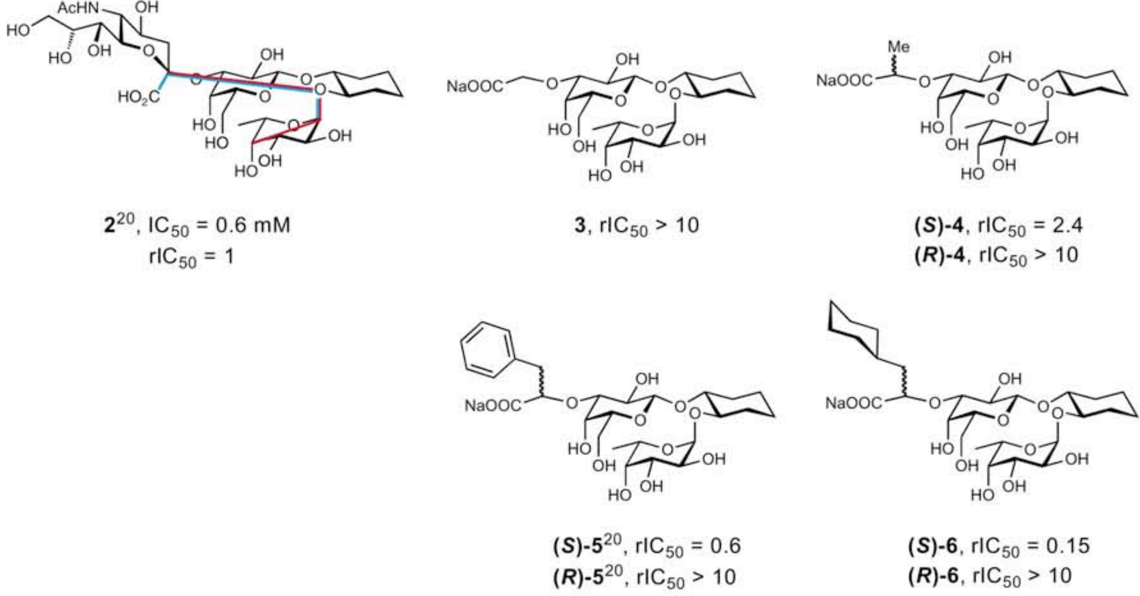


Figure 2. Mimics **3**–**6** of the sLe^x derivative **2** indicate the influence of the acid orientation for affinity.[16] The affinities are given as rel. affinities, that is, relative to mimic **2** which has an IC_{50} of 0.6 mM and rIC_{50} = 1. Core conformation (=relative orientation of L-fucose and D-galactose, indicated in red) and acid orientation (=tilting angle of the carboxylic acid relative to the core, indicated in blue) are shown in **2**.

wells)/stochastic dynamics (MC(JBW)/SD) protocol, affinities of selectin antagonists could be predicted as a function of their pre-organization with respect to core/acid orientation.[16]

From the crystal structure of all three selectins co-crystallized with sLe^x (**1**),[18] it is known that the D-Glc*N*Ac residue solely serves as a linker to orient the D-Gal- and the L-Fuc-moiety in the correct spatial orientation and does not contribute to the binding enthalpy. This is in agreement with the observation that the replacement of the D-Glc*N*Ac moiety by flexible 1,2-diols[16–19] leads to a tremendous loss in affinity as a consequence of the loss in pre-organization. On the other hand, D-Glc*N*Ac replacements by rigid linkers, for example, (*R*, *R*)-cyclohexane-1,2-diol (→**2**,[20] Fig. 2), resulted in equal or even improved affinities.[16,19–21]

Although the importance of the pre-organization of the acid orientation for the affinity of selectin antagonists has been demonstrated,[16] only a few studies to further stabilize the acid orientation in the bioactive conformation have been reported. Since only the carboxylic acid of D-Neu*N*Ac acts as a pharmacophore, the sugar moiety was replaced by non-carbohydrate acids. The first attempt using glycolic acid showed a substantial reduction in affinity due to reduced pre-organization of the carboxylic acid function (**3**, Fig. 2).[16] However, when D-Neu*N*Ac was replaced by (*S*)-lactic acid (→(***S***)-**4**) and lactic acid derivatives (→(***S***)-**5**[20] or (*S*)-**6**), affinity was

regained. The corresponding diastereomers (***R***)-**4** to (***R***)-**6**, however, showed no affinity. A careful analysis of *R*- and *S*-isomers by MC(JBW)/SD simulations clearly indicated that the *S*-isomers are pre-organized in the bioactive conformation, whereas the *R*-isomers are not. For binding to E-selectin, the *R*-isomers have therefore to undergo a conformational change accompanied by substantial entropy costs.[16] In addition to the observed configurational prerequisite, the affinity also depends on the lipophilicity or the bulkiness of the lactic acid substituent, that is, going from glycolate, to lactate, 2-phenyl lactate, and 2-cyclohexyl lactate an increase in affinity could be observed.

From the docking studies[22] of the lowest energy conformation of a Monte-Carlo search of (***S***)-**6** to E-selectin using the *Yeti* program,[23] it becomes evident that the cyclohexyl group is not establishing an additional lipophilic contact with the binding site of the lectin. Therefore, it was assumed that the increase in activity originates predominantly from an increased degree of pre-organization. To further verify the influence of the lipophilicity/bulkiness of the lactic acid substituent, the corresponding adamantyl derivatives (***R***)-**7** and (***S***)-**7** were analyzed by molecular modeling, synthesized, and biologically evaluated.

2. Results and discussion

The analysis of the two diastereomeric adamantyl derivatives (***R***)-**7** and (***S***)-**7** (Fig. 3) was conducted according to the MC(JBW)/SD protocol.[16] The *S*-isomer shows a high degree of pre-organization in the area of the bioactive window (indicated in the core conformation/acid orientation plot by a red square, Fig. 3b). The *R*-isomer of **7**, however, shows a conformational focus point outside the bioactive area (Fig. 3a). Since (***S***)-**7** shows a higher degree of pre-organization than (***S***)-**6**, the most active compound in Figure 2, an improved affinity was expected.

The retrosynthetic analysis for the two diasteromeric adamantyl derivatives (***R***)/(***S***)-**7** (Scheme 1) generated the adamantyl-lactic acid derivatives (***R***)/(***S***)-**8** and the known building blocks **9**[24] and **10**.[19]

Racemic adamantyl-lactic acid (**rac-12**, Scheme 2) was obtained by a Giese-type radical addition of the adamantyl radical generated from 1-adamantyl bromide to the acrylate **11**, followed by hydrolysis of the ethyl ester.[25] Subsequent formation of the benzyl ester (→**rac-13**) followed by preparative chiral resolution on a Chiralpak AD-H column yielded the two enantiomers (***S***)-**13** (ee 99.5%) and (***R***)-**13** (ee 99.6%). The chiral resolution with the corresponding methyl and *p*-nitrobenzyl derivatives was less efficient, because the differences in retention time for the two enantiomers were smaller than in case of the benzyl ester **rac-13**. By transforming the enantiomers (***S***)-**13** and (***R***)-**13** into the corresponding triflates (***S***)-**14** and (***R***)-**14**, the electrophiles needed for the alkylation of the 3-position of the galactose moiety were obtained.

For the assignment of the absolute configuration of the adamantyl building blocks, racemic adamantyl-lactic acid (**rac-12**) was treated with the chiral auxiliary (*S*)-α methylbenzylamine (**15**)[26] to yield the two corresponding diastereomeric amides **16** and **17** (Scheme 3). Since the two diastereomers exhibit significantly different R_f values, they could be separated by standard column chromatography on silica gel. Because diastereomer **16**

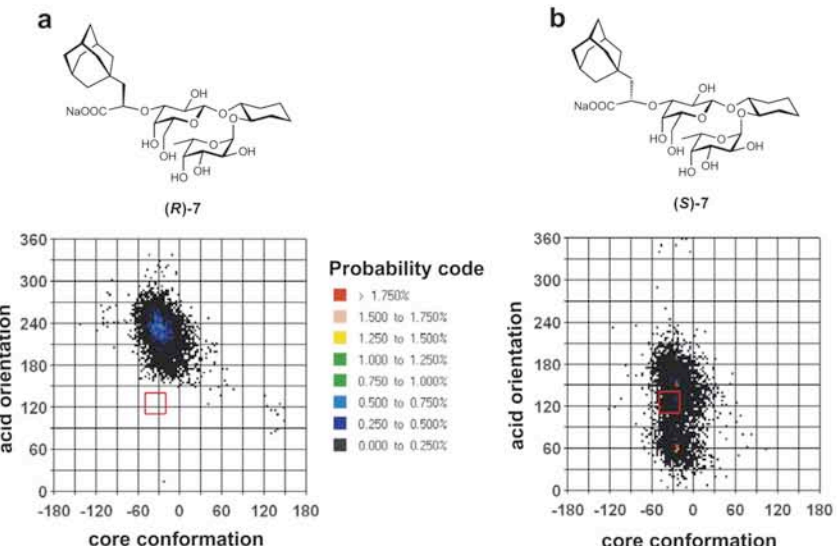


Figure 3. Core conformation/acid orientation plot of both adamantyl bearing E-selectin antagonist (***R***)-**7** and (***S***)-**7**. The bioactive window is indicated with a red square.

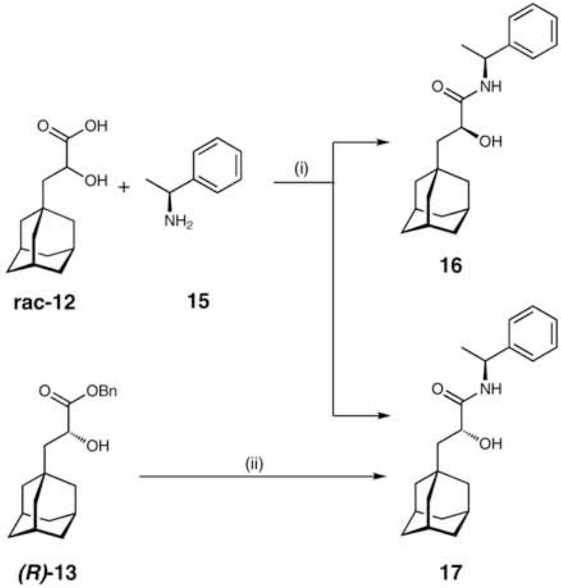
Scheme 1. Retrosynthetic analysis for the target compounds.

Scheme 2. Reagents and conditions: synthesis of the adamantyl-lactic acids. (i) TMSCl, TEA, Et_2O, 0 °C–rt, 3.5 h (85%); (ii) Bu_3SnH, AIBN, 1-adamantyl bromide, PhMe, reflux, 14 h; (iii) KF, H_2O, rt, overnight; (iv) NaOH, H_2O, 50 °C, 12 h; HCl (54% for three steps); (v) Cs_2CO_3, MeOH, H_2O; (vi) BnBr, DMF, rt, 20 h (73% for two steps); (vii) chiral resolution, HPLC Chiralpak AD-H ((*S*)-**13**: ee 99.5%; (*R*)-**13**: ee 99.6%); (viii) Tf_2O, DCM, 2,6-^{t}Bu$_2$–py, 0–20 °C, 3 h ((*S*)-**14**: 96%; (*R*)-**14** 79%).

could be crystallized, the determination of its absolute configuration by X-ray crystallography was possible (Table 1 and Fig. 4). It is noteworthy that the asymmetric unit contains three molecules of which two have a stretched and one a collapsed conformation (Fig. 4). Because (***R***)-**13** could be transformed into **17**, the allocation of the absolute configuration of the adamantyl derivatives (***S***)-**13** and (***R***)-**13** was achieved.

The synthesis of the selectin antagonists **7** started from (*R, R*)-cyclohexane-1,2-diol (**19**) which was α-fucosylated with the thiofucoside donor **18**[17] according to the in situ anomerization procedure developed by Lemieux[27] (Scheme 4). In a second glycosylation step, **10** was galactosylated with thiogalactoside donor **9** using DMTST[28] as promotor. Finally, removal of the benzoate protective groups by transesterification yielded the core molecule **21**.[21]

For the desired antagonist (***S***)-**7**, the regioselective alkylation of the 3-position of the galactose moiety was achieved using the alkylating agent (***R***)-**14** and the mild tin-acetal coupling procedure.[29] Due to the increased steric bulk of the adamantyl substituent, the yield (21%) was rather low. In addition, a significant amount of a side product, benzyl (*S*)-3-(1-adamantyl)-2-fluoro-propionate (**23**, see experimental part for details), was isolated. The formation of **23** is a consequence of the bulkiness of the adamantyl substituent, allowing the stannophilic fluoride ion, which is used to open the stannylene acetal regioselectively, to act also as nucleophile. The sodium salt of the acid (***S***)-**7** was obtained after hydrogenolysis and ion exchange chromatography. Finally, possible traces of the polyanionic ion exchange resin, which would lead to false positive results,[30] were removed by size exclusion chromatography.



Scheme 3. Reagents and conditions: determination of the absolute configuration of the adamantyl derivatives (*S*)-**13** and (*R*)-**13**: (i) dioxane, μW, 180 °C, 15 min (85%); (ii) **15**, dioxane, 80 °C, 48 h (quant.).

Table 1. Crystallographic data of amide **16**

Compound	**16**
CCDC number	626224
Empirical formula	$C_{21}H_{29}NO_2$
Formula weight	327.45
Temperature (K)	100
Wavelength (Å)	1.54184
Crystal system	Orthorhombic
Space group	$P2_12_12_1$
Cell dimensions	
a (Å)	10.386(2)
b (Å)	18.795(4)
c (Å)	28.311(6)
α (°)	90
β (°)	90
γ (°)	90
Volume (Å^3)	5526.1(19)
Z	12
Density calculated (kg/dm^3)	1.181
F_{000}	2136
θ range for data collection (°)	3.12–64.44
Reflections collected	31059
Independent reflections	9154
Data/restraints/parameters	9154/0/667
Goodness of fit on F^2	0.810
Final R indices [$I > 2\sigma(I)$]	$R1 = 0.0323$, $wR2 = 0.0564$
R indices (all data)	$R1 = 0.0512$, $wR2 = 0.0611$
Largest diff. peak and hole (eÅ^3)	0.136 and −0.150

In the synthesis of the second diastereomer (*R*)-**7**, the alkylation of the 3-position of galactose with (*S*)-**14** was accompanied by the formation of lactone **24**. In this case, the lactone is particularly easily formed, because the bulky adamantyl-methyl substituent adopts an equatorial position in the δ-lactone ring (vs an axial position in case of (*R*)-**14**). After hydrogenolysis, the crude product was treated with aqueous sodium hydroxide to open the δ-lactone. Diastereomer (*R*)-**7** was finally obtained after size exclusion chromatography.

The results obtained in a competitive binding assay[31] confirmed our prediction that the *S*-configurated lactic acid substituent leads to active antagonists, whereas the *R*-configuration produces inactive compounds. However, contrary to the predicted properties, (*S*)-**7** (rIC_{50} = 0.32) is slightly less active than (*S*)-**6** (rIC_{50} = 0.15).

3. Conclusion

Docking studies using the crystal structure of E-selectin and a bioactive conformation of (*S*)-**7**, which was extrapolated from the one of **1**,[11] clearly demonstrated that no interaction of the adamantyl substituent with the protein occurs. Thus, the bulky adamantyl substituent solely contributes to the stabilization of the acid orientation. Since the predicted acid orientation in (*S*)-**7** (Fig. 3) is superior to the one in (*S*)-**6**,[16] lower entropy costs upon binding, and therefore an improved affinity to E-selectin was expected. One possible explanation for the discrepancy between our prediction based on molecular modeling and the test results may be related to the lipophilicity of the adamantyl substituent, which could lead to aggregate formation or unfavorable solvation properties.

4. Experimental

4.1. General methods

Analyses of the conformational preferences of the E-selectin antagonists were performed in aqueous solution by applying the systematic pseudo-Monte-Carlo (MC) (SUMM, systematic multiple minimum search) simulation technique[32] and the 'Jumping Between Wells/Stochastic Dynamics' ((JBW)/SD) protocol[16] implemented in MacroModel 5.0.[33] First, the locations of the most relevant energy minima (conformations) of a compound were determined in an internal coordinate systematic pseudo-Monte-Carlo SUMM simulation.[32] 2000 steps were performed for each free-rotatable bond excluding terminal CH_3 groups. All structures within 20 kJ/mol from the energy of the global minimum were retained. The shape of the potential energy surface was then probed in a subsequent JBW/SD simulation, which used the information obtained in the SUMM analysis. Thus, a Boltzmann-weighted ensemble of states was generated in a 10 ns MC(JBW)/SD simulation by jumping between different energy wells, that is, the energetically best 100 conformations found in the preceeding SUMM analysis, and performing stochastic dynamics simulations within each well. All calculations were performed using the AMBER[34] force field augmented by parameters for carbohydrates[16,35] and in conjunction with the GB/SA continuum water model for implicit solvation.[36] This led to a realistic sampling of the conformational space of the structure of interest.

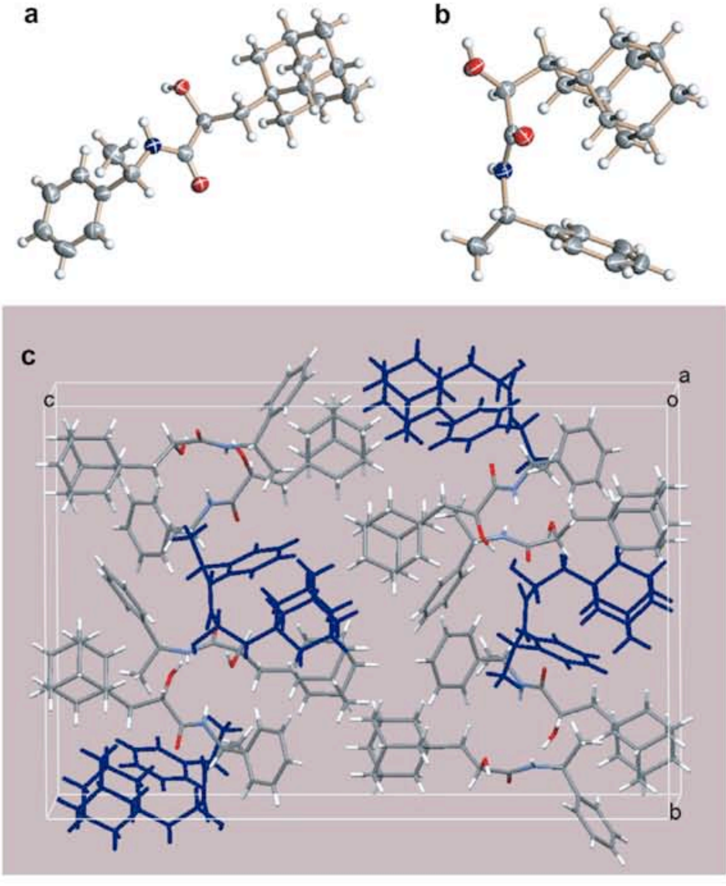


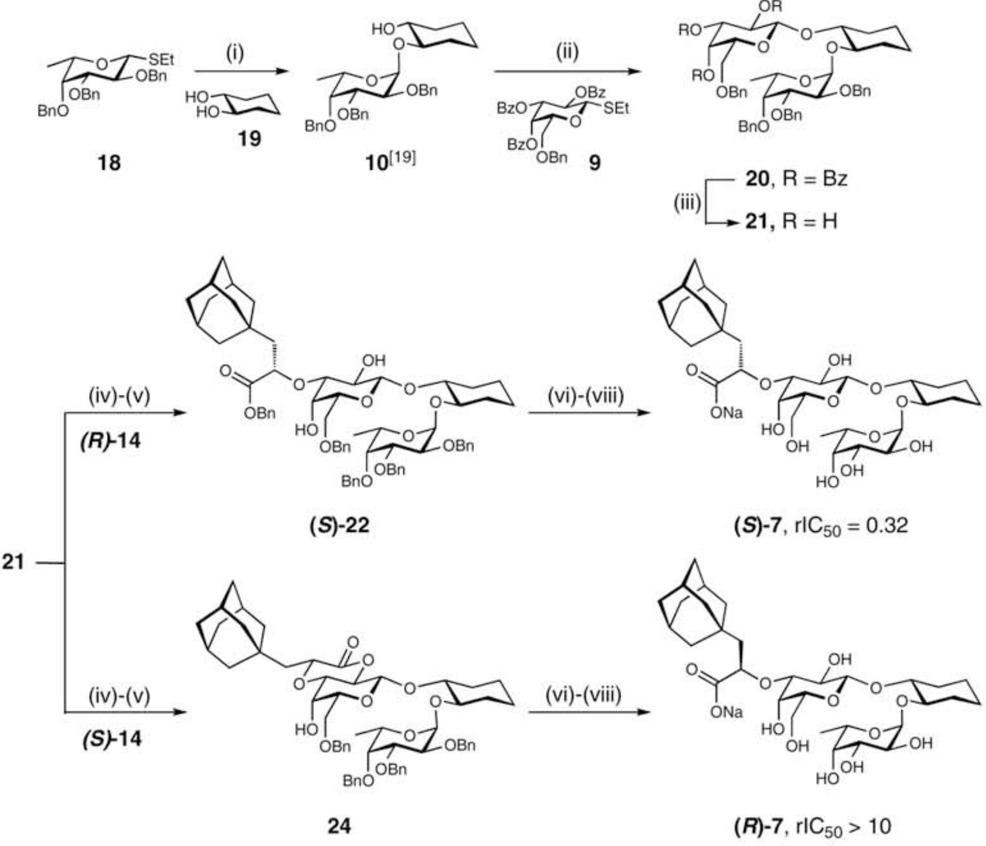
Figure 4. X-ray crystal structure of **16**: The asymmetric unit (c) contains stretched molecules (a) and conformationally collapsed conformers (b).

Nuclear magnetic resonance spectroscopy was performed on a Bruker Avance 500 UltraShield spectrometer at 500.13 MHz (^{1}H) or 125.76 MHz (^{13}C). Chemical shifts are given in ppm and were calibrated on residual solvent peaks[37] or to tetramethyl silane as internal standard. Multiplicities were specified as s (singlet), d (doublet), dd (doublet of a doublet), t (triplet), q (quartet), dq (doublet of a quartet), quint. (quintublet) or m (multiplet). Interpretation of the spectra was performed according to first order[38] and higher order where possible.

The signals were assigned with the help of DEPT-135, ^{1}H,^{1}H-COSY/TOCSY and ^{1}H,^{13}C-HSQC/HMBC experiments. Assignments are indicated according to IUPAC nomenclature. For complex molecules, the following prefixes for substructures are used: Ad (adamantyl), Cy (cyclohexyl), Fuc (fucose), Gal (galactose), and Lac (lactate). C^{i} indicates the *ipso* substituted carbons of aromatic systems.

Optical rotations were measured on a Perkin Elmer 341 polarimeter in the indicated solvents in p.a. quality. Microanalyses were performed at the Department of Chemistry, University of Basel, Switzerland. ESI mass spectra were recorded on a Waters micromass ZQ instrument. High resolution mass spectra were obtained on an ESI Bruker Daltonics micrOTOF spectrometer equipped with a TOF hexapole detector.

TLC was performed using silica gel 60 coated glass plates containing fluorescence indicator (Merck KGaA, Darmstadt, Germany) using either UV light (254 nm) and by charring in aqueous $KMnO_4$ solution or in a molybdate solution (a 0.02 M solution of ammonium cerium sulfate dihydrate and ammonium molybdate tetrahydrate in aqueous 10% H_2SO_4) with heating to 140 °C for 5 min. Column chromatography was performed using silica gel 60 (0.040–0.063 mm) from Fluka. Microwave reactions were performed in a CEM Discover microwave apparatus. Hydrogenation reactions were performed in a shaking apparatus (Parr Instruments Company, Moline, Illinois, USA) in 250 mL or 500 mL bottles with 4 bar H_2 pressure. Solvents were purchased from Fluka and dried prior to use. DCM was dried by filtration through basic aluminum oxide (Fluka). Dioxane, DME, Et_2O, and PhMe were dried by distillation from sodium/benzophenone. DMF was dried by distillation from calcium hydride and MeOH by distillation from sodium methoxide.



Scheme 4. Reagents and conditions: synthesis of selectin antagonists containing adamantyl-lactic acid. (i) Br_2, Bu_4NBr, DCM, DMF, mol. sieves 4 Å, −20 °C–rt, 12 h (58%); (ii) DMTST, DCM, mol. sieves 4 Å, rt, 5 days; (iii) NaOMe, MeOH, rt, overnight (85% for two steps); (iv) Bu_2SnO, MeOH, mol. sieves 3 Å, reflux, 18 h; (v) CsF, DME, rt, 3 d (**22**: 21%; **24**: 25%); (vi) H_2, $Pd(OH)_2/C$, dioxane–H_2O, 4 bar, rt, 4 d; (vii) Na^+–ion exchange; (viii) NaOH, H_2O; (ix) Sephadex-G15 ((*S*)-**7**: 92%; (*R*)-**7**: 31%).

rac-13 was separated using a Gilson preparative HPLC apparatus equipped with a 5 × 50 cm Chiralpak AD column and a UV detector (210 nm). For elution, an isocratic solvent system ethanol/hexanes 5/95 was used (50 mL/min). Analytical chiral HPLC was performed on a Chiralpak AD-H column (250 × 4.6 mm, 1 mL/min, 210 nm). Enantiomeric excess was determined from the peak areas after integration of the analytical chromatograms.

The X-ray crystal structure of **16** was solved at Hoffmann-La Roche, Pharmaceutical Division, Pharma Research 65/308, Basel, Switzerland. The diffraction pattern was measured on a Gemini Ruby diffractometer from Oxford Diffraction, Abingdon, UK, using Cu–K_α-radiation with a wavelength of 1.54184 Å. Structure solution and refinement was performed using the ShelX software[39,40] (G. Sheldrick, Göttingen, Germany).

Crystallographic data (excluding structure factors) for the structure in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary Publication No. CCDC 626224. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK, (fax: +44 (0)1223 336033 or e-mail: deposit@ccdc.cam.ac.uk).

Biological data were obtained using the published ELISA procedure with (*S*)-**6** as reference compound.[31]

4.2. Ethyl 2-trimethylsilyloxy-acrylate (11)

To a solution of ethyl pyruvate (4.78 mL, 43.1 mmol) and chlorotrimethylsilane (6.33 mL, 49.5 mmol) in anhydrous diethylether (50 mL) was added triethylamine (7.19 mL, 51.7 mmol) dropwise over a period of 10 min at 0 °C. After stirring for 1 h at 0 °C, the solution was allowed to warm to rt and stirred for additional 3 h. The reaction mixture was diluted with petrol ether (250 mL), cooled to 0 °C, and washed with cold brine (3 × 50 mL). Drying over Na_2SO_4 and evaporation of the solvent in vacuo gave the volatile crude product **11** as clear yellow oil (8.46 g), which was used in the next step without further purification. ^{1}H NMR ($CDCl_3$): δ 5.45, 4.82 (A, B of AB, $^2J = 1.0$ Hz, 2H, H3), 4.16 (q, $^3J = 7.14$ Hz, 2H, CH_3CH_2–), 1.25 (t, $^3J = 7.15$ Hz, 3H, CH_3CH_2–), 0.17 (s, 9H, $(CH_3)_3Si$–); ^{13}C NMR

($CDCl_3$): δ 164.4 (C1), 147.2 (C2), 103.9 (C3), 61.2 (–CH_2CH_3), 14.1 (–CH_2CH_3), −0.1 ($(CH_3)_3Si$–).

4.3. rac-3-(1-Adamantyl)-lactic acid (rac-12)

To a solution of 1-bromo-adamantane (7.90 g, 36.7 mmol), **11** (13.8 g, 73.4 mmol), and AIBN (1.50 g, 9.18 mmol) in toluene (100 mL), tributyltinhydride (11.7 mL, 44.0 mmol) was added. The reaction mixture was refluxed for 25 h. After cooling to rt, a solution of KF (5.11 g, 88.0 mmol) in water (150 mL) was added and the heterogeneous mixture stirred vigorously overnight. Ethyl acetate (1.5 L) was added, the phases were separated, and the organic layer was washed with water (300 mL) and brine (300 mL). After drying over Na_2SO_4, the solvent was removed in vacuo to give the crude ethyl ester. The residue was dissolved in a mixture of aqueous 1 N NaOH (250 mL) and EtOH (100 mL). After stirring overnight at 50 °C, the ethanol was removed in vacuo and the aqueous phase was diluted with water (1 L), washed with DCM (2 × 350 mL), acidified to pH 0 with aqueous 6 N HCl, and then extracted with DCM (3 × 400 mL). Washing of the combined organic layers with brine (200 mL), drying over Na_2SO_4, and evaporation of the solvent gave **rac-12** as a spectroscopically pure, off-white solid (4.43 g, 54%) which was used without further purification. ^1H NMR ($CDCl_3$): δ 4.40 (d, $^3J = 9.4$ Hz, 1H, H2), 1.98 (br s, 3H, H6), 1.77–1.58 (m, 13H, Ad-CH_2, H3b), 1.41 (A of ABM, $^3J = 9.5$, $^2J = 14.5$ Hz, 1H, H3a); ^{13}C NMR ($CDCl_3$): δ 181.0 (C1), 67.5 (C2), 48.9 (C3), 42.6 (C5), 36.9 (C7), 32.5 (C4), 28.6 (C6); ESI-MS Calcd for $C_{13}H_{21}O_3$ $[M+H]^+$: 225.1; Found: 225.0.

4.4. rac-Benzyl 3-(1-adamantyl)-lactate (rac-13)

To a suspension of **rac-12** (679 mg, 3.03 mmol) in MeOH/water (3:1) was added Cs_2CO_3 (591 mg, 1.82 mmol) under stirring to give a clear solution. The solvents were removed in vacuo. After drying the residue in high vacuum overnight, it was resuspended in anhydrous DMF (2.5 mL). Then benzyl bromide (2.15 mL, 18.2 mmol) was added and the suspension was stirred for 20 h at rt. Removal of the solvent and purification of the crude product by column chromatography on silica (PE/EE 100:0 > 85:15) gave pure **rac-13** as a colorless oil (690 mg, 73%). R_f (PE/EE 4/1) 0.67; ^1H NMR ($CDCl_3$): δ 7.40–7.35 (m, 5H, Ar–H), 5.22, 5.18 (A, B of AB, $^2J = 12.3$ Hz, 2H, PhCH_2), 4.36–4.33 (m, 1H, H2), 1.96 (br s, 3H, H6), 1.71–1.56 (m, 13H, H3b, 12× Ad-CH_2), 1.37 (A of ABM, $^3J = 9.3$, $^2J = 14.0$ Hz, 1H, H3a); ^{13}C NMR ($CDCl_3$): δ 176.2 (C1), 135.3 (Ar–C^i), 128.6 (Ar–C), 128.5 (Ar–C), 128.2 (Ar–C), 67.8 (C2), 67.3 (PhCH_2), 49.0 (C3), 42.7–42.6 (3C, C5), 37.0–36.9 (3C, C7), 32.4 (C4), 28.7–28.5 (3C, C6); ESI-MS calcd for $C_{20}H_{26}NaO_3$ $[M+Na]^+$: 337.1; Found: 337.1; Anal. calcd. for $C_{20}H_{26}O_3$ + 1/4 H_2O: C 75.32, H 8.38; Found: C 75.32, H 8.26.

With preparative HPLC on a 5 × 50 cm Chiralpak AD column the two enantiomers (***R***)-**13** and (***S***)-**13** were separated: **Benzyl (*R*)-3-(1-adamantyl)-lactate ((*R*)-13)**. Analytical chiral HPLC: $t_R = 10.57$ min, optical purity: ee > 99.6%, $[\alpha]_D^{20}$ +3.1 (*c* 2.35, $CHCl_3$); **Benzyl (*S*)-3-(1-adamantyl)-lactate ((*S*)-13)**. Analytical chiral HPLC: $t_R = 12.01$ min, optical purity: ee > 99.5%, $[\alpha]_D^{20} = -4.2$ (*c* 1.67, $CHCl_3$).

4.5. Benzyl (*R*)-3-(1-adamantyl)-2-(trifluoromethyl)sulfonyloxy-propionate ((*R*)-14)

Ester (***R***)-**13** (162 mg, 0.52 mmol) was dissolved in dry DCM (3 mL) under argon and cooled to −20 °C. 2,6-Di-*tert*-butyl pyridine (197 μL, 0.88 mmol) was added, followed by dropwise addition of triflic anhydride (147 μL, 0.88 mmol). After 2 h at −20 °C, the mixture was diluted with DCM (30 mL) and washed with aqueous 1 M KH_2PO_4 solution (25 mL). The aqueous phase was then extracted with DCM (3 × 40 mL) and the combined organic layers were dried over Na_2SO_4. Evaporation of the solvent in vacuo gave the crude product as a colorless oil. Purification by column chromatography on silica (PE/EE 20/1) gave pure (***R***)-**14** as a colorless oil (186 mg, 78%), which was used immediately for the next reaction. R_f (PE/EE 20/1) 0.40; ^1H NMR ($CDCl_3$): δ 7.38–7.35 (m, 5H, Ar–H), 5.27–5.21 (m, 3H, PhCH_2, H2), 1.96 (br s, 3H, H6), 1.83 (A of ABM, $^2J = 15.4$, $^3J = 7.7$ Hz, 1H, H3a), 1.74–1.53 (m, 13H, H3b, 12× Ad-CH_2); ^{13}C NMR ($CDCl_3$): δ 168.1 (C1), 134.3 (Ar–C^i), 128.8, 128.7, 128.6 (3Ar–C), 81.0 (C2), 68.2 (PhCH_2), 45.8 (C3), 42.0 (3C, C5), 36.5 (3C, C7), 32.3 (C4), 28.3 (3C, C6).

4.6. (*S*)-Benzyl 3-(1-adamantyl)-2-(trifluoromethyl)sulfonyloxy-propionate ((*S*)-14)

Two hundred and sixty milligrams (96%) of the title compound was obtained using the same procedure as described for (***R***)-**14**. R_f and $^1H/^{13}C$ NMR see (***R***)-**14**.

4.7. (*S*)-1-Phenylethyl (*S*)-3-(1-adamantyl)-lactamide (16) and (*S*)-1-phenylethyl (*R*)-3-(1-adamantyl)-lactamide (17)

A microwave vial was charged with a magnetic stirring bar, **rac-12** (224 mg, 1.00 mmol), and (*S*)-α-methylbenzylamine (**15**) (140 μL, 1.10 mmol). Dioxane (2 mL) was added and the vial was sealed under argon. The mixture was heated under microwave irradiation to 180 °C for 70 min. After cooling to rt, the solvent was evaporated, the residue was taken up in ethyl acetate (100 mL) and washed with saturated aqueous K_2CO_3 (2 × 10 mL) and aqueous 0.5 N HCl (2 × 20 mL). The organic layer was dried over Na_2SO_4. Evaporation of the solvent gave the crude mixture of **16** and **17** as a light yellow oil (279 mg, 85%). The diastereomers could be separated by column chromatography on silica (PE/EE 3/1). ESI-MS Calcd for $C_{21}H_{29}NNaO_2$ $[M+Na]^+$: 350.2; Found: 350.2.

(*S*)-1-phenylethyl (*S*)-3-(1-adamantyl)-lactamide (16). R_f (PE/EE 4/1) 0.55; $[\alpha]_D^{20}$ −59.7 (*c* 0.53, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.32–7.23 (m, 5H, Ar–H), 6.962 (d, $^3J = 7.15$ Hz, 1H, N*H*), 5.069 (m, 1H, MeC*H*–), 4.188 (M of ABM, $^3J = 9.32$ Hz, 1H, Lac-H2), 3.050 (br s, 1H, O*H*), 1.95 (br s, 3H, Ad-H3), 1.700 (m, 4H, 3× Ad-CH_2, Lac-H3b), 1.623 (A′ of A′B′, $^2J = 11.83$ Hz,

3H, 3× Ad-CH$_2$), 1.576 (s, 6H, Ad-CH$_2$), 1.464 (d, ^3J = 6.93 Hz, 3H, –C*H*$_3$), 1.332 (A of ABM, ^2J = 14.57, ^3J = 9.45 Hz, 1H, Lac-H3a); ^{13}C NMR (CDCl$_3$): δ 174.15 (C1), 143.09 (Ar–C^i), 128.57 (2Ar–C), 127.25 (*p*Ar–C), 126.08 (2 Ar–C), 69.27 (C2), 49.34 (C3), 48.40 (Ph*C*H–), 42.72 (3C, Ad-CH$_2$), 36.88 (3C, Ad-CH$_2$), 32.29 (Ad-C1), 28.57 (3C, Ad-C3), 21.83 (–*C*H$_3$).

(*S*)-1-phenylethyl (*R*)-3-(1-adamantyl)-lactamide (17). R_f (PE/EE 4/1) 0.45; $[\alpha]_D^{20}$ −49.2 (*c* 0.035, CHCl$_3$); ^{1}H NMR (CDCl$_3$): δ 7.35–7.24 (m, 5H, Ar–H), 6.881 (br s, 1H, N*H*), 5.097 (m, 1H, MeC*H*–), 4.241 (M of ABM, ^3J = 9.50 Hz, 1H, Lac-H2), 1.96 (br s, 3H, Ad-H3), 1.713–1.665 (m, 4H, 3× Ad-CH$_2$, Lac-H3a), 1.624 (A′ of A′B′, ^2J = 11.64 Hz, 3H, Ad-CH$_2$), 1.574 (s, 6H, Ad-CH$_2$), 1.492 (d, ^3J = 6.92 Hz, 3H, –C*H*$_3$), 1.297 (B of ABM, ^2J = 14.56, ^3J = 9.68 Hz, 1H, Lac-H3b); ^{13}C NMR (CDCl$_3$): δ 174.11 (C1), 142.97 (Ar–C^i), 128.62 (2Ar–C), 127.29 (*p*Ar–C), 126.03 (2Ar–C), 69.40 (C2), 49.41 (C3), 48.38 (Ph*C*H–), 42.73 (3C, Ad-CH$_2$), 36.87 (3C, Ad-CH$_2$), 32.32 (Ad-C1), 28.57 (3C, Ad-C3), 21.86 (–*C*H$_3$).

4.8. (*1R,2R*)-2-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-cyclohexyl 2,3,4-tri-*O*-benzoyl-6-*O*-benzyl-β-D-galactopyranoside (20)

Fucoside **10** (4.54 g, 8.52 mmol) and galactose donor **9** (5.88 g, 9.37 mmol) were dissolved in dry DCM (140 mL). Powdered activated molecular sieves (4 Å, 15.0 g) were added and the mixture was stirred for 3.5 h under argon at rt. DMTST (25.6 mmol, 6.60 g) was dissolved in dry DCM (40 mL) and powdered activated molecular sieves (4 Å, 3.75 g) were added and this suspension was stirred for 2 h under argon at rt as well. Then the two suspensions were combined and stirred for 5 days at rt under argon. The mixture was filtered over Celite. After washing of the organic layer with 8% aqueous NaHCO$_3$ (30 mL), the aqueous layer was extracted with DCM (2× 40 mL). The combined organic layers were dried over Na$_2$SO$_4$ and the solvent was removed in vacuo. The crude product **20** was obtained as white foam (9.95 g). It was used without further purification. A small sample was purified by column chromatography on silica (PE/EE 5/1) for analyses. R_f (PE/EE 4/1) 0.11; ^{1}H NMR (CDCl$_3$): δ 7.91 (m, 2H, Bz-H2/5), 7.86 (m, 2H, Bz′-H2/5), 7.71 (m, 2H, Bz″-H2/5), 7.48–7.02 (m, 29 H, Ar–H), 5.86 (br s, 1H, Gal-H4), 5.60 (dd, ^3J = 8.1, ^3J = 10.2 Hz, 1H, Gal-H2), 5.43 (dd, ^3J = 3.2, ^3J = 10.4 Hz, 1H, Gal-H3), 4.89 (A of AB, ^2J = 11.5 Hz, 1H, PhCH$_2$), 4.86 (br s, 1H, Fuc-H1), 4.77 (d, ^3J = 7.9 Hz, 1H, Gal-H1), 4.73 (A′ of A′B′, ^2J = 11.7 Hz, 1H, PhCH$_2$), 4.68 (A″ of A″B″, ^2J = 11.0 Hz, 1H, PhCH$_2$), 4.59 (B″ of A″B″, ^2J = 12.0 Hz, 1H, PhCH$_2$), 4.54 (B of AB, B′ of A′B′, ^2J = 11.6 Hz, 2H, PhCH$_2$), 4.89 (q, ^3J = 6.1 Hz, 1H, Fuc-H5), 4.38, 4.25 (A‴,B‴ of A‴B‴, ^2J = 11.9, 2H, PhCH$_2$), 3.99–3.94 (m, 2H, Fuc-H2, Gal-H5), 3.70 (m, 1H, Cy-CH), 3.64 (s, 1H, Fuc-H4), 3.59–3.48 (m, 4H, Fuc-H3, Gal-H6, Cy-CH), 1.86 (br s, 2H, Cy-CH$_2$), 1.55–1.02 (m, 6H, Cy-CH$_2$), 1.22 (d, ^3J = 6.8 Hz, 3H, Fuc-H6); ^{13}C NMR (CDCl$_3$): δ 165.5, 165.4, 165.0 (3C, Ph-*C*O$_2$–), 139.03, 139.01, 138.7, 137.4 (4C, Bn-C^i), 133.4, 133.1 (3C, Bz-C^i), 129.7–127.1 (Ar–C), 99.5 (Gal-C1), 94.1 (Fuc-C1), 79.8 (CH), 79.1, 79.0, 76.4, 74.9 (CH), 74.8 (CH$_2$), 73.6 (CH$_2$), 73.0 (CH$_2$), 72.5 (CH), 72.0 (CH), 70.0 (Gal-C2), 68.5 (Gal-C4), 67.7 (Gal-C6), 66.3 (Fuc-C5), 29.5 (Cy-CH$_2$), 28.5 Cy-(CH$_2$), 22.94 (Cy-CH$_2$), 22.87 (Cy-CH$_2$), 16.7 (Fuc-C6); ESI-MS Calcd for $C_{67}H_{68}NaO_{14}$ [M+Na]$^+$: 1119.5; Found: 1119.6.

4.9. (*1R,2R*)-2-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-cyclohexyl 6-*O*-benzyl-β-D-galactopyranoside (21)

The protected pseudotrisaccharide **20** (9.70 g crude material) was dissolved in dry MeOH (100 mL). When 3 M NaOMe in MeOH (3.5 mL) was added to the solution, a white precipitate appeared which dissolved after a few minutes. The solution was stirred at rt for 16 h and then neutralized with Dowex 50 × 8 ion exchange resin. The mixture was filtered and the solvent evaporated to give the crude product as a colorless oil. Purification by column chromatography on silica (DCM/MeOH, gradient 25/1 to 10/1) yielded pure **21** as a white foam (5.53 g, 85% over two steps from **10**). R_f (DCM/MeOH 8/1) = 0.50; ^{1}H NMR (CDCl$_3$): δ 7.38–7.24 (m, 20 H, Ar–H), 4.97 (d, ^3J = 3.6 Hz, 1H, Fuc-H1), 4.94 (A of AB, ^2J = 11.6 Hz, 1H, PhCH$_2$), 4.81 (A′ of A′B′, ^2J = 11.6 Hz, 1H, PhCH$_2$), 4.75 (A″ of A″B″, ^2J = 11.8 Hz, 1H, PhCH$_2$), 4.68 (B′ of A′B′, ^2J = 11.5 Hz, 1H, PhCH$_2$), 4.67 (B″ of A″B″, ^2J = 11.8 Hz, 1H, PhCH$_2$), 4.59 (B of AB, ^2J = 11.6 Hz, 1H, PhCH$_2$), 4.52 (s, 2H, PhCH$_2$), 4.33 (q, ^3J = 6.3 Hz, 1H, Fuc-H5), 4.32 (d, ^3J = 7.4 Hz, 1H, Gal-H1), 4.02 (dd, ^3J = 3.6, ^3J = 9.4 Hz, 1H, Fuc-H2), 3.97 (s, 1H, Gal-H5), 3.96–3.95 (m, 1H, Fuc-H3), 3.79–3.73 (m, 2H, 1× Cy-CH), 3.69 (dd, 1H, *J* = 5.3, *J* = 9.8 Hz), 3.63 (br s, 1H, Fuc-H4), 3.60–3.53 (m, 4H, 1× Cy-CH, Gal-H2), 2.72 (br s, 3H, OH), 2.07–1.98 (m, 2H, Cy-CH$_2$), 1.70–1.65 (m, 2H, Cy-CH$_2$), 1.42–1.18 (m, 4H, Cy-CH$_2$), 1.09 (d, ^3J = 6.4 Hz, 3H, Fuc-H6); ^{13}C NMR (CDCl$_3$): δ 139.1, 138.8, 138.6, 137.8 (4C, 4 Bn-C^i), 128.5–127.3 (Ar–C), 100.3 (Gal-C1), 94.5 (Fuc-C1), 79.5 (CH), 78.0 (CH), 77.1 (CH), 76.34 (CH), 76.29 (CH), 74.8 (Ph*C*H$_2$), 73.6 (Ph*C*H$_2$), 73.4 (CH), 73.2 (CH), 73.1 (Ph*C*H$_2$), 73.0 (Ph*C*H$_2$), 71.1 (CH), 69.2 (Gal-C6), 68.6 (CH), 66.3 (CH), 30.3, 29.2, 23.3 (4C, 4 Cy-CH$_2$), 16.6 (Fuc-C6); Anal. calcd. for $C_{46}H_{56}O_{11}$ + 2/3 H_2O: C 69.33, H 7.25; Found: C 69.39, H 7.22; ESI-MS Calcd for $C_{46}H_{56}NaO_{11}$ [M+Na]$^+$: 807.4; Found: 807.4.

4.10. Benzyl (*2S*)-3-(1-adamantyl)-2-*O*-{1-*O*-[(*1R,2R*)-2-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-cyclohexyl] 6-*O*-benzyl-β-D-galactopyranos-3-yl}-propionate (22)

Compound **21** (199 mg, 253 μmol) and dibutyltinoxide (69.3 mg, 278 μmol) were dissolved in dry MeOH (15 mL). Activated molecular sieves 3 Å (400 mg) were added to the solution, which was then refluxed under argon for 17 h. Filtration of the suspension over Celite, evaporation of the solvent, and drying for 27 h in high vacuum gave a yellow oily substance (258 mg). This residue was suspended in dry DME (6 mL) under argon.

Extensively dried CsF (50 mg, 0.33 mmol) was added and the reaction mixture was stirred for 25 min at rt. Then, a solution of dry (***R***)-**14** (134 mg, 300 μmol) in dry DME (6 mL) was added to the reaction, which turned turbid upon addition, but cleared again after 2.5 h. After stirring for 4 d at rt, the reaction mixture was concentrated and the residue was purified by two consecutive column chromatographies on silica (1: PE/EE 10/1 to 2/1, 2: DCM/MeOH 50/1) to yield **22** as a white solid (58 mg, 21%) and, as a byproduct, (*S*)-benzyl 3-(1-adamantyl)-2-fluoro-propionate (**23**, 48 mg, 51%). **22**: R_f (PE/EE 2/1) 0.50; $[\alpha]_D^{20}$ −48.3 (*c* 1.05, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.37–7.20 (m, 25 H, Ar–H), 5.18, 5.11 (A, B of AB, ^2J = 12.2 Hz, 2H, Ph*C**H**$_2$*), 4.94 (A′ of A′B′, ^2J = 11.6 Hz, 1H, $PhCH_2$), 4.91 (d, ^3J = 2.5 Hz, 1H, Fuc-H1), 4.80 (A″ of A″B″, ^2J = 11.6 Hz, 1H, $PhCH_2$), 4.75 (A‴ of A‴B‴, ^2J = 12.0 Hz, 1H, $PhCH_2$), 4.68 (m, 2H, $PhCH_2$), 4.60 (B′ of A′B′, ^2J = 11.6 Hz, 1H, $PhCH_2$), 4.54–4.46 (m, 3H, 2H of $PhCH_2$, Lac-H2), 4.43–4.40 (m, 1H, Fuc-H5), 4.28 (d, ^3J = 7.7 Hz, 1H, Gal-H1), 4.04–3.94 (m, 2H, Fuc-H2), 3.85 (d, ^3J = 2.8 Hz, 1H, Gal-H4), 3.79–3.63 (m, 6H, 1H of Cy-CH, Fuc-H3/4, Gal-H2/6a,b), 3.59–3.53 (m, 1H, Cy-CH), 3.48 (t, ^3J = 6.0 Hz, 1H, Gal-H5), 3.27 (dd, ^3J = 3.2, ^3J = 9.2 Hz, 1H, Gal-H3), 2.89 (br s, 1H, OH), 2.48 (br s, 1H, OH), 1.97 (br s, 2H, Cy-CH_2), 1.91 (br s, 3H, Ad-H3), 1.72–1.40 (m, 16H, 1H of Cy-CH_2, 12H of Ad-CH_2, Lac-H3a,b), 1.38–1.09 (m, 4H, 4H of Cy-CH_2), 1.06 (d, ^3J = 6.5 Hz, 3H, Fuc-H6); ^{13}C NMR ($CDCl_3$): δ 174.9 (Lac-C1), 139.2, 138.9, 138.8, 138.2, 135.3 (5C, Bn-C^i), 128.7–126.6 (Ar–C), 100.0 (Gal-C1), 94.9 (Fuc-C1), 81.2 (Gal-C3), 79.7 (CH), 78.1 (CH), 77.2 (CH), 76.32 (CH), 76.28 (CH), 76.1 (Ph*C*H_2), 74.9 (CH), 73.6 (Ph*C*H_2), 73.3 (Gal-C5), 73.0 (Ph*C*H_2), 71.4 (CH), 69.1 (CH_2), 67.1 (Gal-C4), 66.9 (Ph-*C*H_2–O–CO–), 66.1 (Fuc-C5), 47.8 (Lac-C3), 42.5 (3× Ad-C3), 36.8 (3× Ad-C2), 32.5 (Ad-C1), 29.9 (Cy-CH_2), 29.3 (Cy-CH_2), 28.5 (3× Ad-C4), 23.3 (2× Cy-CH_2), 16.6 (Fuc-C6); ESI-MS Calcd for $C_{66}H_{80}NaO_{13}$ $[M+Na]^+$: 1103.6; Found: 1103.8.

Benzyl (*S*)-3-(1-adamantyl)-2-fluoro-propionate (23). $[\alpha]_D^{20}$ −6.4 (*c* 2.3, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.39–7.33 (m, 5H, Ar–H), 5.23, 5.19 (A, B of AB, ^2J = 12.2 Hz, 2H, Ph*C**H**$_2$*), 5.09 (ddd, ^2J(H–F) = 50.2, ^3J = 2.3, ^3J = 9.6 Hz, 1H, H2), 1.97 (br s, 3H, H6), 1.75–1.53 (m, 14H, H3a,b, 12H of Ad-CH_2); ESI-MS Calcd for $C_{20}H_{25}FNaO_2$ $[M+Na]^+$: 339.2; Found: 339.2.

4.11. Lactone derivative of benzyl (*2R*) 3-(1-adamantyl)-2-*O*-{1-*O*-[(*1R*,*2R*) 2-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl) cyclohexyl] 6-*O*-benzyl-β-D-galactopyranos-3-yl}-propionate (24)

Compound **24** was prepared in analogy to **22** starting from 21 (51 mg, 65 μmol). Purification by column chromatography (PE/EE 9/1 > 0/10) yielded the pure lactone **24** (62 mg, 25%): $[\alpha]_D^{20}$ −62.4 (*c* 0.25, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.39–7.26 (m, 20 H, Ar–H), 4.95 (A of AB, ^2J = 13.0 Hz, 1H, $PhCH_2$), 4.93 (d, ^3J = 3.6 Hz, 1H, Fuc-H1), 4.84 (A′ of A′B′, ^2J = 11.7 Hz, 1H, $PhCH_2$), 4.76 (d, A″ of A″B″, ^2J = 11.9 Hz, 1H, $PhCH_2$), 4.70 (B′ of A′B′, ^2J = 12.9 Hz, 1H, $PhCH_2$), 4.67 (B″ of A″B″, ^2J = 12.1 Hz, 1H, $PhCH_2$), 4.61 (B of AB, ^2J = 11.6 Hz, 1H, $PhCH_2$), 4.56–4.50 (m, 2H, Gal-H1, Lac-H2), 4.49 (s, 2H, $PhCH_2$), 4.44 (q, ^3J = 7.1 Hz, 1H, Fuc-H5), 4.37 (t, ^3J = 9.7 Hz, 1H, Gal-H2), 4.15 (s, 1H, Gal-H4), 4.03 (dd, ^3J = 3.5, ^3J = 10.1 Hz, 1H, Fuc-H2), 3.97 (dd, ^3J = 2.7, ^3J = 10.1 Hz, 1H, Fuc-H3), 3.78–3.75 (m, 2H, Cy-CH, Gal-H6a), 3.68–3.59 (m, 4H, Cy-CH, Fuc-H4, Gal-H5/6b), 3.49 (dd, ^3J = 2.9, ^3J = 9.7 Hz, 1H, Gal-H3), 2.36 (br s, 1H, OH), 2.00–1.98 (m, 5H, 2× Cy-CH_2, 3× Ad-H3), 1.84 (dd, ^2J = 14.8, ^3J = 2.0 Hz, 1H, Lac-H3a), 1.73–1.51 (m, 15H, 2H of Cy-CH_2, 12H of Ad-CH_2, Lac-H3b), 1.49–1.17 (m, 4H, 4H of Cy-CH_2), 1.11 (d, ^3J = 6.5 Hz, 3H, Fuc-H6); ^{13}C NMR ($CDCl_3$): δ 169.6 (Lac-C1), 139.2, 138.9, 138.7, 137.5 (4C, Bn-C^i), 128.5–127.2 (Ar–C), 98.1 (Gal-C1), 94.5 (Fuc-C1), 79.7 (Fuc-C3), 78.5 (Cy-CH), 78.2 (Cy-CH), 76.7 (Fuc-C2), 76.4 (Gal-H2), 76.0 (Fuc-H4), 75.7 (Lac-C2), 74.82 (Gal-H3), 74.79 (Ph*C*H_2), 73.7 (CH), 73.0 (2C, Ph*C*H_2), 72.9 (Ph*C*H_2), 68.4 (Gal-C6), 66.9 (Gal-C4), 66.0 (Fuc-C5), 46.9 (Lac-C3), 42.6 (3C, Ad-C3), 36.8 (3C, Ad-C2), 32.4 (Ad-C1), 29.5 (Cy-CH_2), 28.7 (Cy-CH_2), 28.5 (3× Ad-C4), 23.0 (2C, Cy-CH_2), 16.6 (Fuc-C6); ESI-MS Calcd for $C_{59}H_{72}NaO_{12}$ $[M+Na]^+$: 995.5; Found: 995.7.

4.12. Sodium (*2S*) 3-(1-adamantyl)-2-*O*-{1-*O*-[(*1R*,*2R*) 2-*O*-(α-L-fucopyranosyl) cyclohexyl] β-D-galactopyranos-3-yl}-propionate ((*S*)-7)

Compound **22** (26.3 mg, 24 μmol) and $Pd(OH)_2/C$ (20 mg) were suspended in dioxane/water (4/1, 3 mL). This suspension was hydrogenated at rt for 3 d. The solvents were removed in vacuo and the residue taken up in MeOH. Filtration over Celite and evaporation of the solvent gave the crude product (22 mg). Purification by column chromatography on silica (DCM/MeOH/H_2O 10/4/0.8), Dowex 50 × 8 ion exchange chromatography (Na^+ form), Sephadex G-15 size exclusion chromatography, and microfiltration afforded (***S***)-**7** (14.8 mg, 92%) as white solid after a final lyophilization from ^{t}BuOH/H_2O. R_f (DCM/MeOH/H_2O 10/4.8/0.5) 0.34; $[\alpha]_D^{20}$ −64.5 (*c* 0.74, MeOH); ^1H NMR (MeOH-d_4): δ 4.86 (m, 1H, Fuc-H1), 4.58 (q, 1H, ^3J = 6.2 Hz, Fuc-H5), 4.32 (d, 1H, ^3J = 7.8 Hz, Gal-H1), 3.96 (d, 1H, ^3J = 8.9 Hz, Lac-H2), 3.89 (dd, 1H, ^3J = 3.3, ^3J = 10.1 Hz, Fuc-H3), 3.84 (d, 1H, ^3J = 2.3 Hz, Gal-H4), 3.76 (m, 1H, Gal-H6a), 3.73–3.65 (m, 4H, 1× Cy-CH, Fuc-H2/4, Gal-H6b), 3.58–3.52 (m, 2H, Gal-H2, 1× Cy-H), 3.47 (t, 1H, ^3J = 6.1 Hz, Gal-H5), 3.23 (dd, 1H, ^3J = 3.0, ^3J = 9.4 Hz, Gal-H3), 2.09–2.03 (m, 2H, Cy-CH_2), 1.93 (br s, 3H, Ad-H3), 1.74–1.61 (m, 14H, 2× Cy-CH_2, 12× Ad-CH_2), 1.60–1.41 (m, 2H, Lac-H3a,b), 1.39–1.25 (m, 4H, 4× Cy-CH_2), 1.18 (d, 3H, ^3J = 6.5 Hz, Fuc-H6); ^{13}C NMR (MeOH-d_4): δ 184.00 (Lac-C1), 102.6 (Gal-C1), 97.0 (Fuc-C1), 84.5 (Gal-C3), 80.2 (Lac-C2), 79.2 (CH), 77.2 (CH), 75.9 (Gal-C5), 73.8 (CH), 71.7 (CH), 71.5 (Fuc-C3), 69.9 (CH), 67.7 (Gal-C4), 67.4 (Fuc-C5), 63.2 (Gal-C6), 50.1 (Lac-C3), 43.9 (3× Ad-C3), 38.2 (3× Ad-C2), 33.5 (Ad-C1), 30.9 (Cy-CH_2), 30.3 (3× Ad-C4), 30.0 (Cy-CH_2), 24.5 (2× Cy-CH_2), 16.6 (Fuc-C6); HR-MS Calcd for $C_{31}H_{49}O_{13}$ $[M-H]^-$: 629.3173; Found: 629.3195.

4.13. Sodium (2*R*)-3-(1-adamantyl)-2-*O*-{1-*O*-[(1*R*,2*R*)-2-*O*-(α-L-fucopyranosyl) cyclohexyl] β-D-galactopyranos-3-yl}-propionate ((*R*)-7)

Hydrogenation was performed in analogy to (*S*)-7. The obtained lactone was opened by stirring in aqueous NaOH overnight at rt. After column chromatography on silica (DCM/MeOH/H_2O 10/4/0.8), (*R*)-7 (8.2 mg, 31%) was obtained as a white solid, which was taken up in MeOH (2 mL), microfiltered, dried, and lyophilized from ^{t}BuOH/H_2O: R_f (DCM/MeOH/H_2O 10/4.8/0.5) 0.58; $[\alpha]_D^{20}$ −31.9 (*c* 0.42, MeOH); ^{1}H NMR (MeOH-d_4): δ 4.89 (m, 1H, Fuc-H1), 4.53 (q, ^3J = 6.4 Hz, 1H, Fuc-H5), 4.33 (d, ^3J = 7.6 Hz, 1H, Gal-H1), 4.25 (d, ^3J = 5.9 Hz, 1H, Lac-H2), 4.09 (d, ^3J = 3.0 Hz, 1H, Gal-H4), 3.87 (dd, ^3J = 3.3, ^3J = 10.1 Hz, 1H, Fuc-H3), 3.80–3.62 (m, 6H, Cy-CH, Fuc-H2/4, Gal-H2/6a,b), 3.58 (dt, ^3J = 3.9, ^3J = 8.8 Hz, 1H, Cy-CH), 3.42 (t, ^3J = 5.7 Hz, 1H, Gal-H5), 3.37 (dd, ^3J = 3.1, ^3J = 9.8 Hz, 1H, Gal-H3), 2.04 (d, J = 12.8 Hz, 2H, Cy-CH_2), 1.95 (br s, 3H, Ad-H3), 1.75–1.64 (m, 14H, 2H of Cy-CH_2, 12H of Ad-CH_2), 1.57 (d, J = 5.8 Hz, 2H, Lac-H3a,b), 1.45–1.25 (m, 4H, 4H of Cy-CH_2), 1.18 (d, ^3J = 6.6 Hz, 3H, Fuc-H6); ^{13}C NMR (MeOH-d_4): δ 183.7 (Lac-C1), 102.9 (Gal-C1), 97.0 (Fuc-C1), 81.6 (Gal-C3), 79.2 (CH), 77.7 (Lac-C2), 77.0 (Cy-CH), 76.0 (Gal-C5), 73.8 (Fuc-C4), 71.6 (Fuc-C3), 71.5 (Fuc-C2), 70.0 (CH), 67.9 (Gal-C4), 67.4 (Fuc-C5), 62.9 (Gal-C6), 49.5 (Lac-C3), 43.9 (3× Ad-C3), 38.1 (3× Ad-C2), 33.5 (Ad-C1), 30.8 (Cy-CH_2), 30.3 (3C, Ad-C4), 29.7, 24.3, 24.2 (3C, Cy-CH_2), 16.6 (Fuc-C6); ESI-MS Calcd for $C_{31}H_{50}NaO_{13}$ $[M+H]^+$: 653.3; Found: 653.3.

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2.2 Targeting the protein surface for additional enthalpic interactions

To screen for sLe^x mimetics with increased binding affinity *via* additional enthalpic interactions in proximity to the carboxylic acid, a library was designed that permits fast and selective diversification in the last step. A set of molecules was synthesized and tested for their biological affinity.

Probing the Carbohydrate Recognition Domain of E-Selectin by a Click-Chemistry Approach: The Importance of the Acid Orientation in sLe^x Mimetics for Binding

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Keywords

E-selectin, sialyl Lewis^x, pre-organization, antagonist, Neu*N*Ac replacement

1. Introduction

The selectins play a key role in the early inflammatory cascade. The recruitment and extravasation of leukocytes into inflamed tissue mediated by E-, P- and L-selectin, a family of closely related cell adhesion molecules, is an essential mechanism of the immune defense. The selectin-leukocyte interaction leads to the characteristic tethering and rolling process of leukocytes on the vascular endothelium. Upon secondary activation, firm adhesion and migration through the endothelial cell monolayer takes place.[1,2] However, excessive influx of leukocytes into the surrounding tissue can cause acute or chronic reactions as observed in reperfusion injury, stroke or rheumatoid arthritis.[3,4] As a result, the antagonism of the selectins is considered to be a valuable approach for the treatment of inflammatory diseases.

Since the physiological ligands of the selectins bear the terminal tetrasaccharide epitope sialyl Lewis^x (**1**, sLe^x, *figure 1*),[5,6] this motif served as lead structure in the search for selectin antagonists.[7-9] Numerous publications determined the essential pharmacophores of sLe^x for binding to E-selectin, *i.e.* four hydroxyl groups (fucose 3- and 4-OH, galactose 4- and 6-OH) and the carboxylic acid of neuraminic acid.[9,10] The bioactive conformation (*figure 1*) of sLe^x was determined by trNOE NMR experiments[11,12] and was subsequently confirmed by X-ray crystallography[13] of E-, P- and L-selectin co-crystallized with sLe^x. Carbohydrate-protein interactions are usually of low affinity[9] (*e.g.* sLe^x has an IC_{50} of 1 mM, rIC_{50} = 1.0). However, a reduction of the entropy costs upon binding by stabilizing the bioactive conformation of sLe^x and mimics thereof, proved to be a valuable approach to increase binding affinity.[14-17]

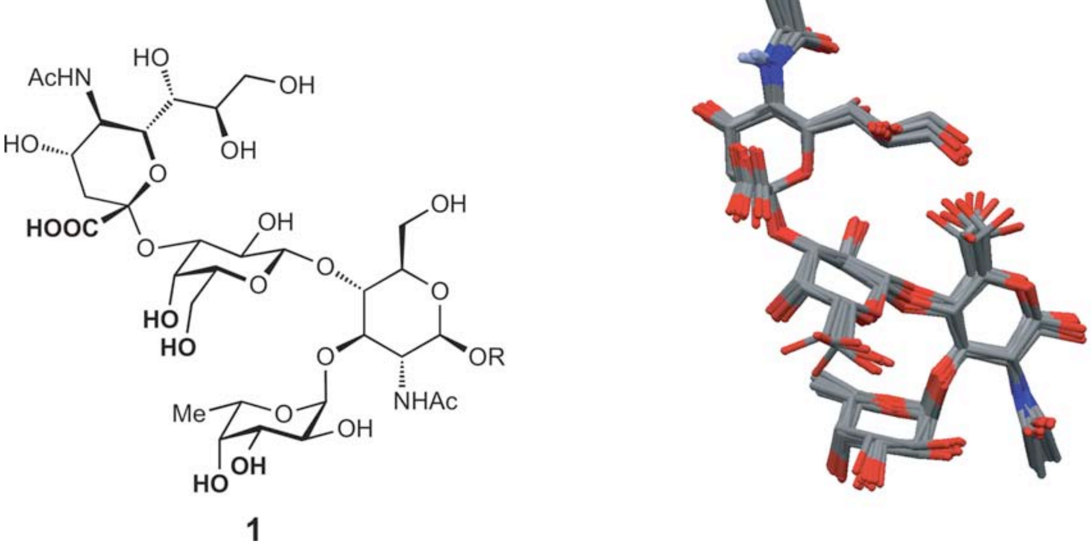


Figure 1: The minimal binding epitope of all three selectins: sialyl Lewis^x (**1**, sLe^x, essential pharmacophores in bold face) and its bioactive conformation as determined by trNOE NMR experiments[11,12]

Numerous modifications of the Le^x core have been studied.[9] Only a few reports, however, are dealing with modifications at the neuraminic acid part of sLe^x.[17] Most of them simply replace Neu*N*Ac by an anionic moiety (*e.g.* sulphate or phosphate) or are focusing on the stabilization of the acid orientation in the bioactive conformation.[16,17] Targeting the receptor surface for additional enthalpic contributions to binding was largely neglected, since only one patent by Kolb is addressing this issue.[18]

To date, there is no crystal structure of a complex of E-selectin with the optimized lead compound CGP69669A[19] available and a docking model suggests that this mimic adopts a similar binding mode to E-selectin as sLe^x itself.[20] Consequently, the protein surface adjacent to the binding region of the carboxylic acid could be suitable for accommodation of additional pharmacophores (*figure 2*).

In the patent of Kolb,[18] a series of isoserine derived ligands was synthesized and biologically evaluated (*figure 3*). Isoserine was used as a neuraminic acid replacement and this motif was diversified *via* amine, amide or sulfonamide linkages. The substituents analyzed varied largely (*e.g.* aryl sulfonamides,

fatty acid amides or polyhydroxylated alkyl chains), but excluded heterocyclic fragments.

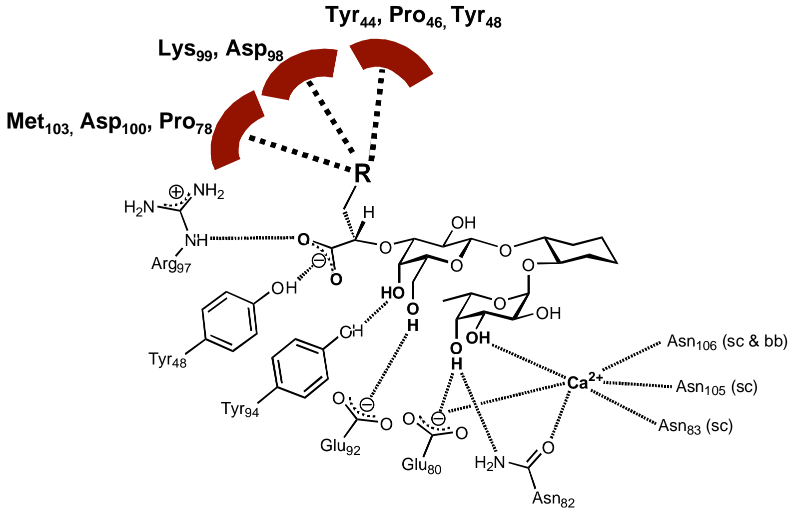


Figure 2: Potential interactions of lactic acid derivatives (R) with amino acids in the E-selectin binding sites (sc: interactions of the side chain, bb: interactions of the backbone)

Biological data reported for these compounds show binding to E-selectin, albeit with rather low affinities. Almost all secondary amines and amides show rIC_{50}s in the range of 0.6 to 2.0. When sulfonamides were introduced, the inhibition potency increases to rIC_{50}s of 0.12-0.26. For the *N*-alkylated amides and tertiary amines, the rIC_{50} values lie within a range of 0.2-0.6. However, none of the analyzed isoserine derivatives was a better ligand to E-selectin than the cyclohexyllactate derivative CGP69669A[14,15] (rIC_{50} = 0.08).

Speculatively interpreted, at least part of these results might originate from the linking functionality. In case of amines, a zwitter ionic species is present at physiological pH, and thus may influence the strength of the crucial salt bridge with the guanidinium of Arg97 in E-selectin (*figure 2*). In case of the amides, the H-bond donating character might influence the conformation of the ligand upon binding to the protein, and therefore alter the optimal conformation for salt-bridge formation, leading to reduced affinity. This would also explain why the *N*-alkylated amides lacking an H-bond donor display better binding when compared to the amides with a free nitrogen bound proton.

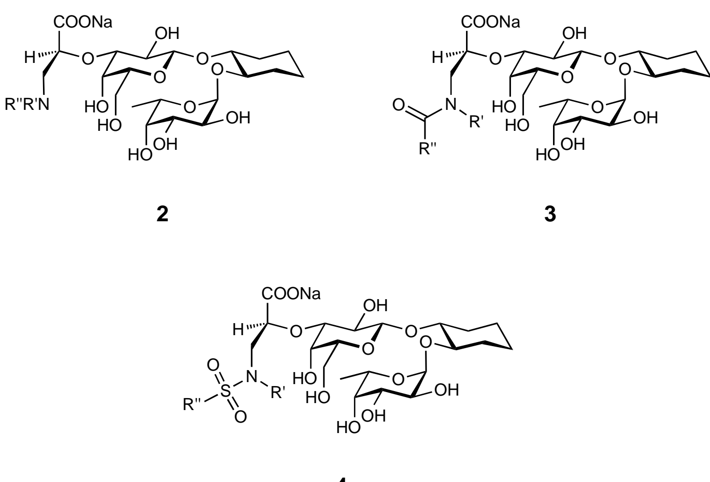


Figure 3: E-selectin ligands with neuraminic acid replacements to target the receptor for additional enthalpic contributions to binding[18]

To address this issue, we report the design, synthesis and biological evaluation of a library of E-selectin antagonists with a diversification as the last step, taking both, the introduction of heterocyclic fragments for receptor targeting, as well as a non-charged and a non-hydrogen bond donating functional group for diversification into account.

2. Results and discussion

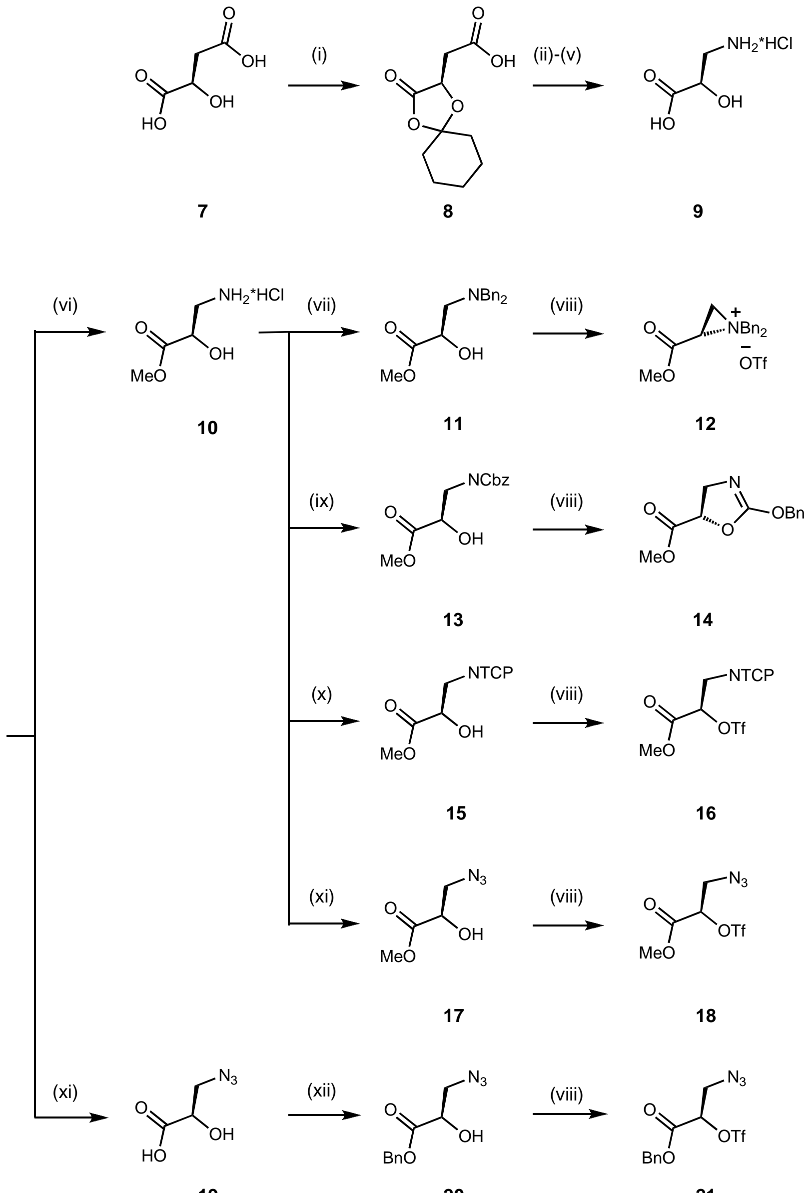
For the selective and quantitative diversification as last step of the synthesis, but also for a linking functionality that meets the desired criteria, a click chemistry[21,22] approach seemed to be the most straight forward. For this purpose, there are various 'spring-loaded' reactions available, *e.g.* the copper-(I) catalyzed Huisgen[23] 1,3-dipolar cycloaddition of azides and alkynes. Here, we introduced an azide functionality into a mimic of sLe^x bearing all necessary pharmacophores (**5**, *scheme 1*). This tetrasaccharide mimetic could then easily be used to obtain selectively derivatized ligands (**6**, *scheme 1*) using an array of commercial terminal alkynes.

Before synthesizing the library, we were carefully analyzing if a ligand bearing an unsubstituted triazol (**6a**, R = H, *scheme 1*) in the 3-position of the (*S*)-lactate derivative would still adopt the bioactive arrangement of the essential pharmacophores in solution using the Monte-Carlo-(jumping-between-

wells)/stochastic dynamics (MC(JBW)/SD) protocol.[14] The *in silico* results (data not shown) nicely demonstrate that antagonist **(*S*)-6a** is well pre-organized in the bioactive window and therefore a suitable candidate as basis for the library.

Scheme 1: E-selectin ligand synthesis *via* click chemistry to target the receptor surface adjacent to the binding site of the carboxylate

For the synthesis of the library precursor **5**, the tin acetal mediated alkylation of the trisaccharide core using a lactate electrophile was chosen. In the present case, the lactate electrophile is derived from (*R*)-isoserine which was obtained from the chiral pool. The hydroxyl group and the 1-carboxylate of (*R*)-malic acid (**7**, *scheme 2*) were protected as cyclohexyl-dioxolane **8** in 98% yield to render the terminal carboxyl group available for selective transformation. Its acyl chloride was obtained by refluxing in thionylchloride. Transformation of the acyl chloride into an acyl azide by treatment with sodium azide gave the precursor for the Curtius rearrangement. In the following rearrangement, an isocyanate is formed which upon acidic hydrolysis intramolecularly reacts with the 2-OH group to form a carbamate. This could be observed by ^{1}H-NMR as a doublet of doublets at 5.00 ppm for H-2, being a downfield shift of 0.8 ppm compared to isoserine hydrochloride. Complete hydrolysis gave (*R*)-isoserine hydrochloride (**9**) in 51% yield over 4 steps.



Scheme 2: (i) Cyclohexanone, BF_3•Et_2O, Et_2O, 0 °C-r.t., 1 d, 98%; (ii) $SOCl_2$, reflux, 30 min; (iii) NaN_3, H_2O, acetone, -20 °C, 1 h; (iv) PhMe reflux, 30 min; (v) 6M HCl reflux, 6 h, 51% (4 steps); (vi) $SOCl_2$, MeOH, reflux, 3.5 h, quant.; (vii) BnBr, NEt_3, DMF, r.t., 36 h, 85%; (viii) Tf_2O, 2,6-$^tBu_2$-py, CH_2Cl_2, -20 °C, 5 h (**16**: 68%, **18**: 48%, **21**: 88%); (ix) Cbz-Cl, $NaHCO_3$, THF/H_2O, 0 °C, 3 h, 69%; (x) TCP-anhydride, DIPEA, PhMe/DMF, reflux, 5 h, 31%; (xi) TfN_3, $CuSO_4$, $NaHCO_3$, PhMe/H_2O/ MeOH, r.t., 24 h (**17**: 58%); (xii) BnBr, NEt_3, DMF, r.t., 1 d, 63% over 2 steps;

Scheme 3: (i) Bu_2SnO, MeOH, mol. sieves 3Å, reflux, 15 h; (ii) **21**, CsF, DME, r.t., 6 d; (iii) $Pd(OH)_2/C$, H_2, THF, H_2O, 4 bar, r.t., 72 h; (iv) TfN_3, $CuSO_4$, $NaHCO_3$, PhMe/H_2O/MeOH, r.t., 22 h; (v) 1. alkyne, Cu, $CuSO_4$, EtOH/H_2O, 24 h; 2. 1 eq NaOH**;** yields (over 3 steps from **23**): **6b** 35%, **6c** 35%, **6d** 15%, **6e** 34%, **6f** 33%; for **6a**: TMS-acetylene, DIPEA, CuCl, MeCN/H_2O, 9 d; (vi) for **6a**: 1. TBAF, HOAc/THF, r.t., 30 min; 2. 1 eq NaOH, 7% (over 4 steps from **23**);

Because the tin-acetal mediated alkylation with lactate triflates usually gives low to moderate yields, we studied several protecting groups for the amine as well as for the carboxylic acid. For this S_N2-type alkylation reaction, small protecting groups are expected to give higher yields because of a better accessibility of the electrophilic carbon. Therefore, the carboxylate was protected as methyl ester (**10**) by refluxing (*R*)-isoserine hydrochloride (**9**) in methanol in the presence of thionylchloride. For the amine protection, a series of standard amine protecting groups was tested (*scheme 2*). Dibenzylation of the amino group in **10** yielded the dibenzylamino product **11** in quantitative

yield. However, when the secondary hydroxyl group was transformed into a leaving group by treatment with triflic anhydride, the tertiary amine underwent an intramolecular cyclization with loss of triflate to form the aziridinium compound **12**. Similar observations have been made recently by Couturier and co-workers by transforming the hydroxyl group of isomeric methyl *N*,*N*-dibenzyl serinate into a mesylate.[24]

To reduce the nucleophilicity of the nitrogen, the amine was protected as electron withdrawing *O*-benzyl carbamate **13**. However, upon treatment of **13** with triflic anhydride cyclization occurred to give the oxazoline **14**. Finally, protection of the amine as tetrachlorophthalimide rendered **15** in moderate yield, which could be transformed into the corresponding stable triflate **16**. Additionally, an azide was installed as protecting group by CH_2Cl_2-free diazo transfer.[25] The resulting azido lactate **17** was then transformed into the corresponding triflate **18**. As alternative protecting group for the carboxylic acid, a benzyl ester (**20**) was installed after introduction of the azide protecting group in **19**. The corresponding electrophile was obtained by transformation of the hydroxyl group into a leaving group (**21**).

23 → (i) → **25**, 86% → (ii) → **6g**, 39%

Scheme 4: (i) 1-benzotriazolyl-prop-2-yne, Cu, MeOH/H_2O, 48 h; (ii) 1. $Pd(OH)_2$/C, H_2, dioxane/H_2O, 4 bar, r.t., 24 h; 2. 1 eq NaOH;

Tin-acetal mediated alkylation of the 3-position of galactoside **22**[16] (*scheme 3*) with the obtained electrophiles **16**, **18** and **21** was in our hands only successful for **21**. In case of the TCP protected amine **16**, steric hindrance may have been the primary cause of failure. For the sterically least demanding azido methyl ester **18**, the water sensitive alkylation probably

failed due to insufficient drying of the volatile triflate. Finally, alkylation with the less volatile benzyl derivative **21** proved successful, although with moderate yield (*scheme 3*).[26,27]

Hydrogenolytic deprotection of tetrasaccharide mimetic **23** gave the amino compound **24**. The azide needed for click chemistry mediated diversification was reinstalled with the CH_2Cl_2-free diazo transfer (**5**, *scheme 3*). Finally, a small library of putative E-selectin antagonists (**6a-f**, *table 1*) was obtained by copper-(I) catalyzed Huisgen 1,3-dipolar cycloaddition with the corresponding alkynes. The tetrahydro-benzotriazolyl compound **6g** was obtained by inverting the reaction sequence, *i.e.* the fully protected azide **23** was first reacted with benzotriazolylpropyne to give **25**, followed by hydrogenolytic cleavage of the benzyl groups and partial hydrogenation of the benzotriazol moiety (*scheme 4*).

The obtained sLe^x mimetics were tested in a static cell-free *in vitro* assay for their binding to E-selectin.[28] The results obtained for the azido lactate derived library showed that all molecules were binding to the protein (**6e** as one exception). However, the rIC_{50}s vary widely. When additional fragments are attached to the triazol linker, the binding was not increased, yet it is in the range of sLe^x. Interestingly, the unsubstituted triazol is the best binder in this series with an rIC_{50} of 0.48. Finally, the molecule tested with the lowest rIC_{50} of 0.29 proved to be the unsubstituted azide **5**.

Table 1: Relative IC_{50}s (rIC_{50}) of the E-selectin antagonists **5** and **6a-g**. IC_{50}s were measured using CGP69669 (rIC_{50} = 0.08) as reference compound and are scaled on sLe^x (rIC_{50} = 1).

Entry	Compound	R =	rIC_{50}
1	**5**	-	0.29
2	**6a**	H	0.48
3	**6b**		1.03
4	**6c**	S	0.88
5	**6d**	N	3.13
6	**6e**	F_3C	n.a.
7	**6f**	N N N	1.18
8	**6g**	N N N	1.22

3. Conclusion

In the present manuscript, we show the design, synthesis and biological evaluation of a small library of E-selectin ligands with neuraminic acid replacements. In these ligands, pharmacophore fragments were introduced to target the receptor for additional enthalpic contributions to binding. A related set of molecules by Kolb[18] based on amine and amide linkers did not improve binding affinity to the receptor. This may have originated from a negative influence on the necessary salt bridge between the ligand carboxylate and Arg97 by the zwitterionic character in the amine series or by additional hydrogen bonding possibilities in the amide series. Therefore, the library molecules in this study were obtained from the azido-lactate mimic **5** of sLe^x, which was used in copper-(I) catalyzed click chemistry with various alkynes to introduce diversity in the last step of the synthesis. The *trans*-triazol generated by this chemistry was chosen as a linker because of its facile introduction as well as the lack of positive charge or hydrogen-bond donating properties, which might have a negative influence on binding. The obtained ligands were tested in an *in vitro* assay and showed binding to E-selectin (with one exception (**6e**)). However, when pharmacophore fragments were added to the triazol (**6b-g**), the observed inhibitory potency dropped when compared to the unsubstituted triazol **6a** or even the azido compound **5**. These data together with the reported affinities of the Kolb library let us conclude that pharmacophores attached in vicinity to the essential carboxylate might have additional enthalpic interactions with the protein but will then alter the acid orientation, which was shown to be essential for binding. This would lead to a weakening of the salt bridge with the protein and consequently result in decreased affinity. Since this interpretation is based on a computational model on the binding mode of sLe^x mimics, solid experimental structural data (*i.e.* X-ray crystallography or NMR spectroscopy) of such a mimetic in complex with E-selectin is needed for further understanding and optimization of the binding potency. Studies addressing these issues are in progress.

4. Experimental

General Methods

Nuclear magnetic resonance spectroscopy was performed on a Bruker Avance 500 UltraShield spectrometer at 500.13 MHz (^{1}H) or 125.76 MHz (^{13}C). Chemical shifts are given in ppm using residual solvent peaks[29] or tetramethyl silane as references. Multiplicities were specified as s (singlet), m (multiplet) or interpreted according to 1st order[30] and higher order where possible.

The signals were assigned with the help of DEPT-135, ^{1}H,^{1}H-COSY/TOCSY and ^{1}H,^{13}C-HSQC/HMBC experiments. Assignments are indicated according to IUPAC nomenclature. For complex molecules, the following prefixes for substructures are used: Cy (cyclohexyl), Fuc (fucose), Gal (galactose), Lac (lactate), and Bt (benzotriazol). C^{i} indicates the *ipso* substituted carbons of aromatic systems.

IR spectra were recorded on a Perkin Elmer Spectrum One as KBr pellets or as films on NaCl plates. Bands are given in cm^{-1} and characterized as s (strong), m (medium) or w (weak). Optical rotations were measured on a Perkin Elmer 341 polarimeter in the indicated solvents in *p.a.* quality. ESI mass spectra were recorded on a Waters micromass ZQ instrument. High resolution mass spectra were obtained on a ESI Bruker Daltonics micrOTOF spectrometer equipped with a TOF hexapole detector.

TLC was performed using silica gel 60 coated glass plates containing fluorescence indicator (Merck KGaA, Darmstadt, Germany) and visualized by using UV light (254 nm) and/or by charring either in aqueous $KMnO_4$ solution or in a molybdate solution (a 0.02 M solution of ammonium cerium sulfate dihydrate and ammonium molybdate tetrahydrate in aqueous 10% H_2SO_4) with heating to 140 °C for 5 min. Column chromatography was performed using silica gel 60 (0.040-0.063 mm, Fluka). Hydrogenation reactions were performed in a shaking apparatus (Parr Instruments Company, Moline, Illinois, USA) in 250 mL or 500 mL bottles with 4 bar H_2 pressure. Solvents were purchased from Fluka and dried prior to use. CH_2Cl_2 was dried by

filtration through basic aluminum oxide (Fluka). Dioxane, DME, Et_2O and PhMe were dried by distillation from sodium/benzophenone. DMF was dried over activated molecular sieves and MeOH by distillation from sodium methoxide.

Biological data were obtained using the published ELISA procedure with **(*S*)-6** as reference compound.[28]

***(5R)*-(2,2-Cyclohexylidene-4-oxo-1,3-dioxolan-5-yl) acetic acid (8).** Compound **8** was prepared in analogy to Hanessian *et al.*[31] To a suspension of **7** (4.00 g, 29.8 mmol) in dry Et_2O (100 mL) was added cyclohexanone (3.09 mL, 29.8 mmol) at 0 °C followed by $BF_3{\cdot}Et_2O$ (5.62 mL, 44.8 mmol) *via* syringe. The mixture was stirred at 0 °C for 1 h and turned into a light red solution. After stirring for another 18 h at r.t., Et_2O (100 mL) was added, and the organic phase was washed with aqueous sodium acetate (3 x 10 mL, 10% w/v). The combined aqueous phases were extracted with Et_2O (2 x 50 mL). The combined organic layers were dried over Na_2SO_4 and the solvent evaporated *in vacuo* affording spectroscopically pure **8** (6.25 g; 98%) as slightly red colored crystals.

^{1}H-NMR (500.1 MHz, $CDCl_3$): δ 10.60 (br s, RCO_2H), 4.72 (X of ABX, ^3J = 3.9, 6.5 Hz, 1H, H-2), 3.00 (A of ABX, ^2J = 17.2, ^3J = 3.9 Hz, 1H, H-3a), 2.85 (B of ABX, ^2J = 17.2, ^3J = 6.5 Hz, 1H, H-3b), 1.90-1.81 (m, 2H, $-C_5H_{10}-$), 1.80-1.60 (m, 6H, $-C_5H_{10}-$), 1.55-1.35 (m, 2H, $-C_5H_{10}-$);

^{13}C-NMR (125.8 MHz, $CDCl_3$): δ 175.1, 172.0 (2 C=O), 112.2 (C-ketal), 70.0 (C-2), 36.2 (2C, C_5H_{10}), 35.3 (C-3), 24.4 (1C, C_5H_{10}), 23.4 (2C, C_5H_{10});

ESI-MS calcd. for $C_{10}H_{13}O_5$ $[M-H]^-$: 213.1; found: 213.0;

IR (KBr): 3208 (m), 2939 (m), 1802 (s), 1733 (s), 1375 (m), 1291 (m), 1197 (s), 932 (m);

$[\alpha]^{20}_D$ = -2.9 (*c* 1.0, $CHCl_3$);

This analytical data matched with the reported NMR spectra.[32]

***(R)*-3-Amino-2-hydroxy-propionic acid hydrochloride (9).** Compound **8** (6.25 g, 29.2 mmol) was dissolved in $SOCl_2$ (48 mL) in a round bottom flask with strong gas evolution. The solution was heated to reflux and the gas evolution ceased after 15 min. After refluxing the dark solution for 1 h, the solvent was evaporated and the residue dried under high vacuum over night. The remaining brownish oil was then dissolved in acetone (56 mL) and cooled to -20 °C. A solution of sodium azide (2.56 g, 39.4 mmol) in water (16 mL) was added dropwise to the solution *via* syringe and the mixture was stirred for 1.5 h. After removal of the acetone *in vacuo* at 0 °C, water (150 mL) was added and the aqueous phase was extracted with PhMe (3 x 200 mL). The combined organic layers were dried (Na_2SO_4) and concentrated to a final volume of ca. 75 mL. The remaining solution was slowly heated to reflux for 1 h, then the solvent was removed and aqueous HCl (6M, 35 mL) was added. The mixture was refluxed for 7 h. The solvent was removed *in vacuo*, the residue was taken up in water (100 mL) and washed with CH_2Cl_2 (1 x 100 mL, 2 x 50 mL). The combined organic phases were extracted with aqueous HCl (3N, 2 x 50 mL). The aqueous phases were pooled and the water removed by evaporation to give a brownish oil (3.05 g). Precipitation from PhMe/EtOAc/MeOH gave **9** as off-white solid (2.1 g, 51%).

R_f (*n*-BuOH/HOAc/H_2O/py, 4:1:2:1) = 0.10;

^{1}H-NMR (500.1 MHz, D_2O): δ 4.52 (X of ABX, ^3J = 4.1, ^3J = 8.4 Hz, 1H, H-2), 3.45 (A of ABX, ^2J = 13.3, ^3J = 4.0 Hz, 1H, H-3a), 3.23 (B of ABX, ^2J = 13.3, ^3J = 8.4 Hz, 1H, H-3b);

^{13}C-NMR (125.8 MHz, DMSO-d6): δ 172.6 (C-1), 67.0 (C-2), 41.7 (C-3);

ESI-MS calcd. for $C_3H_8NO_5$ $[M-Cl]^+$: 106.1; found: 106.0;

IR (film): 3422 (s), 1735 (s), 1623 (m), 1234 (m), 1150 (m), 1079 (m);

$[\alpha]^{20}_D$ = +9.9 (*c* 0.48, H_2O);

Methyl (*R*)-*3*-amino-2-hydroxy-propanoate hydrochloride (10) was synthesized according to a procedure by Lall *et al.*[33] Isoserine

hydrochloride (1.29 g, 9.11 mmol) was dissolved in MeOH (15 mL), cooled to 0 °C and $SOCl_2$ (1.78 mL, 18.2 mmol) was added dropwise *via* syringe. The solution was then heated to reflux for 1 h. Subsequently, the solvent was evaporated and MeOH (15 mL) was added. At 0 °C, $SOCl_2$ (1.78 mL, 18.2 mmol) was added and the mixture refluxed for 2.5 h. Removal of the solvent yielded the title compound (1.42 g, quant.) as a brown spectroscopically pure solid, which was used without further purification.

^{1}H-NMR (500.1 MHz, $CDCl_3$): δ 8.22 (br s, 3H, NH_3^+), 4.39 (X of ABX, ^{3}J = 8.5, ^{3}J = 3.1 Hz, 1H, H-2), 3.67 (s, 3H, MeO), 3.08-3.07 (m, 1H, H-3a), 2.90-2.88 (m, 1H, H-3b);

^{13}C-NMR (125.8 MHz, $CDCl_3$): δ 171.5 (C-1), 66.9 (C-2), 52.1 (MeO), 41.5 (C-3);

ESI-MS calcd. for $C_4H_{10}NO_3$ $[M-Cl]^+$: 120.1; found: 120.0;

IR (KBr): 3502 (s), 3044 (s), 1743 (s), 1513 (m), 1243 (s), 1146 (s);

$[\alpha]_D^{20}$ = +16.3 (*c* 0.60, H_2O);

This analytical data matched with the reported optical rotation and IR spectra.[34]

Methyl (*R*)-*3-N,N*-dibenzylamino-2-hydroxy-propanoate (11). To a solution of amino-ester **10** (121 mg, 0.78 mmol) in MeOH (10 mL), benzyl bromide (0.28 mL, 2.34 mmol) and $NaHCO_3$ (350 mg, 4.17 mmol) were added. The mixture was heated to reflux for 2 h. Volatiles were removed by evaporation. The residue was taken up in water (5 mL) and extracted with EtOAc (3 x 25 mL). The combined organic phases were washed with brine (3 x 20 mL) and dried over Na_2SO_4. After removal of the solvent the residue was purified by column chromatography (petrol ether/EtOAc, gradient of 6/1 to 4/1) to give **11** (232 mg, quant.) as a white solid.

R_f (petrol ether/EtOAc, 2:1) = 0.75;

^{1}H-NMR (500.1 MHz, $CDCl_3$): δ 7.34-7.24 (m, 10H, Ar-H), 4.25 (M of ABM, ^3J = 4.3, ^3J = 6.4 Hz, 1H, H-2), 3.78 (A' of A'B', ^2J = 13.5 Hz, 2H, $PhCH_2$), 3.67 (s, 3H, MeO), 3.52 (B' of A'B', ^2J = 13.5 Hz, 2H, $PhCH_2$), 2.90 (A of ABM, ^2J = 13.4, ^3J = 4.2 Hz, 1H, H-3a), 2.86 (B of ABM, ^2J = 13.4, ^3J = 6.8 Hz, 1H, H-3b);

^{13}C-NMR (125.8 MHz, $CDCl_3$): δ 173.9 (C-1), 138.5 (2C, Ar-C^i), 129.1, 128.4, 127.3 (10C, Ar-CH), 69.4 (C-2), 59.0 (2C, Ph*C*H_2), 56.1 (C-3), 52.3 (MeO);

ESI-MS calcd. for $C_{18}H_{22}NO_3$ $[M+H]^+$: 300.2; found: 300.2;

$[\alpha]_D^{20}$ = +32.7 (*c* 0.79, $CHCl_3$);

Methyl (*R*)-*N*-benzyloxycarbonyl-3-amino-2-hydroxy-propanoate (13). To a solution **10** (200 mg, 1.28 mmol) and sodium carbonate (213 mg, 2.00 mmol) in THF/H_2O (15 mL, 3:1) was added benzyl chloroformate (241 mg, 1.41 mmol) in THF (5 mL) at 0 °C. The reaction was stirred for 3 h. After extraction with EtOAc (3 x 40 mL), the combined organic phases were washed with brine (3 x 15 mL), dried over Na_2SO_4 and the solvent was removed *in vacuo*. The crude product was purified by column chromatography (petrol ether/EtOAc, 2:1) to give **13** (209 mg, 69%) as white solid.

R_f (petrol ether/EtOAc, 2:1) = 0.18;

^{1}H-NMR (500.1 MHz, $CDCl_3$): *δ* 7.37-7.26 (m, 5H, Ar-H), 5.18 (br s, 1H, NH), 5.07 (s, 2H, C*H_2*-Ph), 4.27 (t, ^3J = 4.4 Hz, 1H, H-2), 3.75 (s, 3H, OMe), 3.60-3.46 (m, 2H, H-3);

IR (KBr): 3362 (m), 1724 (s, ester + carbamate), 1258 (s);

This analytical data matched with the reported ^{1}H-NMR spectra of the known (*S*)-enantiomer.[35]

Methyl (*R*)-3-tetrachlorophtalimido-2-hydroxy-propanoate (15). To a solution of **10** (200 mg, 1.28 mmol) and tetrachlorophtalic anhydride (404 mg, 1.41 mmol) in PhMe/DMF (5:1, 12 mL) was added DIPEA (438 μL, 2.56 mmol). The reaction mixture was connected to a Dean Stark water trap

and heated to reflux for 5 h. The solvents were removed *in vacuo*, and the crude product was purified by column chromatography (petrol ether/EtOAc, 4:1) to yield **15** (154 mg, 31%) as white crystalline needles.

R_f (petrol ether/EtOAc, 2:1) = 0.60;

^{1}H-NMR (500.1 MHz, $CDCl_3$): δ 4.47 (X of ABX, ^3J = 5.2 Hz, 1H, H-2), 4.05 (A of ABX, ^3J = 5.1, ^2J = 14.0 Hz, 1H, H-3a), 3.95 (B of ABX, ^3J = 6.8, ^2J = 14.0 Hz, 1H, H-3b), 3. 83 (s, 3H, OMe);

^{13}C-NMR (125.8 MHz, $CDCl_3$): δ 172.9 (C-1), 163.6 (2C, imid-*C*O), 140.6, 130.1, 127.6 (6C, Ar-C), 68.1 (C-2), 53.5 (OMe), 41.7 (C-3);

HR-MS calcd. for $C_{12}H_7Cl_4NO_5Na$ $[M+Na]^+$: 407.8976; found: 407.8991;

IR (KBr): 1713 (s, imide and ester), 1361 (s), 736 (s, ν(C-Cl));

$[\alpha]_D^{20}$ = +4.9 (*c* 0.50, $CHCl_3$);

Methyl (*R*)-3-tetrachlorophtalimido-2-trifluoromethanesulfonyloxy-propanoate (16). To a solution of **15** (154 mg, 0.40 mmol) in dry CH_2Cl_2 (6 mL) was added 2,6-di-*tert.*-butylpyridine (117 µL, 0.52 mmol) at -20 °C under argon. Tf_2O (89 µL, 0.53 mmol) was added dropwise *via* syringe and the mixture was stirred for 3 h at –20 °C. Then a second portion of Tf_2O (13 µL, 0.08 mmol) and 2,6-di-*tert.*-butylpyridine (18 µL, 0.08 mmol) were added. After 1 h the reaction mixture was diluted with CH_2Cl_2 (14 mL) and extracted with aqueous KH_2PO_4 solution (1 M, 30 mL). The phases were separated, and the aqueous phase was extracted with CH_2Cl_2 (2 x 20 mL). The combined organic phases were dried over Na_2SO_4 and the solvent was removed *in vacuo*. Purification of the crude product by column chromatography (petrol ether/EtOAc, gradient of 6:1 to 4:1) gave **16** (142 mg, 68%) as a white crystalline solid.

R_f (petrol ether/EtOAc, 4:1) = 0.30;

^{1}H-NMR (500.1 MHz, $CDCl_3$): δ 5.39 (t, ^3J = 5.9 Hz, 1H, H-2), 4.29 (d, ^3J = 5.9 Hz, 2H, H-3), 3.86 (s, 3H, OMe);

^{13}C-NMR (125.8 MHz, $CDCl_3$): δ 165.1 (C-1), 162.8 (2C, imid-*C*O), 140.8, 130.2, 127.0 (6C, Ar-C), 118.0 (q, ^1J = 320 Hz, CF_3), 78.6 (C-2), 54.2 (OMe), 39.2 (C-3);

ESI-MS calcd. for $C_{13}H_6Cl_4F_3NO_7SNa$ $[M+Na]^+$: 541.8; found: 541.9;

IR (KBr): 1717 (s, imide and ester), 1417 (m), 1137 (m), 737 (m, ν(C-Cl));

Methyl (*R*)-3-azido-2-hydroxy-propanoate (17). Compound **10** (385 mg, 2.47 mmol) and $CuSO_4$•$5H_2O$ (6 mg, 25 μmol) were dissolved in water (6.5 mL). MeOH (22 mL) and $NaHCO_3$ (622 mg, 7.40 mmol) were added subsequently at r.t.. A solution of TfN_3[25] (7.4 mmol in 6.25 mL PhMe) was added dropwise to this solution, and the mixture was stirred vigorously at r.t. for 24 h. After quenching the reaction with benzyl amine (808 μL, 7.41 mmol), the solvents were removed *in vacuo*. The crude product was purified by column chromatography (CH_2Cl_2/MeOH, 20:1) to give the title compound (208 mg, 58%) as an oil.

R_f (CH_2Cl_2/MeOH, 9:1) = 0.70;

^{1}H-NMR (500.1 MHz, $CDCl_3$): *δ* 4.36 (X of ABMX, ^3J = 3.7, ^3J = 4.5 Hz, 1H, H-2), 3.82 (s, 3H, OMe), 3.63 (A of ABX, ^2J = 12.8, ^3J = 3.3 Hz, 1H, H-3a), 3.48 (B of ABX, ^2J = 12.8, ^3J = 4.2 Hz, 1H, H-3b), 3.15 (M of AM, ^3J = 5.1 Hz, 1H, OH);

^{13}C-NMR (125.8 MHz, $CDCl_3$): δ 172.8 (C-1), 70.6 (C-2), 53.9 (C-3), 53.2 (OMe);

This analytical data matched with the reported ^{1}H-NMR spectrum.[36]

Methyl (*R*)-3-azido-2-trifluoromethanesulfonyloxy-propanoate (18). To a solution of **17** (34 mg, 0.23 mmol) in dry CH_2Cl_2 under argon was added 2,6-di-*tert.*-butylpyridine (69 μL, 0.30 mmol) at -20 °C. After dropwise addition of Tf_2O (53 μL, 0.31 mmol), the reaction was stirred for 3 h at -20 °C. After dilution with CH_2Cl_2 (20 mL) the mixture was washed with aqueous KH_2PO_4 solution (1 M, 7.5 mL) and the aqueous phase was extracted with CH_2Cl_2 (2 x

15 mL). The combined organic layers were dried over Na_2SO_4 and the solvent was evaporated *in vacuo*. Purification by column chromatography (petrol ether/EtOAc, 15:1) yielded **18** (31.2 mg, 48%) as colorless oil.

R_f (petrol ether/EtOAc, 8:1) = 0.37;

^{1}H-NMR (500.1 MHz, $CDCl_3$): δ 5.21 (X of ABX, ^3J = 3.6, ^3J = 5.9 Hz, 1H, H-2), 3.87 (s, 3H OMe), 3.84-3.80 (m, 2H, H-3);

^{13}C-NMR (125.8 MHz, $CDCl_3$): δ 165.2 (C-1), 118.6 (q, ^1J = 320 Hz, CF_3), 81.2 (C-2), 54.0 (OMe), 51.7 (C-3);

***(R)*-3-Azido-2-hydroxy propanoic acid (19).** To a solution of **9** (363 mg, 2.56 mmol), $NaHCO_3$ (893 mg, 10.6 mmol) and $CuSO_4$•$5H_2O$ (24 mg, 0.10 mmol) in water (2 mL) were added TfN_3 in PhMe (1 M, 3.1 mL) and MeOH (12 mL). After stirring for 21 h, the organic solvents were removed, and the residue was taken up in water (20 mL). The olive green mixture (pH 8) was washed with CH_2Cl_2 (20 mL) and acidified with 6 N HCl. The resulting light red mixture was extracted with EtOAc (3 x 80 mL). The combined ester phases were dried over Na_2SO_4, filtered and the solvent was removed *in vacuo* to give 635 mg of a 1:1.1 mixture of the **19** (83%) and $TfNH_2$. The material was used in the next step without further purification.

R_f (*n*-BuOH/HOAc/H_2O/pyridine, 4:1:2:1) = 0.60;

^{1}H-NMR (500.1 MHz, DMSO-d_6): δ 4.22 (X of ABX, ^3J = 3.6, ^3J = 5.8 Hz, 1H, H-2), 3.47 (A of ABX, ^2J = 12.8, ^3J = 3.5, 1H, H-3a), 3.40 (B of ABX, ^2J = 12.8, ^3J = 5.9, 1H, H-3b);

^{13}C-NMR (125.8 MHz, DMSO-d_6): δ 173.2 (C-1), 69.8 (C-2), 53.5 (C-3);

ESI-MS calcd. for $C_3H_4N_3O_3$ $[M-H]^-$: 130.0; found: 129.9;

IR (KBr): 2117 (s, N_3), 1733 (s, CO);

This analytical data matched with the reported ^{1}H-NMR spectrum.[37]

Benzyl *(R)*-3-azido-2-hydroxy propanoate (20). To a solution of crude **19** (3.97 g) in DMF (30 mL) was added benzyl bromide (13 mL, 110 mmol) followed by dropwise addition of NEt_3 (19 mL, 137 mmol) at r.t.. Upon addition of the base, the exothermic reaction needed to be cooled with a water bath at r.t.. After stirring for 1 d, the mixture was concentrated, diluted with water (100 mL) and extracted with CH_2Cl_2 (3 x 100 mL). The combined organic phases were dried over Na_2SO_4, filtered and the solvents removed *in vacuo*. The crude product was purified by column chromatography (petrol ether/EtOAc, 4:1). The product (1.92 g, 63% from **9**) was obtained as white solid.

R_f (petrol ether/EtOAc, 4:1) = 0.55;

^{1}H-NMR (500.1 MHz, $CDCl_3$): δ 7.41-7.39 (m, 5H, Ar-H), 5.26 (s, 2H, PhC*H*$_2$), 4.40 (m, 1H, H-2), 3.64 (A of ABX, ^2J = 12.8, ^3J = 3.3 Hz, 1H, H-3a), 3.50 (B of ABX, ^2J = 12.8, ^3J = 4.3 Hz, 1H, H-3b);

^{13}C-NMR (125.8 MHz, $CDCl_3$): δ 172.3 (C-1), 134.9 (Ar-C^i), 129.02, 128.96, 128.67 (5C, Ar-C), 70.4 (C-2), 68.3 (Ph*C*H$_2$), 54.0 (C-3);

ESI-MS calcd. for $C_{10}H_{11}N_3O_3Na$ $[M+Na]^+$: 244.1; found: 243.9;

IR (KBr): 3392 (m), 2110 (s, N_3), 1739 (s, CO), 1199 (s);

$[\alpha]_D^{20}$ = +82.9 (*c* 1.04, $CHCl_3$);

This analytical data matched with the reported ^{1}H-NMR spectrum.[18]

Benzyl *(R)*-3-azido-2-*O*-trifluoromethanesulfonyl-propanoate (21). To a solution of **20** (1.00 g, 4.52 mmol) in dry CH_2Cl_2 (30 mL) under argon was added 2,6-di-*tert.*-butylpyridine (1.73 mL, 7.69 mmol) at -20 °C. After dropwise addition of Tf_2O (1.29 mL, 7.69 mmol), the reaction was stirred for 3 h at -20 °C. Then, the reaction was allowed to come to 0 °C. After stirring for 1 h, additional 2,6-di-*tert.*-butylpyridine (1.73 mL, 7.69 mmol) and Tf_2O (1.29 mL, 7.69 mmol) were added at 0 °C. After stirring for 3 h, the reaction mixture was diluted with CH_2Cl_2 (100 mL), washed with ice-cold aqueous KH_2PO_4 solution (1 M, 50 mL) and the aqueous phase was extracted with CH_2Cl_2 (2 x 100 mL).

The combined organic layers were dried over Na_2SO_4 and the solvent was evaporated *in vacuo*. Purification by column chromatography (petrol ether/EtOAc, 10:1) yielded **21** (1.40 g, 88%) as colorless oil.

R_f (petrol ether/EtOAc, 8:1) = 0.37;

^{1}H-NMR (500.1 MHz, $CDCl_3$): δ 7.35-7.41 (m, 5H, ArH), 5.31, 5.28 (A, B of AB, ^2J = 12.0 Hz, 2H, Ph*CH₂*), 5.23 (X of A'B'X, ^3J = 3.6, ^3J = 6.0 Hz, 1H, H-2), 3.84 (A' of A'B'X, ^2J = 13.9, ^3J = 3.6 Hz, 1H, H-3a), 3.81 (B' of A'B'X, ^2J = 13.9, ^3J = 6.0 Hz, 1H, H-3b);

^{13}C-NMR (125.8 MHz, $CDCl_3$): δ 164.4 (C-1), 133.9 (Ar-C^i), 129.1, 128.8, 128.6 (5C, Ar-C), 118.4 (q, ^1J = 320 Hz, CF_3), 81.0 (C-2), 69.0 (PhCH_2), 51.5 (C-3);

ESI-MS calcd. for $C_{11}H_{10}F_3N_3O_5SNa$ $[M+Na]^+$: 376.0; found: 376.0;

This analytical data matched with the reported ^{1}H- and ^{13}C-NMR spectrum.[18]

Benzyl (*2S*)-3-azido-2-*O*-{1-*O*-[(*1R,2R*)-2-*O*-(2,3,4-tri-*O*-benzyl-α-L-fuco-pyranosyl)-cyclohexyl] 6-*O*-benzyl-β-D-galactopyranos-3-yl} propionate (23). Trisaccharide mimetic **22**[16] (667 mg, 0.85 mmol) and Bu_2SnO (235 mg, 0.94 mmol) were dried in high vacuum for 1 h and subsequently dissolved in dry MeOH (50 mL) under argon with warming. Activated molecular sieves (3Å, 2.00 g) were added to the solution, which was then refluxed under argon for 15 h. Filtration of the suspension over celite, evaporation of the solvent, co-evaporation with dry PhMe and drying for 8 h in high vacuum gave a yellow oily substance (880 mg). This residue was dissolved in dry DME (10 mL) under argon and a solution of dry triflate **21** (384 mg, 1.09 mmol) in dry DME (4 mL) was added. When extensively dried CsF (168 mg, 1.11 mmol) was added, the solution turned slightly turbid. After stirring for 6 d at r.t., TLC indicated a ratio of acceptor to product of ca. 1:1, but no triflate. A solution of KF (10%) in aqueous KH_2PO_4 (1N, 50 mL) was added and after stirring for 1 h, the reaction was extracted with CH_2Cl_2 (3 x 50 mL). The combined organic phases were dried over Na_2SO_4 and the solvents were removed *in vacuo*. Purification of the residue by column chromatography

(CH_2Cl_2/iPrOH, gradient 0 to 4%) yielded **23** (329 mg, 39%) as a white solid.

R_f (PhMe/EtOAc, 3/1) = 0.55;

^{1}H-NMR (500.1 MHz, $CDCl_3$): δ 7.37-7.23 (m, 25 H, Ar-H), 5.23, 5.15 (A, B of AB, ^2J = 12.1 Hz, 2H, PhC*H*2OCO-), 4.96-4.94 (m, unresolved A' of A'B', 2H, Fuc-H1, 1H of PhC*H*2), 4.80 (A'' of A''B'', ^2J = 11.6 Hz, 1H, PhC*H*2), 4.74 (A''' of A'''B''', ^2J = 11.9 Hz, 1H, PhC*H*2), 4.68 (B'' of A''B'', B''' of A'''B''', ^2J = 11.7 Hz, 2H, PhC*H*2), 4.60 (B' of A'B', ^2J = 11.0 Hz, 1H, PhC*H*2), 4.57 (M of ABM, ^3J = 2.2, ^3J = 5.2 Hz, 1H, Lac-H2), 4.52, 4.48 (A'''' and B'''' of A''''B'''', ^2J = 12.0 Hz, 1H, PhC*H*2), 4.38 (q, ^3J = 7.8 Hz, 1H, Fuc-H5), 4.30 (d, ^3J = 7.7 Hz, 1H, Gal-H1), 4.02-3.97 (m, 3H, Fuc-H2, -H3, Gal-H4), 3.80 (t, ^3J = 8.3 Hz, 1H, Gal-H2), 3.76-3.74 (m, 2H, 1H of Cy-CH, Gal-H6a), 3.67 (br s, 1H, Fuc-H4), 3.65-3.50 (m, 5H, 1H of Cy-CH, Gal-H5, -H6b, Lac-H3a/b), 3.43 (dd, ^3J = 2.1, ^3J = 9.3 Hz 1H, Gal-H3), 2.02-1.81 (m, 2H, Cy-CH_2), 1.67-1.60 (m, 2H, Cy-CH_2), 1.39-1.12 (m, 4H, Cy-CH_2), 1.10 (d, ^3J = 6.3 Hz, 3H, Fuc-H6);

^{13}C-NMR (125.8 MHz, $CDCl_3$): δ 170.5 (Lac-C1), 139.2, 138.9, 138.8, 138.0, 134.8 (5C, Ar-C^i), 128.8-127.2 (25C, Ar-CH), 100.2 (Gal-C1), 94.6 (Fuc-C1), 82.8 (Gal-C3), 79.7 (CH), 79.0 (Lac-C2), 78.2 (Fuc-C4), 77.2 (Gal-C2), 76.3 (Cy-CH), 76.1 (CH), 74.9, 73.7 (2C, Ph*C*H2), 73.2 (CH), 73.01, 72.97 (2C, Ph*C*H2), 71.1 (CH), 68.9 (Gal-C6), 67.6 (Ph-*C*H2-O-CO-), 67.3 (CH), 66.3 (Fuc-C5), 52.8 (Lac-C3), 30.1, 29.7, 29.2, 23.3 (4C, Cy-CH_2), 16.6 (Fuc-C6);

ESI-MS calcd. for $C_{56}H_{65}N_3O_{13}Na$ $[M+Na]^+$: 1010.4; found: 1010.5;

IR (KBr): 2923 (s), 2106 (s, N_3), 1742 (s, CO), 1453 (m);

This analytical data matched with the reported ^{1}H-NMR spectrum.[18]

(2*S*)-3-Amino-2-*O*-{1-*O*-[(*1R,2R*)-2-*O*-(α-L-fucopyranosyl)-cyclohexyl]-β-D-galactopyranos-3-yl} propanoic acid (24). To a solution of protected azide **23** (293 mg, 297 µmol) in THF/water (5 mL; 4:1) was added $Pd(OH)_2$/C (50 mg) and the reaction mixture was hydrogenated at 4 bar for 72 h at r.t.. Additional $Pd(OH)_2$/C (100 mg) was added and the mixture hydrogenated at 4 bar for another 48 h. After dilution with MeOH/water (1:1, 20 mL), the

mixture was filtered and the solvents were removed to give crude **24** (144 mg), which was used without further purification.

^{1}H-NMR (500.1 MHz, D_2O): δ 4.99 (d, ^{3}J = 3.9 Hz, 1H, Fuc-H1), 4.63 (q, ^{3}J = 6.6 Hz, 1H, Fuc-H5), 4.52 (d, ^{3}J = 7.8 Hz, 1H, Gal-H1), 4.29 (dd, ^{3}J = 8.2, ^{3}J = 3.9 Hz, 1H, Lac-H2), 4.04 (d, ^{3}J = 2.8 Hz, 1H, Gal-H4), 3.91 (dd, ^{3}J = 10.4, ^{3}J = 6.6 Hz, 1H, Fuc-H3), 3.79 (dd, ^{3}J = 9.3, ^{3}J = 3.0 Hz, 1H, Fuc-H2), 3.77-3.71 (m, 4H, Fuc-H4, Gal-H5, -H6a, 1H of Cy-CH), 3.65-3.60 (m, 2H, Gal-H2, -H6b), 3.55 (dd, ^{3}J = 9.6, ^{3}J = 3.2 Hz, 1H, Gal-H3), 3.53-3.49 (m, 1H, 1H of Cy-CH), 3.41 (dd, ^{2}J = 13.8, ^{3}J = 3.9 Hz, 1H, Lac-H3a), 3.22 (dd, ^{2}J = 13.4, ^{3}J = 8.3 Hz, 1H, Lac-H3b), 2.14-2.04 (m, 2H, Cy-CH_2), 1.69 (br s, 2H, Cy-CH_2), 1.33-1.21 (m, 4H, Cy-CH_2), 1.19 (d, ^{3}J = 6.8 Hz, 3H, Fuc-H6);

^{13}C-NMR (125.8 MHz, D_2O): δ 171.0 (Lac-C1), 100.1 (Gal-C1), 95.9 (Fuc-C1), 82.9 (Gal-C3), 79.0 (Cy-CH), 77.6 (2C, Lac-C2 and Cy-CH), 74.7, 72.4, 70.8 (Fuc-C4, Gal-C2, -C5), 69.9 (Fuc-C3), 68.2 (Fuc-C2), 66.9 (Gal-C4), 66.8 (Fuc-C5), 61.8 (Gal-C6), 42.2 (Lac-C3), 29.9, 29.6, 29.5, 23.5 (4C, Cy-CH_2), 15.6 (Fuc-C6);

HR-MS calcd. for $C_{21}H_{36}NO_{13}$ $[M-H]^-$: 510.2192; found: 510.2197;

Sodium (2*S*)-3-azido-2-*O*-{1-*O*-[(*1R*,*2R*)-2-*O*-(α-L-fucopyranosyl)-cyclohexyl]-β-D-galactopyranos-3-yl} propionate (5). To a solution of crude **24** (126 mg) in MeOH/water (3 mL; 7.5:1) was added a solution of TfN_3[25] in PhMe (0.91 mL, 0.36 M). After stirring for 22 h at r.t., the reaction mixture was concentrated and purified by column chromatography (CH_2Cl_2/MeOH/water, 10/4/0.8) to give **5** (79 mg, contaminated with silica gel). This product was used for the 1,3-dipolar cycloaddition reactions without further purification. For analysis and biological testing, a small sample (6.6 mg) was purified by preparative HPLC to give pure **5** (4.7 mg, 35%, two steps) as free acid. The sodium salt was obtained by addition of an equimolar amount of aqueous NaOH (1 M, 87 µL) to an aqueous solution of the acid. Spectral data for the free acid:

R_f (CH_2Cl_2/MeOH/water, 10:4:0.8) = 0.26;

^{1}H-NMR (500.1 MHz, CD_3OD): δ 4.85 (d, ^3J = 3.8 Hz, 1H, Fuc-H1), 4.62 (q, ^3J = 6.5 Hz, 1H, Fuc-H5), 4.51 (dd, ^3J = 5.4, ^3J = 3.5 Hz, 1H, Lac-H2), 4.30 (d, ^3J = 7.7 Hz, 1H, Gal-H1), 4.00 (d, ^3J = 2.7 Hz, 1H, Gal-H4), 3.87 (dd, ^3J = 9.9, ^3J = 3.4 Hz, 1H, Fuc-H3), 3.78-3.74 (m, 2H, Lac-H3a, Gal-H6a), 3.73-3.63 (m, 5H, Fuc-H2, -H4, Gal-H2, -H6b, 1H of Cy-CH), 3.57-3.51 (m, 2H, 1H of Cy-CH, Lac-H3b), 3.45-3.41 (m, 2H, Gal-H3, -H5), 2.05-2.03 (m, 2H, Cy-CH_2), 1.71 (br s, 2H, Cy-CH_2), 1.41-1.22 (m, 4H, Cy-CH_2), 1.18 (d, ^3J = 6.4 Hz, 3H, Fuc-H6);

^{13}C-NMR (125.8 MHz, CD_3OD): δ 175.1 (Lac-C1), 102.8 (Gal-C1), 97.5 (Fuc-C1), 84.8 (Gal-C3), 80.5 (Lac-C2), 79.4 (CH), 77.5 (Cy-CH), 76.2 (Gal-C5), 74.0 (CH), 72.4 (Gal-C2), 71.7 (Fuc-C3), 70.2 (CH), 68.6 (Gal-C4), 67.6 (Fuc-C5), 62.9 (Gal-C6), 54.3 (Lac-C3), 31.1, 30.2, 24.6 (4C, Cy-CH_2), 16.7 (Fuc-C6);

HR-MS calcd. for $C_{21}H_{34}N_3O_{13}$ $[M-H]^-$: 536.2097; found: 536.2091;

IR (KBr, Na-form of **5**): 3412 (s), 2111 (s, N_3), 1606 (s, CO), 1447 (m);

$[\alpha]^{20}_D$ = -112.1 (*c* 0.24, MeOH);

Sodium (2*S*)-3-*N*-(1,2,3-triazol-1-yl)-2-*O*-{1-*O*-[(*1R*,*2R*)-2-*O*-(α-L-fuco-pyranosyl)-cyclohexyl]-β-D-galactopyranos-3-yl} propionate (6a). A solution of crude **5** (14 mg) and DIPEA (13.7 µL, 80 µmol) in MeCN/water (1.5 mL, 2:1) was thoroughly degassed by bubbling through argon. Subsequently, TMS-acetylene (7.4 µL, 54 µmol) and CuCl (5.3 mg, 80 µmol) were added and the mixture was stirred under argon. After 4 d, additional CuCl (5.3 mg, 80 µmol) and TMS-acetylene (15 µL, 110 µmol) were added and the reaction was stirred for another 5 d. The solvents were removed *in vacuo*, the residue was taken up in MeOH, filtered (0.2 µm) and evaporated. After drying the residue for 2 h in high vacuum, it was taken up in TBAF in THF (1 M, 1 mL) and HOAc was added (0.2 mL). The reaction was stirred for 30 min at r.t.. Evaporation of the solvent and purification of the residue by preparative HPLC, followed by ion-exchange chromatography (Dowex-50, Na-form) and a second preparative HPLC gave 2.0 mg (7%, 4 steps) of pure

6a as the free acid. The sodium salt was obtained by addition of equimolar amounts of aqueous NaOH to an aqueous solution of the acid. Spectral data for the free acid:

^{1}H-NMR (500.1 MHz, D_2O): δ 8.08 (s, 1H, triazol-H), 7.78 (s, 1H, triazol-H), 4.95 (d, ^3J = 3.5 Hz, 1H, Fuc-H1), 4.90 (dd, ^2J = 14.1, ^3J = 3.4 Hz 1H, Lac-H3a), 4.80-4.73 (m, 1H, Lac-H3b), 4.63-4.57 (m, 2H, Fuc-H5, Lac-H2), 4.41 (d, ^3J = 7.0 Hz, 1H, Gal-H1), 4.01 (d, ^3J = 1.7 Hz, 1H, Gal-H4), 3.87 (dd, ^3J = 10.4, ^3J = 3.4 Hz, 1H, Fuc-H3), 3.77-3.65 (m, 5H, Fuc-H2, -H4, Gal-H6a/b, 1H of Cy-CH), 3.54 (dd, ^3J = 7.4, ^3J = 4.2 Hz, 1H, Gal-H5), 3.50-3.43 (m, 3H, Gal-H2, -H3, 1H of Cy-CH), 2.09-2.02 (m, 2H, Cy-CH_2), 1.69-1.63 (m, 2H, Cy-CH_2), 1.27-1.17 (m, 4H, Cy-CH_2), 1.15 (d, ^3J = 6.9 Hz, 3H, Fuc-H6);

^{13}C-NMR (125.8 MHz, D_2O, HCO_2H std.): δ 174.9 (Lac-C1), 99.7 (Gal-C1), 95.5 (Fuc-C1), 82.1 (Gal-C3), 78.3 (CH), 78.1 (CH), 77.2 (Lac-C2), 74.3 (CH), 72.0 (Gal-C5), 70.2 (Fuc-C3), 69.5 (Fuc-C2), 67.9 (Gal-C4), 67.6 (Fuc-C5), 66.4 (2C, CH), 61.3 (Gal-C6), 52.0 (Lac-C3), 29.6, 29.1, 23.1 (4C, Cy-CH_2), 15.2 (Fuc-C6);

HR-MS calcd. for $C_{23}H_{37}N_3O_{13}Na$ $[M+H]^+$: 586.2224; found: 586.2214;

Sodium (2*S*)-3-*N*-[4-benzyl-1,2,3-triazol-1-yl]-2-*O*-{1-*O*-[(*1R*,*2R*)-2-*O*-(α-L-fucopyranosyl)-cyclohexyl]-β-D-galactopyranos-3-yl} propionate (6b). Compound **5** (7.1 mg, 13 µmol) was dissolved in a solution of 1-phenyl-prop-2-yne (16.4 µL, 132 µmol) in EtOH/water (1 mL, 3:2). Copper powder (1.0 mg, 15.7 µmol) and $CuSO_4 \cdot 5H_2O$ (3.5 mg, 14 µmol) were added, and the reaction was vigorously stirred for 24 h at r.t.. Filtration through cotton, evaporation of the solvents, and microfiltration (0.2 µm) of a solution of the residue in MeOH/water (1:1) gave the crude product which was purified by preparative HPLC. Pure **6b** was obtained as free acid (5.4 mg, 35% over 3 steps) and was subsequently transformed in to the sodium salt by addition of equimolar amounts of NaOH. Spectral data for the free acid:

R_f (CH_2Cl_2/MeOH/water, 10:4:0.8) = 0.34;

^{1}H-NMR (500.1 MHz, CD_3OD): δ 8.00 (s, 1H, triazol-H), 7.30-7.22 (m, 4H, Ar-

H), 7.20-7.17 (m, 1H, p-Ar-H), 4.88-4.84 (m, 2H, Fuc-H1, Lac-H3a), 4.74-4.68 (m, 1H, Lac-H3b), 4.62-4.56 (m, 2H, Fuc-H5, Lac-H2), 4.26 (d, ^3J = 7.7 Hz, 1H, Gal-H1), 4.03 (br s, 2H, PhC*H₂*), 3.93 (br s, 1H, Gal-H4), 3.86 (dd, ^3J = 10.0, ^3J = 3.3 Hz, 1H, Fuc-H3), 3.77-3.64 (m, 5H, Fuc-H2, -H4, Gal-H6a/b, 1H of Cy-CH), 3.61-3.52 (m, 2H, Gal-H2, 1H of Cy-CH), 3.41 (m, 1H, Gal-H5), 3.55-3.31 (m, 1H, Gal-H3), 2.07-2.01 (m, 2H, Cy-CH_2), 1.73-1.67 (m, 2H, Cy-CH_2), 1.40-1.22 (m, 4H, Cy-CH_2), 1.18 (d, ^3J = 6.5 Hz, 3H, Fuc-H6);

^{13}C-NMR (125.8 MHz, CD_3OD): δ 174.5 (Lac-C1), 140.7 (triazol-C^i), 131.4 (Ar-C^i), 129.8, 129.7 (4 Ar-CH), 127.6 (2C, triazol-*C*H, Ar-CH), 102.8 (Gal-C1), 97.4 (Fuc-C1), 84.9 (Gal-C3), 79.5 (Lac-C2), 77.5 (Cy-CH), 76.1 (Cy-CH), 74.0 (Gal-C5), 72.1 (Fuc-C4), 71.7 (Gal-C2), 70.5 (Fuc-C3), 68.0 (Fuc-C2), 67.6 (2C, Fuc-C5, Gal-C4), 63.0 (Gal-C6), 53.7 (Lac-C3), 32.7 (Ph*C*H_2), 31.1, 30.9, 24.6 (4C, Cy-CH_2), 16.8 (Fuc-C6);

HR-MS calcd. for $C_{30}H_{43}N_3O_{13}Na$ $[M+H]^+$: 676.2694; found: 676.2696;

$[\alpha]^{20}_D$ = -67.7 (*c* 0.27, MeOH);

Sodium (2*S*)-3-*N*-[4-(3-thiophenyl)-1,2,3-triazol-1-yl]-2-*O*-{1-*O*-[(*1R*,*2R*)-2-*O*-(α-L-fucopyranosyl)-cyclohexyl]-β-D-galactopyranos-3-yl} propionate (6c). In analogy to **6b**, compound **6c** was prepared from **5** (7.1 mg, 13 µmol) and 3-ethynyl-thiophene (12.7 µL, 129 µmol). After HPLC, 5.3 mg (35% over 3 steps) of the free acid were obtained, which was transformed into the sodium salt as described above. Spectral data for the free acid:

R_f (CH_2Cl_2/MeOH/water, 10:4:0.8) = 0.27;

^{1}H-NMR (500.1 MHz, CD_3OD): δ 8.54 (s, 1H, triazol-H), 7.75-7.74 (m, 1H, Ar-H), 7.50-7.48 (m, 2H, Ar-H), 4.98 (dd, ^2J = 14.3, ^3J = 2.9 Hz, 1H, Lac-H3a), 4.84 (d, ^3J = 3.9 Hz, 1H, Fuc-H1), 4.77 (dd, ^2J = 14.2, ^3J = 6.8 Hz, 1H, Lac-H3b), 4.64 (dd, ^3J = 6.7, ^3J = 2.9 Hz, 1H, Lac-H2), 4.58 (q, ^3J = 6.6 Hz, 1H, Fuc-H5), 4.28 (d, ^3J = 7.7 Hz, 1H, Gal-H1), 3.97 (d, ^3J = 2.7 Hz, 1H, Gal-H4), 3.86 (dd, ^3J = 10.1, ^3J = 3.4 Hz, 1H, Fuc-H3), 3.75 (dd, ^2J = 11.4, ^3J = 6.9 Hz, 1H, Gal-H6a), 3.72-3.62 (m, 5H, Fuc-H2, -H4, Gal-H2, Gal-H6b, 1H of Cy-CH), 3.54 (dt, ^3J = 9.4, ^3J = 4.5 Hz, 1H, 1H of Cy-CH), 3.44-3.38 (m, 2H, Gal-

H3, -H5), 2.07-2.01 (m, 2H, Cy-CH_2), 1.74-1.66 (m, 2H, Cy-CH_2), 1.43-1.22 (m, 4H, Cy-CH_2), 1.15 (d, ^3J = 6.7 Hz, 3H, Fuc-H6);

^{13}C-NMR (125.8 MHz, CD_3OD): δ 145.1 (triazol-C^i), 133.2 (Ar-C^i), 127.6, 127.0 (2C, Ar-C), 124.2 (triazol-*C*H), 122.2 (Ar-C), 102.7 (Gal-C1), 97.4 (Fuc-C1), 85.0 (Gal-C3), 79.5 (CH), 77.5 (Cy-CH), 76.0 (Gal-C5), 74.0 (CH), 72.0 (Gal-C2), 71.7 (Fuc-C3), 70.2 (CH), 67.9 (Gal-C4), 67.6 (Fuc-C5), 63.0 (Gal-C6), 54.1 (Lac-C3), 31.1, 30.2, 24.6 (4C, Cy-CH_2), 16.8 (Fuc-C6);

HR-MS calcd. for $C_{27}H_{39}N_3O_{13}SNa$ $[M+H]^+$: 668.2101; found: 668.2100;

$[\alpha]^{20}_D$ = -60.8 (*c* 0.27, MeOH, free acid);

Sodium (2*S*)-3-*N*-[4-(3-pyridyl)-1,2,3-triazolyl]-2-*O*-{1-*O*-[(*1R,2R*)-2-*O*-(α-L-fucopyranosyl)-cyclohexyl]-β-D-galactopyranos-3-yl} propionate (6d). In analogy to **6b**, compound **6d** was prepared from **5** (7.1 mg, 13 µmol) and 3-ethynyl-pyridine (15 mg, 145 µmol). After HPLC, 2.2 mg (15% over 3 steps) of the free acid were obtained, which was transformed into the sodium salt as described above. Spectral data for the free acid:

R_f (CH_2Cl_2/MeOH/water, 10:4:0.8) = 0.22;

^{1}H-NMR (500.1 MHz, CD_3OD): δ 9.04 (br s, 1H, Ar-H), 8.78 (s, 1H, triazol-H), 8.52 (br s, 1H, Ar-H), 8.30 (d, ^3J = 7.6 Hz, 1H, Ar-H), 7.55-7.51 (m, 1H, Ar-H), 5.02 (dd, ^2J = 14.5, ^3J = 2.8 Hz, 1H, Lac-H3a), 4.83 (d, ^3J = 3.5 Hz, 1H, Fuc-H1), 4.79 (dd, ^2J = 14.2, ^3J = 7.1 Hz, 1H, Lac-H3b), 4.72 (dd, ^3J = 7.0, ^3J = 2.7 Hz, 1H, Lac-H2), 4.59 (q, ^3J = 6.7 Hz, 1H, Fuc-H5), 4.27 (d, ^3J = 7.2 Hz, 1H, Gal-H1), 3.98 (d, ^3J = 2.3 Hz, 1H, Gal-H4), 3.85 (dd, ^3J = 9.8, ^3J = 3.1 Hz, 1H, Fuc-H3), 3.75 (dd, ^2J = 11.7, ^3J = 7.2 Hz, 1H, Gal-H6a), 3.71-3.65 (m, 4H, Fuc-H2, -H4, Gal-H6b, 1H of Cy-CH), 3.62 (t, ^3J = 8.0 Hz, 1H, Gal-H2), 3.52 (dt, ^3J = 9.4, ^3J = 4.4 Hz, 1H, 1H of Cy-CH), 3.44-3.38 (m, 2H, Gal-H3, -H5), 2.07-2.00 (m, 2H, Cy-CH_2), 1.72-1.65 (m, 2H, Cy-CH_2), 1.41-1.19 (m, 4H, Cy-CH_2), 1.13 (d, ^3J = 6.5 Hz, 3H, Fuc-H6);

^{13}C-NMR (125.8 MHz, CD_3OD, selected resonances from ^{1}H,^{13}C-HSQC): δ 147.2, 135.1, 126.1, 124.8 (4C, Ar-*C*H), 102.3 (Gal-C1), 97.0 (Fuc-C1), 84.3

(Gal-C3), 78.6 (Lac-C2), 77.0 (CH), 75.9 (CH), 73.1 (CH), 71.9 (CH), 71.3 (Fuc-C3), 71.2 (CH), 67.5 (Gal-C4), 66.7 (Fuc-C5), 62.8 (Gal-C6), 54.1 (Lac-C3), 31.1, 30.2, 24.6 (4C, Cy-CH_2), 16.8 (Fuc-C6);

HR-MS calcd. for $C_{28}H_{40}N_4O_{13}Na$ $[M+H]^+$: 663.2490; found: 663.2490;

$[\alpha]^{20}_D$ = -44.7 (*c* 0.11, MeOH, free acid);

Sodium (2*S*)-3-*N*-[4-(3-trifluoromethyl-phenyl)-1,2,3-triazol-1-yl]-2-*O*-{1-*O*-[(*1R*,*2R*)-2-*O*-(α-L-fucopyranosyl)-cyclohexyl]-β-D-galactopyranos-3-yl} propionate (6e). In analogy to **6b**, compound **6e** was prepared from **5** (7.1 mg, 13 µmol) and 3-ethynyl-trifluoromethylbenzene (18.7 µL, 130 µmol). After HPLC, 5.9 mg (34% over 3 steps) of the free acid were obtained, which was transformed into the sodium salt as described above. Spectral data for the free acid:

R_f (CH_2Cl_2/MeOH/water, 10:4:0.8) = 0.35;

^{1}H-NMR (500.1 MHz, CD_3OD): δ 8.75 (s, 1H, triazol-H), 8.16 (s, 1H, Ar-H), 8.10-8.09 (m, 1H, Ar-H), 7.65-7.61 (m, 2H, Ar-H), 5.01 (dd, ^2J = 13.7, ^3J = 2.3 Hz, 1H, Lac-H3a), 4.83 (d, ^3J = 3.6 Hz, 1H, Fuc-H1), 4.82-4.76 (m, 2H, Lac-H2, -H3b), 4.59 (q, ^3J = 6.7 Hz, 1H, Fuc-H5), 4.27 (d, ^3J = 7.6 Hz, 1H, Gal-H1), 3.99 (d, ^3J = 2.5 Hz, 1H, Gal-H4), 3.85 (dd, ^3J = 10.1, ^3J = 3.3 Hz, 1H, Fuc-H3), 3.75 (dd, ^2J = 11.4, ^3J = 6.9 Hz, 1H, Gal-H6a), 3.72-3.64 (m, 4H, Fuc-H2, -H4, Gal-H6b, 1H of Cy-CH), 3.64-3.61 (m, 1H, Gal-H2), 3.52 (dt, ^3J = 9.4, ^3J = 4.3 Hz, 1H, 1H of Cy-CH), 3.41-3.41 (m, 2H, Gal-H3, -H5), 2.04-1.99 (m, 2H, Cy-CH_2), 1.68 (br s, 2H, Cy-CH_2), 1.41-1.21 (m, 4H, Cy-CH_2), 1.13 (d, ^3J = 6.6 Hz, 3H, Fuc-H6);

^{13}C-NMR (125.8 MHz, CD_3OD): δ 174.5 (Lac-C1), 147.4 (triazol-C^i), 133.2 (Ar-C^i), 131.0, 130.4 (2C, Ar-*C*H), 125.8 (triazol-CH), 125.0, 123.4 (2C, Ar-C), 102.8 (Gal-C1), 97.5 (Fuc-C1), 84.7 (Gal-C3), 79.6 (Lac-C2), 79.0 (Cy-CH), 77.6 (Cy-CH), 76.1 (Gal-C5), 74.0 (Fuc-C4), 72.2 (Gal-C2), 71.7 (Fuc-C3), 70.2 (Fuc-C2), 68.2 (Gal-C4), 67.5 (Fuc-C5), 62.9 (Gal-C6), 53.8 (Lac-C3), 31.1, 30.3, 24.6 (4C, Cy-CH_2), 16.7 (Fuc-C6);

HR-MS calcd. for $C_{30}H_{40}F_3N_3O_{13}Na$ $[M+H]^+$: 730.2411; found: 730.2419;

$[\alpha]^{20}_D$ = -52.9 (*c* 0.27, MeOH, free acid);

Sodium (2*S*)-3-*N*-[4-(benzotriazolylmethyl)-1,2,3-triazol-1-yl]-2-*O*-{1-*O*-[(*1R*,*2R*)-2-*O*-(α-L-fucopyranosyl)-cyclohexyl]-β-D-galactopyranos-3-yl} propionate (6f). In analogy to **6b**, compound **6f** was prepared from **5** (7.1 mg, 13 µmol) and 1-*N*-benzotriazolyl-prop-2-yne (20.7 mg, 132 µmol). After HPLC, 5.4 mg (33% over 3 steps) of the free acid were obtained, which was transformed into the sodium salt as described above. Spectral data for the free acid:

R_f (CH_2Cl_2/MeOH/water, 10:4:0.8) = 0.30;

^{1}H-NMR (500.1 MHz, CD_3OD): δ 8.37 (br s, 1H, triazol-Ar-H), 7.98 (A of ABCD, ^3J = 7.5 Hz, 1H, Bt-H), 7.81 (D of ABCD, ^3J = 7.5 Hz, 1H, Bt-H), 7.55 (C of ABCD, ^3J = 7.5 Hz, 1H, Bt-H), 7.43 (C of ABCD, ^3J = 7.5 Hz, 1H, Bt-H), 6.03 (br s, 2H, Bt-C*H*$_2$-triazol), 4.92-4.86 (m, 2H, Fuc-H1, Lac-H3a), 4.74 (dd, ^3J = 13.5, ^3J = 6.5 Hz, 1H, Lac-H3b), 4.63-4.61 (m, 1H, Lac-H2), 4.59 (q, ^3J = 6.4 Hz, 1H, Fuc-H5), 4.24 (d, ^3J = 7.8 Hz, 1H, Gal-H1), 3.93 (br s, 1H, Gal-H4), 3.87 (dd, ^3J = 10.0, ^3J = 3.5 Hz, 1H, Fuc-H3), 3.77-3.65 (m, 5H, Fuc-H2, -H4, Gal-H6a/b, 1H of Cy-CH), 3.61-3.53 (m, 2H, Gal-H2, 1H of Cy-CH), 3.41-3.38 (m, 1H, Gal-H5), 3.33-3.31 (m, 1H, Gal-H3), 2.08-2.01 (m, 2H, Cy-CH_2), 1.71 (br s, 2H, Cy-CH_2), 1.42-1.22 (m, 4H, Cy-CH_2), 1.18 (d, ^3J = 6.7 Hz, 3H, Fuc-H6);

^{13}C-NMR (125.8 MHz, CD_3OD): δ 176.8 (Lac-C1), 147.2 (Bt-C^i), 142.9 (triazol-C^i), 134.4 (Bt-C^i), 139.1 (Bt-CH), 127.4 (triazol-CH), 125.9 (Bt-CH), 120.1 (Bt-CH), 112.0 (Bt-CH), 102.7 (Gal-C1), 97.4 (Fuc-C1), 85.1 (Gal-C3), 79.5 (Cy-CH), 78.7 (Lac-C2), 77.4 (Cy-CH), 76.0 (Gal-C5), 74.0 (Fuc-C4), 72.8 (Gal-C2), 71.7 (Fuc-C3), 70.2 (Fuc-C2), 67.9 (Gal-C4), 67.6 (Fuc-C5), 63.0 (Gal-C6), 53.8 (Lac-C3), 44.6 (Bt-CH_2), 30.8, 24.4 (4C, Cy-CH_2), 16.8 (Fuc-C6);

HR-MS calcd. for $C_{30}H_{42}N_6O_{13}Na$ $[M+Na]^+$: 717.2708; found: 717.2705;

$[\alpha]^{20}_D$ = -53.7 (*c* 0.27, MeOH);

Benzyl (2*S*)-3-*N*-[4-(benzotriazolylmethyl)-1,2,3-triazol-1-yl]-2-*O*-{1-*O*-[(*1R*,*2R*)-2-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-cyclohexyl] 6-*O*-benzyl-β-D-galactopyranos-3-yl} propionate (25). 3-Benzotriazolyl propyne (95 mg, 60.7 µmol) was dissolved in MeOH/water (1 mL; 9:1) and copper powder (1 mg) was suspended in this solution. A solution of azide **23** (10 mg, 10.1 µmol) in MeOH/water (1 mL, 9:1) was added and the reaction mixture was stirred for 24 h at r.t.. Additional copper powder (5 mg) was added and stirring continued for further 24 h before the solvent was removed *in vacuo*. Purification by column chromatography (PhMe/EtOAc 1:1) yielded **25** (10 mg; 86%) as a white solid.

R_f (PhMe/EtOAc, 1:1) = 0.35;

^{1}H-NMR (500.1 MHz, $CDCl_3$): δ 7.97-7.92 (m, 1H, Bt-H), 7.85 (br s, 1H, triazol-H), 7.62-7.57 (m, 1H, Bt-H), 7.38 (m, 1H, Bt-H), 7.32-7.13 (m, 26H, 25 Ar-H, 1 Bt-H), 5.80 (br s, 2H, Bt-CH_2-triazol), 5.13, 5.06 (A, B of AB, ^2J = 11.9 Hz, 2H, PhCH_2OCO-), 4.88-4.86 (m, 2H, Fuc-H1, 1H of PhCH_2), 4.74-4.70 (m, 2H, 1H of PhCH_2, Lac-H3a), 4.66 (A''' of A'''B''', ^2J = 12.0 Hz, 1H, PhCH_2), 4.61 (B'' of A''B'', B''' of A'''B''', ^2J = 11.7 Hz, 2H, PhCH_2), 4.53 (B' of A'B', ^2J = 11.5 Hz, 1H, PhCH_2), 4.50-4.46 (m, 2H, Lac-H2, -H3b), 4.42, 4.38 (A'''' and B'''' of A''''B'''', ^2J = 12.0 Hz, 1H, PhCH_2), 4.31 (q, ^3J = 6.3 Hz, 1H, Fuc-H5), 4.07 (d, ^3J = 7.7 Hz, 1H, Gal-H1), 3.94 (dd, ^3J = 10.1, ^3J = 3.5 Hz, 1H, Fuc-H2), 3.89 (dd, ^3J = 10.1, ^3J = 2.7 Hz, 1H, Fuc-H3), 3.77 (d, ^3J = 2.5 Hz, 1H, Gal-H4), 3.66-3.60 (m, 3H, Fuc-H4, Gal-H6a, 1H of Cy-CH), 3.57-3.51 (m, 3H, Gal-H2, -H6b, 1H of Cy-CH,), 3.31 (t, ^3J = 6.0 Hz, 1H, Gal-H5), 3.39 (dd, ^3J = 2.7, ^3J = 9.3 Hz, 1H, Gal-H3), 1.97-1.87 (m, 2H, Cy-CH_2), 1.66-1.56 (m, 2H, Cy-CH_2), 1.35-1.13 (m, 4H, Cy-CH_2), 1.01 (d, ^3J = 6.5 Hz, 3H, Fuc-H6);

^{13}C-NMR (125.8 MHz, $CDCl_3$): δ 170.2 (Lac-C1), 139.4, 139.1, 139.0, 138.2, 134.5 (5C, Ar-C^i), 129.1-127.5 (Ar-C), 124.4, 120.0, 110.4 (3C, Bt-Ar-C), 100.7 (Gal-C1), 95.0 (Fuc-C1), 83.6 (Gal-C3), 79.9 (Fuc-C3), 78.4 (Fuc-C4), 77.9 (Cy-CH), 77.4 (Lac-C2), 76.6 (Fuc-C2), 76.3 (Cy-CH), 75.1, 73.8, 73.2 (4C, Ph*C*H_2), 73.0 (Gal-C5), 70.6 (Gal-C2), 69.0 (Gal-C6), 68.4 (Ph*C*H_2-O-CO-), 66.7 (Gal-C4), 66.4 (Fuc-C5), 52.3 (Lac-C3), 44.0 (Bt-*C*H_2-triazol), 30.2,

29.9, 29.4, 23.4 (4C, Cy-CH_2), 16.9 (Fuc-C6);

ESI-MS calcd. for $C_{65}H_{72}N_6O_{13}Na$ $[M+Na]^+$: 1167.5; found: 1167.7;

Sodium (2*S*)-3-*N*-[4-(tetrahydrobenzotriazolylmethyl)-1,2,3-triazol-1-yl]-2-*O*-{1-*O*-[(*1R,2R*)-2-*O*-(α-L-fucopyranosyl)-cyclohexyl]-β-D-galacto-pyranos-3-yl} propionate (6g). To a solution of **25** (10 mg, 8.7 µmol) in dioxane/water (3 mL, 4:1) was added $Pd(OH)_2/C$ (18 mg) and the reaction mixture was hydrogenated at 4 bar for 48 h at r.t.. After filtration and removal of the solvents, the crude product was purified by preparative HPLC to give the free acid of **6g** (2.4 mg, 39%). The sodium salt was obtained as a white powder by addition of stoichiometric amounts of dilute NaOH (1 N, 30 µL) to an aqueous solution of **6g** followed by lyophilization. Spectral data for the free acid:

^{1}H-NMR (500.1 MHz, CD_3OD): δ 8.27 (br s, 1H, triazol-Ar-H), 5.57 (br s, 2H, 4H-Bt-CH_2-triazol), 4.93-4.84 (m, 2H, Fuc-H1, Lac-H3a), 4.75 (dd, ^3J = 14.6, ^3J = 5.9 Hz, 1H, Lac-H3b), 4.64-4.60 (m, 1H, Lac-H2), 4.59 (q, ^3J = 6.8 Hz, 1H, Fuc-H5), 4.27 (d, ^3J = 8.0 Hz, 1H, Gal-H1), 3.94 (br s, 1H, Gal-H4), 3.86 (dd, ^3J = 9.9, ^3J = 3.6 Hz, 1H, Fuc-H3), 3.77-3.65 (m, 5H, Fuc-H2, -H4, Gal-H6a/b, 1H of Cy-CH), 3.61-3.52 (m, 2H, Gal-H2, 1H of Cy-CH), 3.41 (t, ^3J = 5.6 Hz, 1H, Gal-H5), 3.35-3.34 (m, 1H, Gal-H3), 2.70-2.64 (m, 4H, 4H-Bt-H), 2.07-2.02 (m, 2H, Cy-CH_2), 1.87-1.77 (m, 4H, 4H-Bt-H), 1.73-1.68 (m, 2H, Cy-CH_2), 1.41-1.22 (m, 4H, Cy-CH_2), 1.18 (d, ^3J = 6.6 Hz, 3H, Fuc-H6);

HR-MS calcd. for $C_{30}H_{46}N_6O_{13}Na$ $[M+Na]^+$: 721.3021; found: 721.3021;

$[\alpha]^{20}_D$ = -40.8 (*c* 0.12, MeOH);

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26. In the course of the tin-acetal mediated alkylation of the 3-position of galactose in trisaccharide mimic **23**, also the 2-position was partially alkylated. This 2-*O* alkylated product could not be observed in the NMR spectrum of the resulting tetrasaccharide mimic **23**. However, during the following transformations, the 2-*O* alkyl isomer accumulated, resulting in the isolation of 0.5 mg of the 2-*O* isomer of **6e** (3-*O* vs. 2-*O* isomer ratio of 12:1). For the other library members, 2-*O* isomers were also observed, could be separated from the desired 3-*O* isomer but were discarded. Interestingly, the introduction of the lactate group of **6e** in the 2-*O* position led to a significant change of the ligand's internal conformation, as observed by the chemical shift of the Fuc-H5 proton in **6e**. In the bioactive conformation, the Fuc-H5 proton is deshielded by the β-face of galactose, resulting in a chemical shift of 4.59 ppm. For the 2-*O* isomer of **6e**, the chemical shift of the corresponding Fuc-H5 proton has shifted by 1 ppm and is now found at 3.61 ppm. This value corresponds closely to 3.80-3.68 ppm of methyl fucosides reported by Yang[27] in 1991, indicating a total loss of stacking of the fucose α-face with the galactose β-face which is essential for binding to E-selectin.

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2.2b A safe and convenient method for the preparation of triflyl azide, and its use in diazo transfer reactions to primary amines

In the course of the studies for the synthesis of the library precursor (*vide supra, chapter 2.2*), an improved methodology for the transformation of primary amines into azides *via* the copper-catalyzed diazo transfer was found.

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A safe and convenient method for the preparation of triflyl azide, and its use in diazo transfer reactions to primary amines

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Abstract—A safe and convenient method for the copper(II)-catalyzed diazo transfer from triflyl azide to primary amines is reported. By replacing CH_2Cl_2 by toluene the formation of hazardous side products, for example, azido-chloromethane and diazidomethane can be avoided.
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1. Introduction

Azides are versatile functional groups that undergo a variety of reactions, such as 1,3-dipolar cycloadditions and rearrangement processes, or act as precursors for nitrene chemistry.[1] In addition, azides are widely used as convenient protecting groups for primary amines. Traditionally, the introduction of azides has been achieved by substituting appropriate leaving groups with inorganic azide. However, in the last 15 years, diazo transfer from triflyl azide (TfN_3) to primary amines has found wide use. Although this approach has already been published by Cavender and Shiner[2] in 1972, it was rarely applied until the early 1990s.[3]

In 1996, Wong and co-workers introduced[4] a mild transition metal-catalyzed modification of the original diazo transfer procedure, and in 2002, he elucidated[5] its copper(II)-catalyzed mechanism. A successful application in aminoglycoside synthesis was published by the same group.[6]

However, a general drawback of diazo transfer is the formation of hazardous intermediates, for example, TfN_3, which can only be handled in solution.[2] Furthermore, in all published procedures for the preparation of triflyl azide and its application in the diazo transfer to primary amines, triflic anhydride and sodium azide are used in a biphasic system of CH_2Cl_2/H_2O. Under these reaction conditions, nucleophilic substitutions on CH_2Cl_2 leading to azido-chloromethane and/or diazidomethane tend to occur. Thus, the formation of perilous diazidomethane has been reported by Hassner et al.[7,8] upon shaking an azide anion source in CH_2Cl_2. When this reaction mixture was pipetted or used for IR spectroscopy (films between NaCl plates) explosions occurred.[8] As a consequence of the formation of diazidomethane several additional explosions were reported, leading to the destruction of facilities and severely injured staff. Therefore, several laboratories reported that they banned the use of halogenated solvents in combination with sodium azide.[9–11]

2. Results and discussion

In order to avoid the formation of azido-chloromethane and diazidomethane, as well as high concentrations thereof, we tested various replacements for CH_2Cl_2. Toluene was identified as a solvent replacement for the preparation of TfN_3 to avoid the formation of hazardous diazidomethane and potentially hazardous azido-chloromethane byproducts in the typical solvent used, and furthermore, for the application of TfN_3 in the diazo transfer reaction. Toluene is inert towards sodium azide under the employed conditions and, because of its boiling point, high concentrations of TfN_3 can easily be avoided (Fig. 1).

Wong reported that carefully adjusted homogeneous reaction conditions are a prerequisite for a successful diazotransfer.[4,5] Therefore, three different solvent ratios leading to monophasic mixtures (see ternary phase

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Tf_2O → (NaN_3, toluene/H_2O, 0 - 10 °C, 2.5 h) → TfN_3

1 → (TfN_3, $CuSO_4$, $NaHCO_3$, toluene/MeOH/H_2O, 14 h, r.t.) → 2, quant.

Figure 1. Preparation of TfN_3 and diazo transfer to a primary amine using toluene as co-solvent.

diagram[13]) for H_2O/PhMe/MeOH were tested (Table 1, entries 1–3), using methyl 6-amino-6-deoxy-α-D-glucopyranoside (**1**) as starting material. Under all three conditions, the desired azide **2** was obtained in good to excellent yield. Furthermore, the diazo transfer also turned out to be successful under heterogeneous conditions (Table 1, entry 4).

Table 1. Diazo transfer reaction **1** → **2** using different solvent ratios

Entry	Solvent ratios (H_2O/PhMe/MeOH)	Yield[a] (%)
1	1/1.7/6.7, homogeneous	Quant.
2	1.3/1/5.4, homogeneous	74
3	1/4.3/10, homogeneous	Quant.
4	1/1/1, heterogeneous	96

[a] Isolated yields.

To test the scope of this modified version of the diazo transfer reaction, a number of primary amines were

Table 2. Results of the diazo transfer under homogeneous conditions using H_2O/PhMe/MeOH 1/1.7/6.7 (see also Refs. 12 and 14–17)

Entry	Amine	Azide	Time (h)	Yield[a] (%)
1	**1**	**2**[12]	14	Quant.
2	**3**	**4**[b,3]	21	72
3	**5**	**6**[b,3]	36	50
4	**7**	**8**[b,3]	36	19
5	**9**	**10**[14]	14	Quant.
6	**11**	**12**[15]	14	83
7	**13**	**14**[16]	14	93
8	**15**	**16**[17]	14	52

[a] Isolated yields.

[b] For purification the crude products were acetylated.

investigated. The reactions were carried out in a monophasic solvent mixture of H_2O/PhMe/MeOH 1/1.7/6.7. The triflyl azide solution in toluene was added to a mixture of the amine, sodium hydrogencarbonate and copper(II) sulfate in water, followed by methanol to obtain a homogeneous turquoise solution. The TLC indicated the reactions to be complete within 14 h, except for the amines **3**, **5** and **7**. Even after 21 and 36 h, respectively, they yielded the corresponding azides **4**, **6** and **8** only in moderate yields (Table 2, entries 2–4). The decrease in yield for **4**, **6** and **8** is probably due to the increasing steric hindrance, which is in agreement with results reported by Vasella et al.[3]

In conclusion, we have shown that (1) toluene can be used instead of dichloromethane for the generation of triflyl azide and (2) the TfN_3/toluene solution can directly be applied to the diazo transfer to primary amines. This method is therefore significantly safer than the previously reported procedure, because highly hazardous diazidomethane can no longer be formed. Furthermore, the reaction proceeds smoothly under homogeneous, but also heterogeneous conditions (Table 1). This is a significant improvement, considering the necessity of the reported delicate adjustment of the solvent ratio using CH_2Cl_2.[5] Because the safety risk originating from the formation of azido-chloromethane and diazidomethane can be avoided, even an industrial application of this novel diazo transfer methodology is possible.

3. Experimental

3.1. Preparation of the triflyl azide stock solution

After sodium azide (545 mg, 8.38 mmol) was dissolved in water (1.37 mL), toluene (1.37 mL) was added. The mixture was cooled to 0 °C under vigorous stirring. After the dropwise addition of triflic anhydride (896 μL, 4.19 mmol) and further vigorous stirring for 30 min at 0 °C, the temperature was raised to 10 °C and the biphasic mixture was stirred for 2 h. A saturated aqueous solution of sodium hydrogencarbonate was added dropwise until gas evolution had ceased. The two phases were separated and the aqueous layer was extracted with toluene (2 × 1.37 mL). The combined organic layers were used in the subsequent diazo transfer reactions.

3.2. Typical procedure for the diazo transfer reaction

The amine (0.23 mmol), sodium hydrogencarbonate (78 mg, 0.93 mmol) and copper(II) sulfate pentahydrate (2.2 mg, 0.01 mmol) were dissolved in water (0.3 mL). Triflic azide stock solution (0.51 mL) was added, followed by the addition of methanol (1.98 mL) to yield a homogeneous system. Subsequently, the blue mixture was stirred vigorously at room temperature. Complete consumption of the amine was monitored by TLC and is also indicated by a colour change of the reaction mixture from blue to green. Solvents were removed in vacuo with a rotary evaporator keeping the temperature strictly below 25 °C. The residue was purified by chromatography on silica gel (eluant: dichloromethane–methanol). Fully unprotected sugars were transformed into their acetates with Ac_2O/pyridine prior to chromatography.

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2.3 Pre-organization of the carboxylic acid by covalent means

The insufficient biological results of the library directed towards additional enthalpic interactions prompted me to tackle the problem of pre-organization first and then screen for additional pharmacophores. Inspired by the work of Wong[182] and Thoma[183], a covalently pre-organized carboxylic acid should allow optimal salt-bridge formation with Arg97 supported by Tyr48. Secondly, this scaffold could then be exploited for the attachment of additional pharmacophores without disturbing the geometry of the acid orientation.

Thoma's approach (**21**, chapter 1.4.2) led to a covalent orientation of the carboxylic acid, although outside the bioactive conformation and, therefore, was not successful as selectin ligand. In the Wong group (**(*S*)-23**, **(*R*)-23**, chapter 1.4.2), the carboxylic acid was introduced into a galactoside derived disaccharyl ketone using D- and L-cysteine, respectively. The angle of the carboxylic acid was covalently fixed through a thiazine ring. However, the results published by Wong's group show that both diastereomers, obtained from D- and L-cysteine, exhibit similar biological activities towards the selectins. They claim that the orientation of the carboxylic acid is not of special importance. Furthermore, it is reported that the main contribution to the increased affinity of the spirocyclic antagonists originates from the lipophilicity of the sulphur in both diastereomers, when compared to the acyclic carboxymethyl analog (**22**[184,185], chapter 1.4.2).

The two spirocyclic diastereomers synthesized by Wong and co-workers additionally showed different behavior in chemical stability. Whereas the L-cysteine derived compound **(*R*)-23** (chapter 1.4.2), with the acid oriented in the 'wrong' orientation, was reported to be a moderately stable molecule, the D-cysteine analog **(*S*)-23** with the 'correct' orientation of the acid was not. Rapid isomerization *via* inversion of the orientation of the spirocyclic thiazine ring was reported.

This isomerization, or even hydrolysis of the labile thiazine ring is not surprising. The stability of cysteine derived thiazines was extensively studied by Riemschneider,[186,187] Belikov[188] and Butvin[189]. Unsubstituted thiazines derived from formaldehyde, 2-monosubstituted thiazines from other aldehydes and 2,2-disubstituted thiazines from ketones were analyzed for their solubility and stability in different solvents. Although formaldehyde derived thiazines were stable, 2,2-disubstituted analogs obtained from ketones were very labile especially in polar solvents like water or methanol.[186] Belikov reported an immediate hydrolysis of the 2,2-dimethyl thiazine from L-cysteine upon contact with water.[188]

The approach of the Wong group to fix the acid orientation *via* a spirocyclic ring was a very valuable idea, although the exact nature of the thiazine compounds tested remains obscure. When taking the literature on thiazine stability in aqueous solvents into account, it can be assumed that the similar biological activity of the two diastereomeric thiazines might be a result of hydrolysis rather than similar intrinsic activity of the pure individual compounds.

Consequently, the idea of a spirocyclic ring for the locking of the acid orientation was continued, however, by using a stable 5-membered ring as locking-motif. For this purpose the *spiro*-tetrahydrofuranyl galactoside derivative **17** (*scheme 1*) was chosen as target structure.

The retrosynthetic analysis (*scheme 1*) of **17** leads *via* a retro-Williamson disconnection to the acyclic precursor **18**. The leaving group necessary for ring closure should be introduced from acrylate **19**, which leads to the 3*C*-allyl galactoside motif **20** by retro-Grubbs olefin metathesis. This carbon-linked allyl derivative **20** gives the ketone **21** by retro-Grignard disconnection, which leads *via* a series of protecting group transformations to the known building block **24**[190] as available starting material.

To study the transformations at the galactose residue of **24**, a simpler and commercially available starting material was chosen. Galactoside **25** (*scheme*

2) was first selectively *O*-allylated in the 3-position with the help of a tin-acetal intermediate using a modified procedure of Pieters[191] and co-workers.

Scheme 1: Retrosynthetic analysis of the desired spiro target structure **17**

Subsequently, the remaining hydroxyl groups in **26** were protected as benzoates to give **27** in quantitative yield. Removal of the allyl ether by palladium mediated allyl transfer to methanol gave the free alcohol **28**. Chromium-VI mediated oxidation of the secondary alcohol gave the desired ketone **29** in 86% yield. The carbon-linked allyl group was introduced in **30** by a Barbier-type reaction using an allyl-zinc reagent. This reaction proceeded smoothly with a diastereomeric excess of more than 98%.

To confirm the desired axial substitution, ROESY NMR spectroscopy was performed. Unfortunately, ROE's between Gal-H1/H5 and the allylic protons were not detected, as observed by Hindsgaul[192] and co-workers for similar galactose derivatives. Because the epimer of **30** was not accessible *via* this route due to a high degree of stereoselectivity, a control to unambiguously determine the stereochemistry in **30** was not available. Consequently, **30** was

Scheme 2: Synthesis of the acrylate precursor in the benzoate test series: (i) DBTO, MeCN, PhMe, µW, 7 min., 150 °C; (ii) AllBr, TBAI, 10 d, r.t.; (iii) BzCl, C_5H_5N, 18 h, 0 °C – r.t.; (iv) cat. $PdCl_2$, MeOH, 22 h, 40 °C; (v) PDC, HOAc, mol. sieves, CH_2Cl_2, 3 h, 0 °C – r.t.; (vi) Zn (powder), AllBr, cat. I_2, dry THF, 4 h, r.t.; (vii) methyl acrylate, Grubbs 2nd gen., CH_2Cl_2, reflux, 2 h;

crystallized and the structure of *gulo*-derivative **30** could be confirmed by X-ray crystallography (*figure 14*, *table 1*). Although the configuration of **30** was the *gulo*- and not the desired *galacto*-epimer, it was transformed into acrylate **31** for further synthetic studies using Grubbs olefin cross-metathesis in very good yield (*scheme 2*).

An epoxide introduced *via* asymmetric epoxidation (AE)[193] could serve as leaving group in the subsequent cyclization to form the 5-membered ring in **17** (*scheme 1*) and give rise to a free hydroxyl group as attachment handle for further derivatization in a library derived from **17**. AE of acrylates using the novel electron-deficient fructose-derived Shi auxiliary[194] promised to be a valuable approach to stereoselectively introduce an epoxide. Therefore, the auxiliary was synthesized according to the acidic[195] acylation conditions used by Vidal-Ferran[196] in four steps from fructose. However, when **31** was exposed to epoxidation conditions with the fructose-derived auxiliary, epoxide product could not be detected neither using the Shi[194] conditions at pH 8-10, nor under Vidal-Ferran[196] conditions in a buffered system at pH 6. Other mostly nucleophilic epoxidation conditions (*e.g.* H_2O_2/NaOH,[197] mCPBA, mCPBA/base, NaOCl/C_5H_5N or ^{t}BuOOH/base) were applied to **31**, but did

either not react or led to complex mixtures because of the labile benzoate protecting groups.

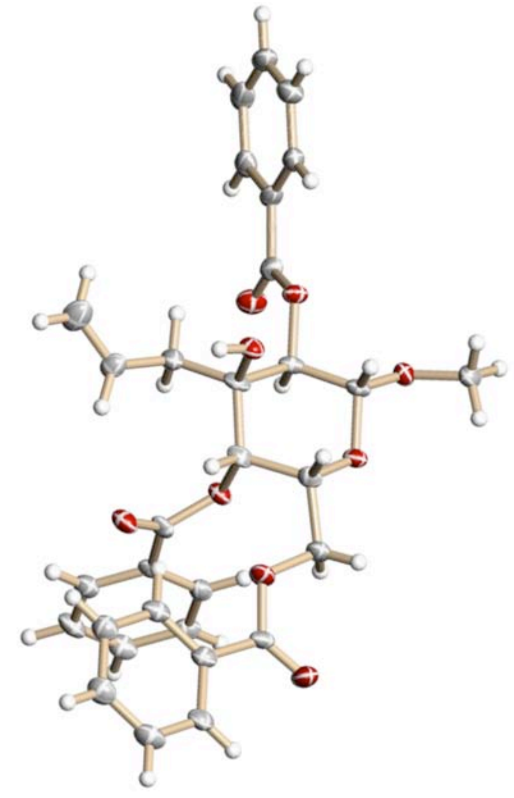

Figure 14: ORTEP plot of the X-ray structure of **30** proving the *gulo* stereochemistry

Alternatively, an asymmetric dihydroxylation (AD) using Sharpless conditions with AD-mix beta should lead to a diastereomerically pure diol.[198] Since the pK_a of the α-hydroxyl group is much more acidic then the one of the remaining hydroxyl groups, it could be transformed selectively into a leaving group suitable for cyclization.[199] When the AD reaction was performed with **31**, no product was formed. Since AD and AE failed on **31**, but were successful on methyl cinnamate in control experiments, it can be concluded that the double bond in **31** is not accessible by the catalysts and can therefore not be oxidized using these methods. Dihydroxylation of **31** without a chiral catalyst was possible and the corresponding mixture of syn-diols could be obtained (61%, d.r. 1:1.7, data not shown). Cyclization of the diol to the desired THF ring was studied under a variety of conditions ($CeCl_3$,[200] Tf_2O/2,6-$^tBu_2$-C_5H_3N/THF, Tf_2O/2,6-$^tBu_2C_5H_3N/CH_2Cl_2$), but did not react or led to complex mixtures of unidentified products.

Table 1: Crystallographic data of gulose derivative **30**

Compound	**30**
CCDC number	not deposited
Empirical formula	$C_{31}H_{30}O_9$
Formula weight	546.55
Temperature (K)	100
Wavelength (Å)	1.5418
Crystal system	Monoclinic
Space group	$P2_1$
Cell dimensions	
a (Å)	8.26620(10)
b (Å)	11.1738(2)
c (Å)	14.5223(2)
α (°)	90
β (°)	92.2730
γ (°)	90
Volume ($Å^3$)	1340.29(3)
Z	2
Density calculated (kg/dm^3)	1.354
F_{000}	576
θ range for data collection (°)	3.05-58.93
Reflections collected	5729
Independent reflections	3138
Data/restraints/parameters	3138/1/304
Goodness of fit on F^2	1.121
Final R indices [$I > 2\sigma(I)$]	R1 = 0.0830, wR2 = 0.2073
R indices (all data)	R1 = 0.0910, wR2 = 0.2184
Largest diff. peak and hole (e $Å^3$)	0.624 and -0.644

Because of the undesired *gulo* stereochemistry in **30** and the difficulties with the oxidation of the double bond in **31**, protecting groups were changed from benzoates to benzyl ethers. This allowed both, zinc mediated Barbier-type allylation and a more reactive Grignard allylation to extend the galactose

moiety. Furthermore, a reduction of the acrylate to an allylic alcohol for Sharpless epoxidation was now possible with these protecting groups.

Scheme 3: Synthesis of the epoxide precursor in the benzyl test series: (i) DBTO, MeCN, PhMe, µW, 7 min., 150 °C; (ii) AllBr, TBAI, 3 d, 60 °C; (iii) BnBr, NaH, DMF, 4 h, 0 °C – r.t.; (iv) cat. $PdCl_2$, MeOH, 3.5 h, 40 °C; (v) $(CO)_2Cl_2$, DMSO, NEt_3, CH_2Cl_2, 5 h, -78 °C – r.t.; (vi) Zn (powder), AllBr, cat. I_2, dry THF, 4 h, r.t.; (vii) methyl acrylate, Grubbs 2nd gen., CH_2Cl_2, reflux, 2 h; (viii) DIBAl-H, PhMe, -78 °C, 1 h; (ix) (-)-DET, $Ti(OiPr)_4$, ^{t}BuOOH, mol. sieves, -20 °C - r.t., 2 h;

Again, methyl galactoside **25** served as the starting material for studying the reaction sequence. Selectively protected galactoside **32** (*scheme 3*) was obtained after 3-*O* allylation as performed for **26** (*scheme 2*), but with increased temperature (60 °C) during the allylation. This led to a shortened reaction time of 3 days and an increased yield after benzylation of the remaining hydroxyl groups. Deallylation of **32** using Pd(II) and methanol gave the free alcohol **33**, which was used for oxidation to the ketone **34** under Swern conditions. Barbier-type allylation of **34** gave an inseparable mixture of diastereomers of **35** (d.r. 4.3:1) which was exposed to olefin metathesis conditions to give **36** in 90% yield over 2 steps.

Scheme 4: (i) NaOMe, MeOH, r.t., 12 h, r.t. (ii) H_2, Pd/C, MeOH, 12 h, r.t.

To verify the stereochemistry in **35**, Overhauser effects were analyzed by ROESY NMR and could only be observed between Gal-H1/H5 and the allylic protons of the side chain for the minor diastereomer. These results indicate again a preference of the zinc reagent for the *gulo* type product over the desired *galacto* type product. To verify these assignments, both benzoate protected **30** and the mixture of benzyl protected **35** were transformed into 3-*C* propyl derivative **43** (*scheme 4*). Because the major diastereomer of the product **43** obtained from **35** showed the same NMR data as the one obtained from diastereomerically pure **30**, a *gulo* preference could be unambiguously assigned for the Barbier type allylation to obtain **35**.

Acrylate **36** could now be reduced to give the allylic alcohol **37**, which was diastereoselectively epoxidized under Sharpless conditions to give epoxide **38** (*scheme 3*). Upon exposure of **38** to camphor sulfonic acid in methylene chloride, the expected cyclization to give the spiro isomer **39** could not be observed (*scheme 5*). An unexpected benzyl group migration from 2-*O* to the primary terminal hydroxyl group took place, followed by a nucleophilic attack of the less sterically hindered 2-OH to the epoxide to give an annulated THF ring in **41** in 23% yield as the only observed product. Similar selective sulfonic acid mediated cleavage of more labile 2-*O* p-methoxybenzyl groups in galactosides has rarely been reported in the literature.[201,202] The two hydroxyl groups in **41** were subsequently derivatized with 3,5-dinitrobenzoyl chloride to give the diester **42**.

Careful NMR analysis of **41** and its derivative **42** further confirmed the proposed structure (*figure 15*). In **41**, the absence of a HMBC signal between C-2 and benzylic protons indicated loss of the 2-*O*-benzyl group, and additionally the large coupling constants (10.4 and 12.7 Hz) of H_{ax} of the methylene group adjacent to C-3 originate from the gemial coupling with H_{eq}

Scheme 5: Cyclization of epoxide **38** and derivatization for structure assignment: (i) CSA, CH_2Cl_2, r.t., 12 h; (ii) 3,5-$(NO_2)_2$BzCl, DMAP, CH_2Cl_2, r.t., 4 h;

and a trans-oriented vicinal proton. This ^3J trans coupling is only present in the 5-membered ring of **41**, in a possible isomeric 6-membered ring the two methylene protons would both have a gauche orientation to the neighboring proton and thus show a ^3J coupling constant in the low single digit Hertz range (1-3 Hz) as derived from the Karplus equation. In **42**, the chemical shift of C-3 has moved downfield by 11.5 ppm in the carbon-13 NMR when compared to its chemical shift in **41**. This is in accordance with the difference of the chemical shifts of the quaternary carbons in *tert.*-BuOH and *tert.*-butyl acetate (+ 11.6 ppm).[203] Furthermore, the methine proton adjacent to the ester

function in the side chain of **42** has shifted downfield by 0.95 ppm, when compared with its chemical shift in **41**.

Figure 15: Assignment of the structure of **41** and its derivative **42** by NMR

With the lessons learnt from these test reactions on monosaccharide **25** (*schemes 2-5*), the synthesis of **17** was now started from the core moiety **44**[190] (*scheme 6*). Selective *O*-allylation of the 3-position of the galactose moiety in **44** gave **45** in good yield. Subsequently, the free hydroxyls were protected as benzyl ethers in **46** and then, the free 3-OH group in **47** was obtained by palladium-(II) mediated deallylation. Swern oxidation of **47** gave the ketone **48** in very good yield. The allylation under Grignard conditions could be optimized to give an almost equimolar ratio (1.2:1) of the undesired but mechanistically preferred *gulo*-**49** to the desired *galacto*-**49** epimer in 77% yield by adding **48** dropwise at 0 °C to a large excess (40 equiv.) of the allyl Grignard reagent in Et_2O. *Galacto*-**49** was subsequently transformed into acrylate **50** in 91% yield *via* olefin metathesis using only 5 mol-% of the Grubbs 2nd generation catalyst. The reduction of **50** to allylic alcohol **51** with

44

45, 64%

46, 90%

47, 94%

48, 88%

49, 77%, d.r. 1:1.2

50, 91%

51, 76%

52, 44%, d.r. approx 1:1

Scheme 6: Synthesis of the epoxide precursor **52**: (i) DBTO, MeCN, PhMe, µW, 7 min., 150 °C; (ii) AllBr, TBAI, 12 h, 75 °C; (iii) BnBr, NaH, DMF, 7 h, r.t.; (iv) cat. $PdCl_2$, MeOH, 2 h, 50 °C; (v) $(CO)_2Cl_2$, DMSO, NEt_3, CH_2Cl_2, 10 h, -78 °C – r.t.; (vi) Mg (turnings), AllBr, cat. I_2, dry Et_2O, 3.5 h, 0 °C; (vii) methyl acrylate, Grubbs 2nd gen., CH_2Cl_2, reflux, 1 h; (viii) DIBAl-H, PhMe, -78 °C, 1 h; (ix) (-)-DET, $Ti(OiPr)_4$, ^{t}BuOOH, mol. sieves, -20 °C – +10 °C, 2 h;

DIBAl-H was performed in 76% yield, but also DIBAl-H mediated debenzylation of the galactose-2-*O*-benzyl group was observed as reported for benzylated carbohydrates by Lecourt *et al.*.[204] In the following Sharpless epoxidation of **51**, epoxide **52** was obtained almost as a 1:1 mixture of diastereomers. This probably results from a mismatch situation of the substrate **51** with the (-)-diethyl tartarate complex of titanium-(IV), because homo-allylic alcohols, as also present in **51**, have been reported to be suitable substrates for asymmetric epoxidation *via* the Sharpless route. Match and mismatch in the Sharpless epoxidation of similar allylic – homoallylic systems have been studied in detail by Takano and co-workers and can lead to either

complete selectivity for one diastereomer, or in case of mis-match to equimolar diastereomeric mixtures of products.[205] Furthermore, partial Lewis acid mediated 2-*O* debenzylation occurred during the epoxidation and was responsible for the low yield (44%) in the synthesis of **52**.

Because acidic conditions led to 2-*O* debenzylation as observed for the monosaccharide **38**, but also for **51** and **52** when exposed to Lewis acids, basic conditions were chosen to test the cyclization of the mixture of epoxides **52**. The diastereomeric mixture **52** was exposed to sodium hydride in dry DMF at room temperature and a mixture of isomeric products with correct molecular mass (as observed from mass spectrometry) was obtained (*scheme 7*). Cyclization was successful and the *spiro*-THF compound **53** derived from the undesired epoxide **(*S*,*S*)-52** was obtained in 21% yield. The second product obtained from this base-catalyzed cyclization was a mixture of oxetanes **54** resulting from the desired epoxide **(*R*,*R*)-52** and partially from its diastereomer **(*S*,*S*)-52**. In fact, base-catalyzed isomerization of 4-hydroxy-1,2-epoxybutanes to the corresponding oxetanes has been described in a few reports in the literature, usually for systems where the 5-membered ring product would not be accessible because of high steric hindrance or ring strain of other rings in the molecules.[206-215] The structure of THF **53** and the oxetane mixture **54** was determined by derivatization as bis-dinitrobenzoates **55** and **56**, respectively and analysis of the downfield shifts of the protons adjacent to the ester groups when compared to their precursors **53** and **54**. The stereochemistry of the *spiro*-THF compounds **53** and **55** was determined with the help of ROEs observed between THF-$H4^{syn}$ to Gal-H1 and THF-$H4^{anti}$ to Gal-H5. The peaks of THF-$H4^{syn}$ and THF-$H4^{anti}$ were assigned due to their characteristic difference in ^3J-coupling with THF-H3. THF-$H4^{syn}$ does not have a significant coupling to THF-H3, whereas THF-$H4^{anti}$ has a ^3J coupling of 5.5-6.5 Hz in **53** and **55**.

In accordance with Murphy's law, epoxide **(*S*,*S*)-52** cyclized to the desired spiro compound, however, with the wrong stereochemistry at the THF ring (**53**). Probably an unfavored transition state in the course of the ring-closure leads to the 4-membered ring in **54** for epoxide **(*R*,*R*)-52**. Since **(*S*,*S*)-52** gives

both 5-membered and 4-membered ring products **53** and **54**, a prolonged reaction time was estimated to lead to an isomerization[215] of the oxetanes to the corresponding more stable *spiro*-THF products. Unfortunately, this attempt only resulted in a lower yield of **53** and hydrolysis of the oxetanes, probably due to the presence of NaOH in the NaH used. Interestingly, in a test reaction (10 mg scale) where $NaHCO_3$ was used instead of NaH for the cyclization, no oxetanes were observed by TLC but an additional spot appeared on the TLC plate together with a the spot running at the same R_f value as **53**.

52

(i)

53, 21%

54, 41%

(ii)

(ii)

55, quant.

56, 70%

Scheme 7: Cyclization of epoxides **52** and derivatization of the products for structure assignment: (i) NaH, DMF, r.t., 12 h; (ii) 3,5-$(NO_2)_2$BzCl, DMAP, CH_2Cl_2, r.t., 3-12 h;

Selective oxidation of the primary alcohol in **53** with TEMPO/NaOCl[216] and final deprotection are expected to proceed smoothly to give the derivative of **17** with the undesired stereochemistry. Further studies on the improvement of both, epoxidation and cyclization and on the completion of the synthesis of the derivative of **17** with the undesired stereochemistry could not be

performed due to a lack of time and material. Nevertheless, the proof of principle for the synthesis of a *spiro*-THF sLe^x analog was given.

Experimental

General experimental information is given in the publications of the preceding sections (2.1, 2.2 and 2.4) and this also applies to chapter 2.3.

Methyl 3-*O*-allyl-β-D-galactoside (26). Five batches according to the following procedure were prepared: methyl β-D-galactoside (**25**, 500 mg, 2.57 mmol) and dibutyltin oxide (705 mg, 2.83 mmol) were suspended in a mixture of MeCN/PhMe (6 mL, 1/5 v/v) in a microwave vial. The sealed vial was then exposed to microwave irradiation at a controlled temperature of 150 °C for 7 min. The obtained homogeneous solutions of the five batches were transferred to one round bottom flask. TBAI (13.8 g, 35.4 mmol) and allyl bromide (5.55 mL, 64.4 mmol) were added to the solution, which was protected from light and flushed with argon. After stirring for 10 days at r.t., the solvents were removed *in vacuo* and the residue was purified by column chromatography (SiO_2, CH_2Cl_2/MeOH, gradient of 4-10%). The selectively 3-*O* allylated product **26** (2.81 g) was obtained as a colorless oil containing 16.6 mol-% TBAI (as judged by NMR, this corresponds to a yield of 70%) and was used for the next step without further purification. The NMR spectra of the product corresponded to those reported in the literature.[217]

Methyl 3-*O*-allyl-2,4,6-tri-*O*-benzoyl-β-D-galactoside (27). After compound **26** (2.05 g, 8.75 mmol) was dissolved in pyridine (45 mL) under argon, benzoyl chloride (6.09 mL, 52.5 mmol) was added dropwise at 0 °C. DMAP (53 mg, 0.44 mmol) was added and the reaction stirred for 3 h at 0 °C and then 2.5 days at r.t.. After the solvent was evaporated, the residue was co-evaporated with PhMe and then dried under vacuum. Resuspension of the residue in EtOAc (300 mL) and washing with aqueous HCl (0.5 N, 100 mL), saturated aqueous $NaHCO_3$ (100 mL) and brine (50 mL) gave the crude product after drying over Na_2SO_4 and removal of the solvent. Purification by

column chromatography (SiO_2, petrol ether/EtOAc, 5/1) gave pure **27** (4.72 g, 99%) as white foam.

R_f (petrol ether/EtOAc, 2/1) = 0.47;

The NMR spectra of the product corresponded to those reported in the literature.[218]

Methyl-2,4,6-tri-*O*-benzoyl-β-D-galactoside (28). Compound **27** (4.57 g, 8.36 mmol) was dissolved in dry MeOH (70 mL) and upon addition of $PdCl_2$ (74 mg, 0.42 mmol), the solution became a heterogeneous black mixture. After stirring for 19 h at 45 °C, the solvent was removed and the black residue was taken up in EtOAc (350 mL) and filtered through a celite plug. The EtOAc solution was washed with saturated aqueous sodium $NaHCO_3$ (60 mL), aqueous HCl (0.5 N, 60 mL) and brine (60 mL). Drying over Na_2SO_4 and evaporation of the solvent gave the crude product as white foam (4.36 g), which was purified by column chromatography (SiO_2, petrol ether/EtOAc, 2/1). Pure **28** (3.44 g, 81%) was obtained as white foam.

R_f (petrol ether/EtOAc, 2/1) = 0.32;

The NMR spectra of the product corresponded to those reported in the literature.[219]

Methyl-2,4,6-tri-*O*-benzoyl-β-D-*xylo*-hex-3-ulopyranoside (29). Compound **28** (2.84 g, 5.60 mmol) was dissolved in dry CH_2Cl_2 (50 mL) and activated powdered molecular sieves were added under argon. The mixture was then cooled to 0 °C and 1 drop glacial acetic acid was added. After stirring for 10 min, PDC (2.32 g, 6.16 mmol) was added and the reaction was allowed to warm to r.t. during 3 h. After stirring for further 21 h at r.t., the reaction was filtered through a plug of celite, the volatiles were removed *in vacuo* and the residue was taken up in EtOAc (300 mL). The organic phase was washed with half saturated aqueous $NaHCO_3$ (100 mL) and water (80 mL). The combined aqueous layers were extracted with CH_2Cl_2 (2 x 100 mL) and then, the combined organic layers were dried over Na_2SO_4, filtered and the volatiles were removed *in vacuo*. The residue was purified by column chromatography

(SiO_2, petrol ether/EtOAc, gradient of 25-33%) to give pure **29** (2.41 g, 86%, its hydrate was also detected by ESI-MS, the presence of additional peaks in the NMR and by decreased percentage of carbon and increased percentage of hydrogen in the microanalysis) as white foam.

R_f (petrol ether/EtOAc, 2/1) = 0.38;

^{1}H-NMR (500.1 MHz, $CDCl_3$): δ 8.15-8.02 (m, 6H, Ar-H), 7.64-7.56 (m, 3H, Ar-H), 7.50-7.43 (m, 6H, Ar-H), 5.75 (d, ^3J = 7.7 Hz, 1H, H-2), 5.66 (d, ^3J = 1.6 Hz, 1H, H-4), 4.87 (d, ^3J = 7.7 Hz, 1H, H-1), 4.77 (dd, ^2J = 11.4, ^3J = 6.4 Hz, 1H, H-6a), 4.59 (dd, ^2J = 11.4, ^3J = 6.6 Hz, 1H, H-6b), 4.29 (td, ^3J = 6.5, ^3J = 1.6 Hz, 1H, H-5), 3.65 (s, 3H, MeO-);

^{13}C-NMR (125.8 MHz, $CDCl_3$): δ 194.6 (C-3), 165.9, 164.9, 164.8 (3C, Ar-*C*OOR), 134.0, 133.6, 133.4 (3C, Ar-C^i), 130.2, 130.0, 129.7, 128.7, 128.5, 128.4 (15C, Ar-CH), 103.3 (C-1), 77.0 (C-2), 74.7 (C-4), 72.3 (C-5), 61.6 (C-6), 57.4 (MeO);

elemental analysis calcd. for $C_{28}H_{24}O_9$ (504.49): C 66.66, H 4.79;
found: C 66.16, H 4.87;

ESI-MS calcd. for $C_{28}H_{24}O_9Na$ $[M+Na]^+$: 527.1; found: 527.0;

$[\alpha]^{20}_D$ = -11.1 (*c* 0.50, $CHCl_3$);

Methyl-3-*C*-allyl-2,4,6-tri-*O*-benzoyl-β-D-guloside (30). To a suspension of Zn powder (1.19 g, 18.1 mmol) in dry THF (13.2 mL) one I_2 crystal was added under argon, followed by dropwise addition of allyl bromide (0.69 mL, 7.97 mmol). After completion of the addition, the mixture was stirred for 30 min at r.t.. Then, the remaining Zn was allowed to settle and a first part of the supernatant (4.00 mL) was added to a solution of compound **29** (1.00 g, 1.99 mmol) in dry THF (13.2 mL) under stirring. After stirring for 1 h, a second fraction of Zn-allyl solution (4.00 mL) was added, and after stirring for another 2 h, a third addition of Zn-allyl solution (4.00 mL) led to completion of the reaction after 4 h in total. The reaction was quenched with saturated aqueous NH_4Cl (44.0 mL), and after stirring for 10 min, the aqueous phase was

extracted with CH_2Cl_2 (3 x 100 mL). The combined organic layers were dried over Na_2SO_4, filtered and the volatiles were removed *in vacuo*. The residue was purified by column chromatography (SiO_2, petrol ether/EtOAc, gradient of 10-50%) to give diastereomerically pure **30** (885 mg, 81%) as white foam. A small sample of **30** was recrystallized from CH_2Cl_2/heptane by evaporation for X-ray analysis.

R_f (petrol ether/EtOAc, 4/1) = 0.55;

^{1}H-NMR (500.1 MHz, $CDCl_3$): δ 8.18-8.07 (m, 6H, Ar-H), 7.66-7.44 (m, 9H, Ar-H), 5.88-5.80 (m, 1H, allyl-CH), 5.45 (d, ^3J = 8.0 Hz, 1H, H-2), 5.39 (br s, 1H, H-4), 5.18-5.03 (m, 2H, allyl-CH_2), 5.00 (d, ^3J = 8.0 Hz, 1H, H-1), 4.70-4.66 (m, 1H, H-5), 4.52 (dd, ^2J = 11.4, ^3J = 7.0 Hz, 1H, H-6a), 4.43 (dd, ^2J = 11.4, ^3J = 5.8 Hz, 1H, H-6b), 3.55 (s, 3H, MeO-), 2.48 (dd, ^2J = 14.4, ^3J = 6.8 Hz, 1H, 1H of allyl-CH_2), 2.31 (dd, ^2J = 14.4, ^3J = 8.2 Hz, 1H, 1H of allyl-CH_2);

^{13}C-NMR (125.8 MHz, $CDCl_3$): δ 166.2, 165.6, 165.2 (3C, Ar-*C*OOR), 133.6, 133.3, 132.9 (3C, Ar-C^i), 130.7 (allyl-CH), 130.7-128.3 (15C, Ar-CH), 121.4 (allyl-CH_2), 100.4 (C-1), 74.2 (C-3), 73.1 (C-2), 71.6 (C-4), 70.6 (C-5), 63.4 (C-6), 57.4 (MeO), 39.3 (allyl-CH_2);

ESI-MS calcd. for $C_{31}H_{30}O_9Na$ $[M+Na]^+$: 569.2; found: 569.2;

elemental analysis calcd. for $C_{31}H_{30}O_9$ (546.57): C 68.12, H 5.53;
found: C 68.00, H 5.60;

$[\alpha]^{20}_D$ = +20.3 (*c* 0.50, $CHCl_3$);

Methyl-3-*C*-(*E*-1-methoxycarbonyl-prop-1-en-3-yl)-2,4,6-tri-*O*-benzoyl-β-D-guloside (31). A round bottom flask equipped with a reflux condenser was charged with a solution of compound **30** (184 mg, 0.34 mmol) in dry CH_2Cl_2 (4 mL). Grubbs catalyst 2nd generation (28.6 mg, 0.03 mmol) was added, immediately followed by addition of methyl acrylate (303 µL, 3.37 mmol). After heating the reaction to reflux under argon and stirring for 2 h, the volatiles were removed *in vacuo*. The residue was purified by column chromatography (SiO_2, petrol ether/EtOAc, gradient of 20-33%) to give **31** (179 mg, 88%) as

white foam.

R_f (petrol ether/EtOAc, 3/1) = 0.25;

^{1}H-NMR (500.1 MHz, $CDCl_3$): δ 8.15-8.06 (m, 6H, Ar-H), 7.64-7.33 (m, 9H, Ar-H), 6.95-6.88 (m, 1H, propenyl-H2), 5.67 (d, ^{3}J = 15.6 Hz, 1H, propenyl-H1), 5.44 (d, ^{3}J = 8.0 Hz, 1H, H-2), 5.42 (br s, 1H, H-4), 4.95 (d, ^{3}J = 8.0 Hz, 1H, H-1), 4.68-4.64 (m, 1H, H-5), 4.55 (dd, ^{2}J = 11.5, ^{3}J = 7.0 Hz, 1H, H-6a), 4.38 (dd, ^{2}J = 11.5, ^{3}J = 5.8 Hz, 1H, H-6b), 3.52, 3.49 (2 s, 6H, 2 MeO-), 2.55 (dd, ^{2}J = 14.8, ^{3}J = 7.1 Hz, 1H, 1H of allyl-CH_2), 2.45 (dd, ^{2}J = 14.9, ^{3}J = 8.3 Hz, 1H, 1H of allyl-CH_2);

^{13}C-NMR (125.8 MHz, $CDCl_3$): δ 166.1, 165.8, 165.3, 165.2 (4C, -*C*OOR), 140.9 (propenyl-CH), 133.8, 133.6, 133.1 (3C, Ar-C^{i}), 130.2-128.4 (15C, Ar-CH), 125.3 (propenyl-CH), 100.4 (C-1), 75.0 (C-3), 73.1 (C-2), 71.7 (C-4), 70.6 (C-5), 62.7 (C-6), 57.1, 51.4 (2C, 2 MeO), 38.6 (propenyl-CH_2);

HR-MS calcd. for $C_{33}H_{32}O_{11}Na$ $[M+Na]^+$: 627.1837; found: 627.1861;

elemental analysis calcd. for $C_{33}H_{32}O_{11}$ (604.60): C 65.56, H 5.33;
found: C 65.31, H 5.42;

$[\alpha]^{20}_{D}$ = + 27.1 (*c* 0.50, $CHCl_3$);

Methyl 3-*O*-allyl-2,4,6-tri-*O*-benzyl-β-D-galactoside (32). Galactoside **25** (1.80 g, 9.25 mmol) was 3-*O*-allylated as described above, with two modifications. Less TBAI (3.20 g, 8.68 mmol) was used and the allylation step was run at 60 °C for 3 d. The crude material was purified by column chromatography and gave the TBAI rich product **26**. This material was dissolved in dry DMF (10.0 mL), cooled to 0 °C and NaH (4.44 g, 92.6 mmol, w = 50%) was added portionwise under argon. Benzyl bromide (6.60 mL, 55.8 mmol) was added dropwise over 10 min at 0 °C. The reaction was allowed to warm slowly to r.t. and was stirred for 4 h at r.t.. Then, the reaction was quenched with H_2O (75.0 mL) and extracted with CH_2Cl_2 (4 x 50 mL). The combined organic layers were dried over Na_2SO_4, filtered and the volatiles removed *in vacuo*. Purification of the residue by column

chromatography (SiO_2, petrol ether/EtOAc, gradient of 5-50%) gave pure **32** (3.65 g, 78%, 2 steps) as white foam.

R_f (petrol ether/EtOAc, 6/1) = 0.30;

^{1}H-NMR (500.1 MHz, $CDCl_3$): δ 7.39-7.26 (m, 15H, Ar-H), 5.97-5.90 (m, 1H, allyl-CH), 5.34-5.16 (m, 2H, allyl-CH_2), 4.93 (A of AB, ^2J = 11.7 Hz, 1H, 1H of $PhCH_2$), 4.87 (A' of A'B', ^2J = 11.0 Hz, 1H, 1H of $PhCH_2$), 4.75 (B' of A'B', ^2J = 11.3 Hz, 1H, 1H of $PhCH_2$), 4.61 (B of AB, ^2J = 11.6 Hz, 1H, 1H of $PhCH_2$), 4.45, 4.41 (A'', B'' of A''B'', ^2J = 11.7 Hz, 2H, 2H of $PhCH_2$), 4.26 (d, ^3J = 7.7 Hz, 1H, H-1), 4.22-4.17 (m, 2H, 2H of allyl-CH_2), 3.86 (d, ^3J = 2.4 Hz, 1H, H-4), 3.74 (dd, ^3J = 7.7, ^3J = 9.7 Hz, 1H, H-2), 3.60 (dd, ^2J = 6.3, ^3J = 1.9 Hz, 1H, H-6a), 3.55-3.51 (m, 5H, H-5, H-6b, MeO), 3.42 (dd, ^3J = 9.8, ^3J = 2.9 Hz, 1H, H-3);

^{13}C-NMR (125.8 MHz, $CDCl_3$): δ 138.8, 138.6, 137.9 (3C, Ar-C^i), 134.9 (allyl-CH), 128.4-127.5 (15C, Ar-CH), 116.6 (allyl-CH_2), 104.9 (C-1), 81.7 (C-3), 79.5 (C-2), 75.1, 74.3, 73.5 (3C, Ph*C*H$_2$), 73.3 (C-5), 73.2 (C-4), 71.9 (allyl-CH_2), 68.8 (C-6), 57.0 (MeO);

ESI-MS calcd. for $C_{31}H_{36}O_6Na$ $[M+Na]^+$: 527.2; found: 527.1;

Methyl-2,4,6-tri-*O*-benzyl-β-D-galactoside (33). Compound **32** (3.34 g, 6.62 mmol) was deallylated at 40 °C for 3.5 h as described for **28**. Pure **32** (2.30 g, 75%) was obtained as white foam.

R_f (petrol ether/EtOAc, 3/1) = 0.20;

ESI-MS calcd. for $C_{28}H_{32}O_6Na$ $[M+Na]^+$: 487.2; found: 487.1;

The NMR spectra of the product corresponded to those reported in the literature.[217]

Methyl-2,4,6-tri-*O*-benzyl-β-D-*xylo*-hex-3-ulopyranoside (34). To a solution of oxalyl chloride (1.90 mL, 21.8 mmol) in dry CH_2Cl_2 (100 mL) at -78 °C was added DMSO (3.80 mL, 54.0 mmol) dropwise under strong gas evolution. After stirring for 10 min at -78 °C, a solution of compound **33** (2.30 g,

4.95 mmol) in dry CH_2Cl_2 (50 mL) was added dropwise. After stirring for 2 h at -78 °C, TEA (5.5 mL, 40.0 mmol) was added dropwise and the color of the reaction changed from light red to colorless. The mixture was allowed to warm to r.t. during 3 h and was finally poured into aqueous saturated $NaHCO_3$ (200 mL). The phases were separated and the aqueous layer was extracted with CH_2Cl_2 (2 x 100 mL). The combined organic layers were dried over Na_2SO_4, filtered and the volatiles removed *in vacuo*. The residue was purified by column chromatography (SiO_2, petrol ether/EtOAc, gradient of 10-45%) to give pure **34** (1.80 g, 80%) as white foam.

R_f (petrol ether/EtOAc, 2/1) = 0.40;

^{1}H-NMR (500.1 MHz, $CDCl_3$): δ 7.40-7.14 (m, 15H, Ar-H), 4.70, 4.66 (A, B of AB, ^2J = 11.8 Hz, 2H, 2H of $PhCH_2$), 4.54, 4.47 (A', B' of A'B', ^2J = 11.9 Hz, 2H, 2H of $PhCH_2$), 4.41 (d, ^3J = 7.6 Hz, 1H, H-1), 4.39-4.36 (m, 2H, H-2, 1H of $PhCH_2$), 4.29 (B'' of A''B'', ^2J = 11.8 Hz, 1H, 1H of $PhCH_2$), 3.88 (d, ^3J = 1.3 Hz, 1H, H-4), 3.76-3.70 (m, 3H, H-5, H-6a, H-6b), 3.57 (s, 3H, MeO-);

^{13}C-NMR (125.8 MHz, $CDCl_3$): δ 204.0 (C-3), 137.8, 137.3, 136.5 (3C, Ar-C^i), 128.5-127.7 (15C, Ar-CH), 105.1 (C-1), 82.2 (C-2), 80.8 (C-4), 73.7 (C-5), 73.6, 73.5, 72.3 (3C, $PhCH_2$), 67.7 (C-6), 57.3 (MeO);

elemental analysis calcd. for $C_{28}H_{30}O_6$ (462.54): C 72.71, H 6.54;
found: C 72.55, H 6.63;

HR-MS calcd. for $C_{28}H_{30}O_6Na$ $[M+Na]^+$: 485.1940; found: 485.1938;

$[\alpha]^{20}_D$ = -82.5 (*c* 2.50, $CHCl_3$);

Methyl-3-*C*-(*E*-1-methoxycarbonyl-prop-1-en-3-yl)-2,4,6-tri-*O*-benzyl-β-D-guloside (*gulo*-36) and methyl-3-*C*-(*E*-1-methoxycarbonyl-prop-1-en-3-yl)-2,4,6-tri-*O*-benzyl-β-D-galactoside (*galacto*-36). Ketone **34** (213 mg, 0.46 mmol) was allylated using the Zn-allyl method as described for **30**.

HR-MS calcd. for $C_{31}H_{36}O_6Na$ $[M+Na]^+$: 527.2410; found: 527.2422;

The crude product was directly used in the subsequent olefin metathesis as

described for **31**. Purification by column chromatography (SiO_2, petrol ether/EtOAc, gradient of 20-33%) gave the inseparable title compounds **36** (233 mg, 90%, 2 steps, d.r. 4.3:1) as white foam.

R_f (petrol ether/EtOAc, 2/1) = 0.40;

HR-MS calcd. for $C_{33}H_{38}O_8Na$ $[M+Na]^+$: 585.2464; found: 585.2465;

Methyl-3-*C*-(*E*-1-methoxycarbonyl-prop-1-en-3-yl)-2,4,6-tri-*O*-benzyl-β-D-guloside (gulo-**36**):

^{1}H-NMR (500.1 MHz, $CDCl_3$): δ 7.29-7.11 (m, 15H, Ar-H), 7.04-6.92 (m, 1H, propenyl-H2), 4.85 (A of AB, ^2J = 11.1 Hz, 1H, $PhCH_2$), 4.62-4.38 (m, 6H, H-1, 5H of $PhCH_2$), 4.16 (t, ^3J = 6.9 Hz, 1H, H-5), 3.63-3.33 (m, 6H, H-4, H-6a,b, MeO), 3.27 (d, ^3J = 7.7 Hz, 1H, H-2), 2.55 (dd, ^2J = 15.3, ^3J = 6.1 Hz, 1H, propenyl-CH_2a), 2.41 (dd, ^2J = 15.3 Hz, ^3J = 8.3 Hz, 1H, propenyl-CH_2b);

^{13}C-NMR (125.8 MHz, $CDCl_3$): δ 166.3 (COOR), 144.7 (propenyl-CH), 138.4, 137.8, 137.5 (3C, Ar-C^i), 128.3-127.4 (15C, Ar-CH), 123.5 (propenyl-CH), 103.0 (C-1), 78.6 (C-2), 77.3 (C-4), 76.3 (C-3), 74.8, 74.7, 73.3 (3C, Ph*C*H_2), 71.7 (C-5), 68.3 (C-6), 56.9, 51.4 (2C, OMe), 37.8 (propenyl-C3);

Methyl-3-*C*-(*E*-1-methoxycarbonyl-prop-1-en-3-yl)-2,4,6-tri-*O*-benzyl-β-D-galactoside (*galacto*-**36**):

^{1}H-NMR (500.1 MHz, $CDCl_3$): δ 7.29-7.11 (m, 15H, Ar-H), 7.04-6.92 (m, 1H, propenyl-H2), 4.75 (A of AB, ^2J = 11.5 Hz, 1H, $PhCH_2$), 4.62-4.38 (m, 5H, 5H of $PhCH_2$), 4.27 (d, ^3J = 7.8 Hz, 1H, H-1), 3.71 (t, ^3J = 6.4 Hz, 1H, H-5), 3.63-3.33 (m, 7H, H-2, H-4, H-6a,b, MeO), 2.83 (dd, ^2J = 15.3, ^3J = 4.9 Hz, 1H, propenyl-CH_2a), 2.22 (dd, ^2J = 15.4, ^3J = 9.5 Hz, 1H, propenyl-CH_2b);

^{13}C-NMR (125.8 MHz, $CDCl_3$): δ 166.3 (COOR), 144.4 (propenyl-CH), 138.4, 137.8, 137.5 (3C, Ar-C^i), 128.3-127.4 (15C, Ar-CH), 123.6 (propenyl-CH), 103.0 (C-1), 81.9 (C-2), 78.0 (C-4), 75.7 (C-3), 75.5, 75.1, 73.3 (3C, Ph*C*H_2), 71.6 (C-5), 68.6 (C-6), 56.9, 51.4 (2C, OMe), 34.7 (propenyl-C3);

Methyl-3-*C*-(*E*-1-hydroxymethyl-prop-1-en-3-yl)-2,4,6-tri-*O*-benzyl-β-D-guloside (*gulo*-37) and Methyl-3-*C*-(*E*-1-hydroxymethyl-prop-1-en-3-yl)-2,4,6-tri-*O*-benzyl-β-D-galactoside (*galacto*-37). A solution of compound **36** (209 mg, 0.37 mmol) in PhMe (20 mL) was cooled to -78 °C under argon and subsequently treated dropwise with DIBAl-H in cyclohexane (2.00 mL, 2.0 mmol, 1 M). After 1 h at -78 °C, saturated aqueous NH_4Cl (1 mL) was added dropwise. Then, the mixture was diluted with CH_2Cl_2, dried (Na_2SO_4), filtered and concentrated. Purification of the residue by column chromatography (SiO_2, petrol ether/EtOAc, gradient of 10-30%) gave the diastereomeric mixture of allylic alcohols **37** (166 mg, 84%, d.r. approx. 5:1, *gulo*:*galacto*).

Methyl-3-*C*-(*E*-1-hydroxymethyl-prop-1-en-3-yl)-2,4,6-tri-*O*-benzyl-β-D-guloside (*gulo*-**37**):

^{1}H-NMR (500.1 MHz, $CDCl_3$): δ 7.33-7.14 (m, 15H, Ar-H), 5.64-5.48 (m, 2H, propenyl-CH), 4.89 (A of AB, ^2J = 11.0 Hz, 1H, $PhCH_2$), 4.57-4.46 (m, 6H, H-1, 5H of $PhCH_2$), 4.31 (t, ^3J = 6.9 Hz, 1H, H-5), 3.92 (d, ^3J = 5.6 Hz, 2H, CH_2OH), 3.61-3.55 (m, 2H, H-6a,b), 3.48 (s, 3H, OMe), 3.42 (s, 1H, H-4), 3.28 (d, ^3J = 7.8 Hz, 1H, H-2), 2.48 (dd, ^2J = 14.6, ^3J = 6.2 Hz, 1H, propenyl-CH_2a), 2.34 (dd, ^2J = 14.7, ^3J = 7.4 Hz, 1H, propenyl-CH_2b);

^{13}C-NMR (125.8 MHz, $CDCl_3$): δ 138.4, 138.3, 138.0 (3C, Ar-C^i), 133.1, (propenyl-CH), 128.5, 128.5, 128.4, 128.3, 128.0, 127.6, 127.1 (16C, 15 Ar-CH, 1 propenyl-CH), 103.3 (C-1), 79.2 (C-2), 77.3 (C-4), 76.2 (C-3), 75.0, 74.8, 73.5 (3 Ph*C*H_2), 72.0 (C-5), 68.7 (C-6), 63.4 (*C*H_2OH), 57.1 (OMe), 37.6 (propenyl-CH_2);

Methyl-3-*C*-(*E*-1-hydroxymethyl-prop-1-en-3-yl)-2,4,6-tri-*O*-benzyl-β-D-galactoside (*galacto*-**37**):

^{1}H-NMR (500.1 MHz, $CDCl_3$): δ 7.33-7.14 (m, 15H, Ar-H), 5.72-5.55 (m, 2H, propenyl-CH), 4.77, 4.62 (A,B of AB, ^2J = 11.5 Hz, 2H, $PhCH_2$), 4.57-4.46 (m, 4H, 4H of $PhCH_2$), 4.31 (d, ^3J = 7.9 Hz, 1H, H-1), 3.99 (d, ^3J = 5.6 Hz, 2H, C*H_2*OH), 3.75 (t, ^3J = 6.6 Hz, 1H, H-5), 3.61-3.55 (m, 3H, H-4, H-6a,b), 3.46

(s, 3H, OMe), 3.41 (d, ^3J = 7.7 Hz, 1H, H-2), 2.70 (dd, ^2J = 15.2, ^3J = 3.5 Hz, 1H, propenyl-CH_2a), 2.11 (dd, ^2J = 15.1, ^3J = 9.0 Hz, 1H, propenyl-CH_2b);

^{13}C-NMR (125.8 MHz, $CDCl_3$): δ 138.9, 138.0, 137.9 (3C, Ar-C^i), 133.0 (propenyl-CH), 128.6, 128.3, 128.0, 127.9, 127.6, 127.1 (16C, 15 Ar-CH, 1 propenyl-CH), 103.4 (C-1), 82.5 (C-2), 78.0 (C-4), 75.9 (C-3), 75.5, 75.3, 75.0 (3C, Ph*C*H_2), 71.7 (C-5), 69.0 (C-6), 63.5 (*C*H_2OH), 57.1 (OMe), 37.6 (propenyl-CH_2);

ESI-MS calcd. for $C_{32}H_{38}O_7Na$ $[M+Na]^+$: 557.3; found: 557.4;

Methyl-3-*C*-(*trans*-1-hydroxy-2*S*,3*S*-epoxy-but-4-yl)-2,4,6-tri-*O*-benzyl-β-D-guloside (38). A mixture of freshly activated molecular sieves (45 mg, 4Å, powder) and dry CH_2Cl_2 (0.8 mL) was cooled to -20 °C under argon. (-)-DET (4.0 µL, 23.5 µmol) and Ti(OiPr)$_4$ (5.8 µL, 19.5 µmol) were added and the mixture was stirred for 25 min. Then, a solution of compound **37** (20.9 mg, 38.1 µmol) in dry CH_2Cl_2 (0.4 mL) was added and the mixture was stirred for additional 30 min. Then, ^{t}BuOOH in decane (13.6 µL, 78.2 µmol, 5.5 M) was added dropwise at -20 °C and the mixture was allowed to warm to 0 °C during 2 h. The mixture was quenched with H_2O (40 µL) and after 30 min aqeous NaOH (10 µL, w = 30%) was added. After stirring for further 30 min at 0 °C, brine was added (5 mL), the phases were separated and the aqueous layer was extracted with CH_2Cl_2 (3 x 10 mL). The combined organic layers were dried over Na_2SO_4, filtered and the volatiles were removed *in vacuo*. The residue was purified by column chromatography (SiO_2, petrol ether/Et_2O, gradient of 50-100%) to give the pure *gulo*-**38** (18.2 mg, 85%) compound as white foam.

^{1}H-NMR (500.1 MHz, CD_2Cl_2): δ 7.30-7.15 (m, 15H, Ar-H), 4.83 (A of AB, ^2J = 11.0 Hz, 1H, PhCH_2), 4.57 (A' of A'B', ^2J = 11.4 Hz, 1H, PhCH_2), 4.53 (d, ^3J = 7.8 Hz, 1H, H-1), 4.51-4.43 (m, 4H, PhCH_2), 4.18 (t, ^3J = 6.9 Hz, 1H, H-5), 3.62-3.55 (m, 4H, H-4, H-6a,b, 1H of CH_2OH), 3.42 (s, 3H, OMe), 3.35-3.30 (m, 1H, 1H of CH_2OH), 3.14 (d, ^3J = 7.7 Hz, 1H, H-2), 2.78 (ddd, ^3J = 2.4, ^3J = 3.7, ^3J = 7.3 Hz, 1H, butyl-H3), 2.72 (s, 1H, OH), 2.67 (dt, ^3J = 2.6, ^3J = 4.9

Hz, 1H, butyl-H2), 1.89 (dd, ^2J = 15.4, ^3J = 4.3 Hz, 1H, butyl-H4a), 1.76 (dd, ^2J = 14.9, ^3J = 7.5 Hz, 1H, butyl-H4b);

^{13}C-NMR (125.8 MHz, CD_2Cl_2): δ 139.0, 138.7, 138.6 (3C, Ar-C^i), 128.9, 128.8, 128.7, 128.5, 128.4, 128.3, 128.2 (15C, Ar-CH), 103.6 (C-1), 79.4 (C-2), 78.0 (C-4), 76.5 (C-3), 75.3, 75.3, 73.9 (3C, Ph*C*H_2), 72.0 (C-5), 69.1 (C-6), 62.2 (*C*H_2OH), 58.2 (butyl-C2), 57.1 (OMe), 52.4 (butyl-C3), 36.6 (butyl-C4);

ESI-MS calcd. for $C_{32}H_{38}O_8Na$ $[M+Na]^+$: 573.2; found: 573.2;

Annulated tetrahydrofuranyl guloside 41. A solution of **38** (44.4 mg, 80.6 µmol) in CH_2Cl_2 (3 mL) was treated with CSA (5 mg) at r.t.. After 12 h at r.t. the solution was quenched with Et_3N (10 µL) and concentrated. The residue was eluted from a column of silica gel (petrol ether/Et_2O, 10-100%) to give pure **41** (10.4 mg, 23%).

R_f (petrol ether/Et_2O, 1/9) = 0.31;

^{1}H-NMR (500.1 MHz, $CDCl_3$): δ 7.32-7.17 (m, 15H, Ar-H), 4.79 (d, ^3J = 7.9 Hz, 1H, H-1), 4.56-4.38 (m, 6H, $PhCH_2$), 4.27 (t, ^3J = 6.7 Hz, 1H, H-5), 4.17 (d, ^3J = 7.4 Hz, 1H, H-α), 4.01 (d, ^3J = 9.7 Hz, 1H, H-β), 3.70 (d, ^3J = 0.8 Hz, 1H, H-4), 3.60 (d, ^3J = 6.9 Hz, 2H, H-6a,b), 3.47 (s, 3H, OMe), 3.41-3.39 (m, 2H, H-2, 1H of CH_2OH), 3.21 ('t', ^3J = ^2J = 9.2 Hz, 1H, 1H of CH_2OH), 2.87 (s, 1H, OH), 2.15 (dd, ^2J = 12.7, ^3J = 10.4 Hz, 1H, H-γa), 1.70 (dd, ^2J = 12.8, ^3J = 2.7 Hz, 1H, H-γb);

^{13}C-NMR (125.8 MHz, $CDCl_3$): δ 138.4, 138.1, 137.5 (3C, Ar-C^i), 128.8, 128.6, 128.5, 128.0, 127.9 (15C, Ar-CH), 101.3 (C-1), 79.5 (C-2), 77.69 (C-4), 77.68 (C-3), 77.6 (C-β), 75.0 ($PhCH_2$), 73.8 (C-5), 73.7, 73.6 (2C, Ph*C*H_2), 71.3 (*C*H_2OH), 71.1 (C-α), 69.1 (C-6), 56.7 (OMe), 33.7 (C-γ);

ESI-MS calcd. for $C_{32}H_{38}O_8Na$ $[M+Na]^+$: 573.2; found: 573.1;

Bis-dinitrobenzoyl annulated tetrahydrofuranyl guloside 42. Compound **41** (10.4 mg, 18.9 µmol) was dissolved in dry CH_2Cl_2 (0.6 mL) under argon.

3,5-Dinitrobenzoyl chloride (19.2 mg, 83 μmol) and DMAP (10.2 mg, 83 μmol) was added at r.t.. After stirring for 4 h, the reaction was concentrated and the residue was purified by column chromatography (SiO_2, petrol ether/Et_2O, gradient of 20-100%) to give **42** (10.5 mg, 59%) as white foam.

R_f (petrol ether/Et_2O, 3:7) = 0.60;

^{1}H-NMR (500.1 MHz, $CDCl_3$): δ 9.17, 9.14, 8.99, 8.93 (4 s, 6H, Ar-H), 7.33-6.99 (m, 15H, Ar-H), 5.15-5.10 (m, 1H, H-α), 4.95 (d, ^3J = 8.0 Hz, 1H, H-1), 4.62, 4.55 (A, B of AB, ^2J = 11.5 Hz, 2H, $PhCH_2$), 4.56 (s, 1H, H-4), 4.39-4.30 (m, 5H, H-β, 4H of $PhCH_2$), 3.89-3.86 (m, 2H, H-2, 1H of $C\mathit{H_2}OBz$), 3.84-3.80 (m, 1H, H-5), 3.68-3.63 (m, 1H, 1H of $C\mathit{H_2}OBz$), 3.59 (s, 3H, OMe), 3.56-3.41 (m, 2H, H-6a,b), 2.56 (d, ^3J = 13.7, 1H, H-γa), 2.50-2.45 (m, 1H, H-γb);

^{13}C-NMR (125.8 MHz, $CDCl_3$): δ 162.3, 161.4 (2C, COOR), 148.9, 148.8, 137.7, 137.5, 137.0, 134.2, 133.3 (9C, Ar-C^i), 129.9-127.6 (19C, Ar-CH), 123.1, 122.9 (2C, Ar-CH), 100.0 (C-1), 89.1 (C-3), 80.1 (C-2), 76.0 (C-α), 75.6 ($PhCH_2$), 75.5 (C-β), 74.0 ($Ph\mathit{C}H_2$), 73.6, 73.5 (2C, C-4, C-5), 73.4 ($Ph\mathit{C}H_2$), 69.0 ($\mathit{C}H_2OBz$), 68.7 (C-6), 56.9 (OMe), 32.2 (C-γ);

HR-MS calcd. for $C_{46}H_{42}N_4O_{18}Na$ $[M+Na]^+$: 961.2392; found: 961.2389.

$[\alpha]^{20}_D$ = +10.1 (*c* 0.50, $CHCl_3$);

Methyl 3-*C*-propyl-β-D-guloside (43). Compound **30** (55.0 mg, 0.10 mmol) was dissolved in dry MeOH (2 mL) under argon. NaOMe in MeOH (0.5 M, 0.5 mL) was added and the reaction was stirred over night at r.t.. After neutralization with Dowex-H^+, the mixture was filtered and the volatiles were removed *in vacuo*. The residue was taken up in dry MeOH (10 mL) and Pd/C (35 mg, w = 10%) was added. The reaction was vigorously stirred in a H_2 atmosphere (1 atm) over night. The mixture was then filtered through a celite plug, and the volatiles were removed *in vacuo*. The crude product was purified by preparative HPLC (C18, H_2O/MeCN 5-95%) to give pure **43** (14.2 mg, 60%, 2 steps) as colorless oil.

^{1}H-NMR (500.1 MHz, CD_3OD): δ 4.44 (d, ^3J = 7.9 Hz, 1H, H-1), 3.96-3.94 (m, 1H, H-5), 3.72 (dd, ^2J = 11.4, ^3J = 6.8 Hz, 1H, H-6a), 3.67 (dd, ^2J = 11.4, ^3J = 5.2 Hz, 1H, H-6b), 3.52 (s, 1H, H-4), 3.51 (s, 3H, MeO), 3.30 (d, ^3J = 7.9 Hz, 1H, H-2), 1.77-1.71 (m, 1H, propyl-H1a), 1.67-1.61 (m, 1H, propyl-H1b), 1.48-1.39 (m, 2H, propyl-H2), 0.94 (t, ^3J = 7.3 Hz, 3H, propyl-H3);

^{13}C-NMR (125.8 MHz, CD_3OD): δ 104.5 (C-1), 76.5 (C-3), 75.6 (C-5), 73.5 (C-2), 70.8 (C-4), 63.3 (C-6), 57.2 (MeO), 37.8 (propyl-C1), 16.2 (propyl-C2), 15.2 (propyl-C3);

ESI-MS calcd. for $C_{10}H_{20}O_6Na$ $[M+Na]^+$: 259.1; found: 259.0;

$[\alpha]^{20}_D$ = -27.9 (*c* 0.71, MeOH);

(*1R,2R*)-2-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-cyclohexyl 3-*O*-allyl-6-*O*-benzyl-β-D-galactopyranoside (45). A suspension of trisaccharide mimetic **44**[190] (1.30 g, 1.66 mmol) and dibutyltin oxide (435 mg, 1.82 mmol) in MeCN/PhMe (13 mL, 1:5) were heated to 150 °C under microwave irradiation under argon for 7 min. TBAI (611 mg, 1.66 mmol) and allyl bromide (717 µL, 8.28 mmol) were added to the clear solution, and the mixture was stirred at 75 °C for 12 h under argon and protected from daylight. After removal of the solvents, the residue was purified by column chromatography (SiO_2, petrol ether/EtOAc, gradient of 10-100%) to give pure **45** (876 mg, 64%) as white foam.

R_f (petrol ether/EtOAc, 1/2) = 0.75;

^{1}H-NMR (500.1 MHz, $CDCl_3$): δ 7.31-7.15 (m, 20 H, Ar-H), 5.92-5.83 (m, 1H, allyl-C*H*), 5.27-5.22 (m, 1H, 1H of allyl-C*H₂*), 5.16-5.13 (m, 1H, 1H of allyl-C*H₂*), 4.88-4.85 (m, 2H, Fuc-H1, 1H of PhC*H₂*), 4.74 (A of AB, ^2J = 11.6 Hz, 1H, 1H of PhC*H₂*), 4.67 (A' of A'B', ^2J = 12.0 Hz, 1H, 1H of PhC*H₂*), 4.62-4.58 (m, 2H, 2H of PhC*H₂*), 4.53 (B'' of A''B'', ^2J = 11.6 Hz, 1H, 1H of PhC*H₂*), 4.42 (br s, 2H, 2H of PhC*H₂*), 4.34 (q, ^3J = 6.3 Hz, 1H, Fuc-H5), 4.25 (d, ^3J = 7.7 Hz, 1H, Gal-H1), 4.15-4.12 (m, 2H, 2H of allyl-C*H₂*), 3.97 (br s, 1H, Gal-H4), 3.94 (dd, ^3J = 3.2, ^3J = 10.2 Hz, 1H, Fuc-H2), 3.90 (dd, ^3J = 2.3, ^3J = 10.1 Hz, 1H, Fuc-H3), 3.73-3.66 (m, 2H, 1H of Cy-CH, Gal-H6a), 3.62-3.57 (m, 3H,

Fuc-H4, Gal-H2, -H6b), 3.53-3.49 (m, 1H, 1H of Cy-CH), 3.48-3.45 (m, 1H, Gal-H5), 3.28 (dd, ^3J = 3.2, ^3J = 9.4 Hz, 1H, Gal-H3), 2.46, 2.30 (2 br s, 2H, 2 OH), 1.99-1.88 (m, 2H, Cy-CH_2), 1.63-1.56 (m, 2H, Cy-CH_2), 1.36-1.10 (m, 4H, Cy-CH_2), 1.01 (d, ^3J = 6.5 Hz, 3H, Fuc-H6);

^{13}C-NMR (125.8 MHz, $CDCl_3$): δ 139.1, 138.9, 138.7, 137.8 (4C, Ar-C^i), 134.4 (allyl-*C*H), 128.3-127.2 (20C, Ar-CH), 117.7 (allyl-*C*H_2), 100.5 (Gal-C1), 94.6 (Fuc-C1), 79.9 (Gal-C3), 79.6 (Fuc-C3), 78.1 (Fuc-C4), 77.5 (Cy-CH), 76.3 (Fuc-C2), 75.9 (Cy-CH), 74.8, 73.6 (2C, Ph*C*H_2), 73.0 (Gal-C5), 72.92, 72.87 (2C, Ph*C*H_2), 71.0 (allyl-CH_2), 70.6 (Gal-C2), 68.8 (Gal-C6), 66.1 (Gal-C4), 66.1 (Fuc-C5), 29.9, 29.1, 23.2 (4C, Cy-CH_2), 16.6 (Fuc-C6);

HR-MS calcd. for $C_{49}H_{60}O_{11}Na$ $[M+Na]^+$: 847.4033; found: 847.4039;

$[\alpha]^{20}_D$ = -42.7 (*c* 0.76, $CHCl_3$);

(*1R,2R*)-2-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-cyclohexyl 3-*O*-allyl-2,4,6-tri-*O*-benzyl-β-D-galactopyranoside (46). Compound **45** (4.40 g, 5.34 mmol) was dissolved in dry DMF (75 mL) and NaH (1.00 g, 21.3 mmol, w = 50%) was added in three portions under argon at r.t.. The suspension was stirred for 2 min and then BnBr (2.52 mL, 21.3 mmol) was added dropwise while keeping the temperature at r.t. with a water bath. After stirring for 7 h, the reaction was quenched with aqueous $NaHCO_3$ and extracted with CH_2Cl_2 (2 x 200 mL). The combined organic layers were dried over Na_2SO_4 and evaporated to dryness. The residue was purified by column chromatography (SiO_2, petrol ether/EtOAc, gradient of 0-40%) to give pure **46** as white foam (4.84 g, 90%).

R_f (petrol ether/EtOAc, 3/1) = 0.60;

^{1}H-NMR (500.1 MHz, $CDCl_3$): δ 7.33-7.10 (m, 30 H, Ar-H), 5.91-5.83 (m, 1H, allyl-C*H*), 5.29-5.23 (m, 1H, 1H of allyl-C*H_2*), 5.12-5.08 (m, 1H, 1H of allyl-C*H_2*), 4.86-4.82 (m, 2H, 2H of PhC*H_2*), 4.77 (br s, 1H, Fuc-H1), 4.67-4.48 (m, 7H, Fuc-H5, 6H of PhC*H_2*), 4.42 (A of AB, ^2J = 10.9 Hz, 1H, PhC*H_2*), 4.35-4.26 (m, 3H, Gal-H1, 2H of PhC*H_2*), 4.16-4.11 (m, 3H, 2H of allyl-C*H_2*, 1H of PhC*H_2*), 3.85 (br s, 3H, Fuc-H2, -H3, Gal-H4), 3.63-3.47 (m, 5H, 2H of Cy-

CH, Gal-H2, -H6a, -H6b), 3.44-3.39 (m, 1H, Gal-H5), 3.34 (dd, ^3J = 2.1, ^3J = 9.6 Hz, 1H, Gal-H3), 3.26 (br s, 1H, Fuc-H4), 2.01-1.88 (m, 2H, Cy-CH_2), 1.64-1.57 (m, 2H, Cy-CH_2), 1.33-1.09 (m, 4H, Cy-CH_2), 0.99 (d, ^3J = 6.3 Hz, 3H, Fuc-H6);

^{13}C-NMR (125.8 MHz, $CDCl_3$): δ 139.5, 139.4, 139.2, 139.0, 138.1 (6C, Ar-C^i), 135.3 (allyl-*C*H), 128.7-127.4 (30C, Ar-CH), 116.6 (allyl-*C*H_2), 101.3 (Gal-C1), 94.7 (Fuc-C1), 82.2 (Gal-C3), 80.1 (2C, Fuc-C3, Gal-C2), 78.9 (Fuc-C4), 78.2 (Cy-CH), 76.3 (Fuc-C2), 75.9 (Cy-CH), 75.5, 75.2 (3C, Ph*C*H_2), 74.1 (Gal-C4), 73.7, 73.1, 72.8 (3C, Ph*C*H_2), 72.7 (Gal-C5), 72.1 (allyl-CH_2), 68.5 (Gal-C6), 66.1 (Fuc-C5), 30.3, 29.5, 23.6 (4C, Cy-CH_2), 16.6 (Fuc-C6);

HR-MS calcd. for $C_{63}H_{72}O_{11}Na$ $[M+Na]^+$: 1027.4972; found: 1027.4999;

$[\alpha]^{20}_D$ = -58.8 (*c* 1.05, $CHCl_3$);

(*1R,2R*)-2-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-cyclohexyl 2,4,6-tri-*O*-benzyl-β-D-galactopyranoside (47). Compound **46** (4.84 g, 4.81 mmol) was dissolved in dry MeOH (100 mL) and $PdCl_2$ (85.0 mg, 0.48 mmol) was added. The suspension was stirred at 50 °C under argon for 105 min. The volatiles were removed *in vacuo* and the residue was purified by column chromatography (SiO_2, petrol ether/EtOAc, gradient of 5-50%) to give pure **47** (4.38 g, 94%) as white foam.

R_f (petrol ether/EtOAc, 2/1) = 0.45;

^{1}H-NMR (500.1 MHz, $CDCl_3$): δ 7.31-7.12 (m, 30 H, Ar-H), 4.96 (A of AB, ^2J = 11.2 Hz, 1H, 1H of PhC*H*$_2$), 4.78 (d, ^3J = 2.8 Hz, 1H, Fuc-H1), 4.76 (A' of A'B', ^2J = 11.1 Hz, 1H, 1H of PhC*H*$_2$), 4.70-4.66 (m, 3H, 3H of PhC*H*$_2$), 4.58 (B'' of A''B'', ^2J = 12.1 Hz, 1H, 1H of PhC*H*$_2$), 4.55-4.51 (m, 4H, Fuc-H5, 3H of PhC*H*$_2$), 4.45 (B' of A'B', ^2J = 11.1 Hz, 1H, 1H of PhC*H*$_2$), 4.37-4.34 (m, 2H, Gal-H1, 1H of PhC*H*$_2$), 4.28 (B''' of A'''B''', ^2J = 11.9 Hz, 1H, 1H of PhC*H*$_2$), 4.22 (B'''' of A''''B'''', ^2J = 11.5 Hz, 1H, 1H of PhC*H*$_2$), 3.87-3.85 (m, 3H, Fuc-H2, -H3, Gal-H4), 3.66-3.62 (m, 1H, Cy-C*H*), 3.61-3.56 (m, 2H, Gal-H3, -H6a), 3.55-3.48 (m, 3H, Gal-H5, -H6b, Cy-C*H*), 3.42 (dd, ^3J = 7.6, ^3J = 9.6 Hz, 1H, Gal-H2), 3.33 (br s, 1H, Fuc-H4), 2.23 (br s, 1H, OH), 2.03-1.87 (m, 2H,

Cy-CH_2), 1.65-1.56 (m, 2H, Cy-CH_2), 1.33-1.11 (m, 4H, Cy-CH_2), 1.01 (d, ^3J = 6.5 Hz, 3H, Fuc-H6);

^{13}C-NMR (125.8 MHz, $CDCl_3$): δ 139.2, 138.9, 138.5, 137.9 (6C, Ar-C^i), 128.5-127.2 (30C, Ar-CH), 101.7 (Gal-C1), 94.8 (Fuc-C1), 80.1 (Gal-C2), 79.9 (Fuc-C3), 78.7 (Fuc-C4), 78.1 (Cy-CH), 76.2 (Fuc-C2), 75.6 (Cy-CH), 75.5 (Gal-C4), 75.3, 75.0, 74.8 (3C, Ph*C*H_2), 74.2 (Gal-C3), 73.4, 72.9 (2C, Ph*C*H_2), 72.8 (Gal-C5), 72.7 (Ph*C*H_2), 68.2 (Gal-C6), 65.9 (Fuc-C5), 30.0, 29.3, 23.3 (4C, Cy-CH_2), 16.7 (Fuc-C6);

HR-MS calcd. for $C_{60}H_{68}O_{11}Na$ $[M+Na]^+$: 987.4659; found: 987.4696;

$[\alpha]^{20}_D$ = -51.8 (*c* 1.00, $CHCl_3$);

(*1R*,*2R*)-2-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-cyclohexyl 2,4,6-tri-*O*-benzyl-β-D-*xylo*-hex-3-ulopyranoside (48). To a solution of oxalyl chloride (1.66 mL, 19.3 mmol) in dry CH_2Cl_2 (100 mL) at -78 °C was added DMSO (3.42 mL, 48.2 mmol) dropwise under strong gas evolution. After stirring for 10 min at -78 °C, a solution of compound **47** (4.65 g, 4.81 mmol) in dry CH_2Cl_2 (100 mL) was added dropwise. After stirring for 2.5 h at -78 °C, NEt_3 (4.74 mL, 33.7 mmol) was added dropwise and the color of the reaction changed from light yellow to colorless. The mixture was stirred another 75 min at -78 °C, was then allowed to warm to r.t. during 6 h and finally poured into aqueous saturated $NaHCO_3$ (200 mL). The phases were separated and the aqueous layer was extracted with CH_2Cl_2 (3 x 150 mL). The combined organic layers were dried over Na_2SO_4, filtered and the volatiles removed *in vacuo*. The residue was purified by column chromatography (SiO_2, petrol ether/EtOAc, gradient of 10-100%) to give pure **48** (4.10 g, 88%) as white foam.

R_f (petrol ether/EtOAc, 3/1) = 0.40;

^{1}H-NMR (500.1 MHz, $CDCl_3$): δ 7.33-6.97 (m, 30 H, Ar-H), 4.81 (d, ^3J = 3.3 Hz, 1H, Fuc-H1), 4.74-4.54 (m, 8H, 8H of PhC*H*$_2$), 4.50 (d, ^3J = 7.5 Hz, 1H, Gal-H1), 4.42 (q, ^3J = 6.2 Hz, 1H, Fuc-H5), 4.37 (A of AB, ^2J = 12.0 Hz, 1H, 1H of PhC*H*$_2$), 4.28, 4.26 (A', B' of A'B', ^2J = 12.6 Hz, 2H, 2H of PhC*H*$_2$), 4.21

(A'' of A''B'', ^2J = 11.2 Hz, 1H, 1H of PhC*H*$_2$), 4.17-4.15 (m, 2H, Gal-H1, 1H of PhC*H*$_2$), 3.89 (dd, ^3J = 3.4, ^3J = 10.1 Hz, 1H, Fuc-H2), 3.87-3.84 (m, 2H, Fuc-H3, Gal-H4), 3.68-3.63 (m, 2H, Gal-H6a, Cy-C*H*), 3.61-3.59 (m, 1H, Gal-H6b), 3.56-3.52 (m, 2H, Gal-H5, Cy-C*H*), 3.34 (br s, 1H, Fuc-H4), 1.96-1.87 (m, 2H, Cy-CH$_2$), 1.65-1.57 (m, 2H, Cy-CH$_2$), 1.33-1.10 (m, 4H, Cy-CH$_2$), 0.85 (d, ^3J = 6.5 Hz, 3H, Fuc-H6);

^{13}C-NMR (125.8 MHz, CDCl$_3$): δ 204.2 (Gal-C3), 139.1, 139.0, 138.9, 137.7, 137.4, 136.7 (6C, Ar-C^i), 128.4-127.1 (30C, Ar-CH), 102.3 (Gal-C1), 94.5 (Fuc-C1), 82.4 (Gal-C2), 81.0 (Gal-C4), 79.8 (Fuc-C3), 79.0 (Cy-CH), 78.3 (Fuc-C4), 76.2 (Fuc-C2), 75.4 (Cy-CH), 74.9, 73.7, 73.3 (3C, Ph*C*H$_2$), 73.0 (Gal-C5), 72.9, 72.8, 72.4 (3C, Ph*C*H$_2$), 67.0 (Gal-C6), 65.9 (Fuc-C5), 29.7, 28.9, 23.1 (4C, Cy-CH$_2$), 16.4 (Fuc-C6);

HR-MS calcd. for $C_{60}H_{66}O_{11}Na$ [M+Na]$^+$: 985.4503; found: 985.4539;

$[\alpha]^{20}_D$ = -47.5 (*c* 0.15, CHCl$_3$);

(*1R,2R*)-2-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-cyclohexyl 3-*C*-allyl-2,4,6-tri-*O*-benzyl-β-D-gulopyranoside (*gulo*-49) and (*1R,2R*)-2-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-cyclohexyl 3-*C*-allyl-2,4,6-tri-*O*-benzyl-β-D-galactopyranoside (*galacto*-49). A round bottom flask equipped with a reflux condenser was charged with Mg powder (3.84 g, 160 mmol), dry Et$_2$O (100 mL) and cat. I$_2$ under argon. Allyl bromide (13.8 mL, 160 mmol) was added dropwise. After half of the allyl bromide was added, the reaction started and the remaining bromide was added dropwise to keep the reaction refluxing. Finally, the mixture was heated to reflux for 20 min. After cooling to 0 °C, a solution of compound **48** (3.85 g, 4.00 mmol) in dry Et$_2$O (100 mL) was added dropwise and the resulting suspension was stirred for 3.5 h. Then, the suspension was quenched with ice and saturated aqueous NH$_4$Cl (200 mL). The phases were separated and the aqueous layer was extracted with CH$_2$Cl$_2$ (3 x 150 mL). The combined organic layers were dried over Na$_2$SO$_4$, filtered and the volatiles were removed *in vacuo*. The residue was purified by column chromatography (SiO$_2$, petrol ether/Et$_2$O, gradient of 10-80%) to give first ***galacto*-49** (1.43 g, 36%) as white foam and second ***gulo*-**

49 (1.63 g, 41%).

(*1R*,*2R*)-2-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-cyclohexyl 3-*C*-allyl-2,4,6-tri-*O*-benzyl-β-D-galactopyranoside (***galacto*-49**):

R_f (petrol ether/Et_2O, 2/1) = 0.48;

^{1}H-NMR (500.1 MHz, $CDCl_3$): δ 7.32-7.14 (m, 30 H, Ar-H), 5.90-5.81 (m, 1H, allyl-CH), 5.09-5.01 (m, 2H, allyl-CH_2), 4.86 (A of AB, ^2J = 11.5 Hz, 1H, 1H of PhCH_2), 4.80 (d, ^3J = 2.9 Hz, 1H, Fuc-H1), 4.75-4.65 (m, 3H, 3H of PhCH_2), 4.61-4.54 (m, 4H, 4H of PhCH_2), 4.52 (q, ^3J = 6.6 Hz, 1H, Fuc-H5), 4.46 (d, ^3J = 7.9 Hz, 1H, Gal-H1), 4.45-4.39 (m, 2H, 2H of PhCH_2), 4.32-4.27 (m, 2H, 2H of PhCH_2), 3.91-3.85 (m, 2H, Fuc-H2, -H3), 3.73-3.70 (m, 1H, Gal-H5), 3.63 (br s, 1H, Gal-H4), 3.62-3.47 (m, 4H, Gal-H6a, -H6b, 2H of Cy-C*H*), 3.37 (d, ^3J = 8.1 Hz, 1H, Gal-H2), 3.35 (br s, 1H, Fuc-H4), 2.73 (dd, ^2J = 15.0, ^3J = 5.0 Hz, 1H, 1H of allyl-CH_2), 2.26 (br s, 1H, OH), 2.13 (dd, ^2J = 15.1, ^3J = 9.3 Hz, 1H, 1H of allyl-CH_2), 1.98-1.87 (m, 2H, Cy-CH_2), 1.63-1.55 (m, 2H, Cy-CH_2), 1.34-1.08 (m, 4H, Cy-CH_2), 1.00 (d, ^3J = 6.5 Hz, 3H, Fuc-H6);

^{13}C-NMR (125.8 MHz, $CDCl_3$): δ 139.2, 139.0, 138.9, 138.4, 138.0 (6C, Ar-C^i), 133.3 (allyl-*C*H), 128.5-127.2 (30C, Ar-CH), 118.4 (allyl-*C*H_2), 99.8 (Gal-C1), 94.6 (Fuc-C1), 82.8 (Gal-C2), 79.9 (Fuc-C3), 78.7 (Fuc-C4), 78.3 (Cy-CH), 78.0 (Gal-C4), 76.3 (Fuc-C2), 76.0 (Gal-C3), 75.7 (Ph*C*H_2), 75.7 (Cy-CH), 75.4, 75.0, 73.2, 73.0, 72.8 (5C, Ph*C*H_2), 70.9 (Gal-C5), 68.5 (Gal-C6), 65.9 (Fuc-C5), 36.6 (allyl-*C*H_2), 30.0, 29.2, 23.3 (4C, Cy-CH_2), 16.7 (Fuc-C6);

HR-MS calcd. for $C_{63}H_{72}O_{11}Na$ $[M+Na]^+$: 1027.4972; found: 1027.4977;

$[\alpha]^{20}_D$ = -41.4 (*c* 0.48, $CHCl_3$);

(*1R*,*2R*)-2-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-cyclohexyl 3-*C*-allyl-2,4,6-tri-*O*-benzyl-β-D-gulopyranoside (***gulo*-49**):

R_f (petrol ether/Et_2O, 2/1) = 0.37;

^{1}H-NMR (500.1 MHz, $CDCl_3$): δ 7.30-7.12 (m, 30 H, Ar-H), 5.92-5.84 (m, 1H, allyl-CH), 5.07-4.96 (m, 3H, 2H of allyl-CH_2, 1H of PhCH_2), 4.77 (br s, 1H,

Fuc-H1), 4.70 (d, ^{3}J = 7.7 Hz, 1H, Gul-H1), 4.67-4.56 (m, 6H, Fuc-H5, 5H of PhC*H*$_2$), 4.49-4.41 (m, 4H, 4H of PhC*H*$_2$), 4.33 (A of AB, ^{2}J = 12.0 Hz, 1H, 1H of PhC*H*$_2$), 4.15-4.11 (m, 2H, Gul-H5, 1H of PhC*H*$_2$), 3.87-3.83 (m, 2H, Fuc-H2, -H3), 3.66-3.60 (m, 1H, Cy-CH), 3.57-3.54 (m, 2H, Gul-H6a, -H6b), 3.50-3.46 (m, 2H, Gul-H4, Cy-CH), 3.25 (br s, 1H, Fuc-H4), 3.19 (d, ^{3}J = 7.6 Hz, 1H, Gul-H2), 2.58 (dd, ^{2}J = 14.6, ^{3}J = 6.0 Hz, 1H, 1H of allyl-C*H*$_2$), 2.49 (dd, ^{2}J = 14.6, ^{3}J = 8.1 Hz, 1H, 1H of allyl-C*H*$_2$), 2.18 (br s, 1H, OH), 2.04-1.87 (m, 2H, Cy-CH$_2$), 1.64-1.55 (m, 2H, Cy-CH$_2$), 1.36-1.08 (m, 4H, Cy-CH$_2$), 1.03 (d, ^{3}J = 6.5 Hz, 3H, Fuc-H6);

^{13}C-NMR (125.8 MHz, CDCl$_3$): δ 139.3, 139.2, 138.9, 138.6, 138.2, 138.0 (6C, Ar-C^i), 133.7 (allyl-*C*H), 128.5-127.2 (30C, Ar-CH), 118.7 (allyl-*C*H$_2$), 99.4 (Gal-C1), 94.8 (Fuc-C1), 79.9 (Fuc-C3), 79.5 (Gal-C2), 78.9 (Fuc-C4), 78.1 (Cy-CH), 76.8 (Gal-C4), 76.2 (Gal-C3), 76.1 (Fuc-C2), 75.9 (Cy-CH), 75.1, 75.0, 74.8, 73.3, 72.9, 72.7 (6C, Ph*C*H$_2$), 71.0 (Gal-C5), 68.3 (Gal-C6), 65.9 (Fuc-C5), 39.5 (allyl-*C*H$_2$), 30.3, 29.4, 23.5 (4C, Cy-CH$_2$), 16.6 (Fuc-C6);

$[\alpha]^{20}_D$ = -65.0 (*c* 1.12, CHCl$_3$);

(*1R*,*2R*)-2-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-cyclohexyl 3-*C*-(*E*-1-methoxycarbonyl-prop-1-en-3-yl)-2,4,6-tri-*O*-benzyl-β-D-galactopyranoside (50). A round bottom flask equipped with a reflux condenser was charged with a solution of compound ***galacto*-49** (1.29 g, 1.28 mmol) in dry CH$_2$Cl$_2$ (40 mL). Grubbs catalyst 2nd generation (54.5 mg, 0.06 mmol) was added, immediately followed by addition of methyl acrylate (1.16 mL, 12.8 mmol). After heating the reaction to reflux under argon and stirring for 1 h, the volatiles were removed *in vacuo*. The residue was purified by column chromatography (SiO$_2$, petrol ether/EtOAc, gradient of 10-100%) to give **50** (1.24 g, 91%) as white foam.

R$_f$ (petrol ether/EtOAc, 5/2) = 0.33;

^{1}H-NMR (500.1 MHz, CDCl$_3$): δ 7.31-7.13 (m, 30 H, Ar-H), 7.02 (ddd, ^{3}J = 5.3, ^{3}J = 9.3, ^{3}J = 15.3 Hz, 1H, propenyl-H2), 5.79 (d, ^{3}J = 16.1 Hz, 1H, propenyl-H1), 4.84 (A of AB, ^{2}J = 11.4 Hz, 1H, 1H of PhC*H*$_2$), 4.80 (d, ^{3}J = 3.3 Hz, 1H,

Fuc-H1), 4.75 (A' of A'B', ^{2}J = 11.5 Hz, 1H, 1H of PhC*H*$_2$), 4.71 (A'' of A''B'', ^{2}J = 11.5 Hz, 1H, 1H of PhC*H*$_2$), 4.67 (A''' of A'''B''', ^{2}J = 12.1 Hz, 1H, 1H of PhC*H*$_2$), 4.59-4.54 (m, 4H, 4H of PhC*H*$_2$), 4.48 (q, ^{3}J = 6.3 Hz, 1H, Fuc-H5), 4.45-4.42 (m, 2H, Gal-H1, 1H of PhC*H*$_2$), 4.37 (A'''' of A''''B'''', ^{2}J = 12.0 Hz, 1H, 1H of PhC*H*$_2$), 4.32-4.28 (m, 2H, 2H of PhC*H*$_2$), 3.90 (dd, ^{3}J = 3.3, ^{3}J = 10.1 Hz, 1H, Fuc-H2), 3.86 (dd, ^{3}J = 2.5, ^{3}J = 10.1 Hz, 1H, Fuc-H3), 3.68-3.47 (m, 9H, Gal-H4, -H5, -H6a, -H6b, 2H of Cy-C*H*, CH_3O-), 3.36 (br s, 1H, Fuc-H4), 3.34 (d, ^{3}J = 7.9 Hz, 1H, Gal-H2), 2.80 (ddd, ^{2}J = 15.4, ^{3}J = 5.2, ^{4}J = 1.4 Hz, 1H, propenyl-H3a), 2.30 (br s, 1H, OH), 2.24 (dd, ^{2}J = 15.4, ^{3}J = 9.4 Hz, propenyl-H3b), 1.97-1.85 (m, 2H, Cy-CH_2), 1.64-1.54 (m, 2H, Cy-CH_2), 1.33-1.07 (m, 4H, Cy-CH_2), 0.99 (d, ^{3}J = 6.5 Hz, 3H, Fuc-H6);

^{13}C-NMR (125.8 MHz, $CDCl_3$): δ 166.4 (CO_2R), 144.5 (propenyl-C2), 139.2, 139.1, 138.8, 138.6, 138.0, 137.8 (6C, Ar-C^{i}), 128.6-127.1 (30C, Ar-CH), 123.7 (propenyl-C1), 99.7 (Gal-C1), 94.6 (Fuc-C1), 82.5 (Gal-C2), 79.8 (Fuc-C3), 78.6 (Fuc-C4), 78.4 (Cy-CH), 78.0 (Gal-C4), 76.3 (Fuc-C2), 76.0 (Gal-C3), 75.8 (Ph*C*H$_2$), 75.6 (Cy-CH), 75.5, 75.0, 73.2, 73.0, 72.8 (5C, Ph*C*H$_2$), 70.9 (Gal-C5), 68.2 (Gal-C6), 65.9 (Fuc-C5), 51.4 (MeO), 35.0 (propenyl-C3), 29.7, 29.1, 23.2 (4C, Cy-CH_2), 16.7 (Fuc-C6);

HR-MS calcd. for $C_{65}H_{74}O_{13}Na$ $[M+Na]^+$: 1085.5027; found: 1085.5030;

$[\alpha]^{20}_{D}$ = -26.3 (*c* 1.47, $CHCl_3$);

(*1R,2R*)-2-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-cyclohexyl 3-*C*-(*E*-1-hydroxymethyl-prop-1-en-3-yl)-2,4,6-tri-*O*-benzyl-β-D-galactopyranoside (51). Compound **50** (42 mg, 40.3 µmol) was dissolved in dry PhMe (2.5 mL) and cooled to -78 °C under argon. DIBAl-H (200 µL, 0.20 mmol, 1M in cyclohexane) was added dropwise. After stirring for 1 h, the reaction was quenched with saturated aqueous NH_4Cl (0.5 mL), diluted with CH_2Cl_2 and directly dried over Na_2SO_4. After filtration, the volatiles were removed *in vacuo*. The residue was purified by column chromatography (SiO_2, petrol ether/EtOAc, gradient of 13-100%) to give **51** (31.8 mg, 76%) as white foam.

R_f (petrol ether/EtOAc, 4/1) = 0.20;

^{1}H-NMR (500.1 MHz, $CDCl_3$): δ 7.31-7.12 (m, 30 H, Ar-H), 5.72-5.55 (m, 2H, propenyl-H1, -H2), 4.85 (A of AB, ^2J = 11.5 Hz, 1H, 1H of PhC*H₂*), 4.80 (d, ^3J = 3.2 Hz, 1H, Fuc-H1), 4.76-4.70 (m, 2H, 2H of PhCH_2), 4.67 (A' of A'B', ^2J = 12.1 Hz, 1H, 1H of PhCH_2), 4.59-4.54 (m, 4H, 4H of PhCH_2), 4.51 (q, ^3J = 5.4 Hz, 1H, Fuc-H5), 4.45 (d, ^3J = 8.0 Hz, 1H, Gal-H1), 4.43 (A'' of A''B'', ^2J = 11.2 Hz, 1H, 1H of PhCH_2), 4.37 (A''' of A'''B''', ^2J = 11.9 Hz, 1H, 1H of PhCH_2), 4.32-4.28 (m, 2H, 2H of PhCH_2), 4.00-3.97 (m, 2H, HOCH_2), 3.90 (dd, ^3J = 3.2, ^3J = 10.1 Hz, 1H, Fuc-H2), 3.86 (dd, ^3J = 2.5, ^3J = 10.1 Hz, 1H, Fuc-H3), 3.71-3.67 (m, 1H, Gal-H5), 3.63-3.57 (m, 2H, Gal-H4, Cy-C*H*), 3.55-3.48 (m, 3H, Gal-H6a, -H6b, Cy-C*H*), 3.36 (br s, 1H, Fuc-H4), 3.36 (d, ^3J = 7.8 Hz, 1H, Gal-H2), 2.68 (dd, ^2J = 14.6, ^3J = 4.2 Hz, 1H, propenyl-H3a), 2.24 (br s, 1H, OH), 2.13 (dd, ^2J = 15.1, ^3J = 9.1 Hz, 1H, propenyl-H3b), 1.99-1.86 (m, 2H, Cy-CH_2), 1.63-1.48 (m, 2H, Cy-CH_2), 1.33-1.08 (m, 4H, Cy-CH_2), 1.00 (d, ^3J = 6.5 Hz, 3H, Fuc-H6);

^{13}C-NMR (125.8 MHz, $CDCl_3$): δ 139.2, 139.1, 138.9, 138.8, 138.3, 137.8 (6C, Ar-C^i), 137.8 (propenyl-C1), 128.3-127.2 (31C, 30 Ar-CH, propenyl-C2), 99.9 (Gal-C1), 94.6 (Fuc-C1), 82.8 (Gal-C2), 79.9 (Fuc-C3), 78.7 (Fuc-C4), 78.3 (Cy-CH), 77.7 (Gal-C4), 76.3 (Fuc-C2), 76.0 (Gal-C3), 75.7 (Ph*C*H_2), 75.6 (Cy-CH), 75.4, 74.9, 73.3, 73.0, 72.9 (5C, Ph*C*H_2), 70.7 (Gal-C5), 68.3 (Gal-C6), 65.9 (Fuc-C5), 63.6 (*C*H_2OH), 35.1 (propenyl-C3), 30.0, 29.2, 23.3 (4C, Cy-CH_2), 16.7 (Fuc-C6);

HR-MS calcd. for $C_{64}H_{74}O_{12}Na$ $[M+Na]^+$: 1057.5078; found: 1057.5078;

$[\alpha]^{20}_D$ = -32.2 (*c* 1.59, $CHCl_3$);

(*1R,2R*)-2-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-cyclohexyl 3-*C*-(*trans*-1-hydroxy-2,3-epoxy-but-4-yl)-2,4,6-tri-*O*-benzyl-β-D-galactopyranoside (52). A mixture of freshly activated molecular sieves (100 mg, 4Å, powder) and dry CH_2Cl_2 (8 mL) was cooled to -20 °C under argon. (-)-DET (40 µL, 0.23 mmol) and $Ti(OiPr)_4$ (57 µL, 0.19 mmol) were added and the mixture was stirred for 30 min. Then, a solution of compound **51** (400 mg, 0.38 mmol) in dry CH_2Cl_2 (8 mL) was added and the mixture was stirred for additional 30 min. Then, ^{t}BuOOH in decane (281 µL, 1.55 mmol, 5.5 M) was added

dropwise at -20 °C and the mixture was allowed to warm to 10 °C during 2 h. The mixture was cooled to 0 °C and first, H_2O (0.40 mL) and after 30 min NaOH aq. (0.10 mL, w = 30%) was added. After stirring for further 30 min at 0 °C, brine was added (50 mL), the phases were separated and the aqueous layer was extracted with CH_2Cl_2 (3 x 100 mL). The combined organic layers were dried over Na_2SO_4, filtered and the volatiles were removed *in vacuo*. The residue was purified by column chromatography (SiO_2, petrol ether/EtOAc, gradient of 10-100%) to give **52** (177 mg, 44%) as a mixture of diastereomers as white foam.

R_f (petrol ether/EtOAc, 2/5) = 0.80;

^{1}H-NMR (500.1 MHz, $CDCl_3$): δ 7.31-7.12 (m, 30 H, Ar-H), 4.86-4.19 (m, 2H, Fuc-H1, -H5, Gal-H1, 12H of PhC*H*$_2$), 3.92-3.85 (m, 2H, Fuc-H2, -H3), 3.81-3.78 (m, 1H, Gal-H5, major isomer), 3.74, 3.46 (2 m, 2H, butyl-H1, major isomer), 3.57, 3.49 (2 m, 2H, butyl-H1, minor isomer), 3.70-3.48 (m, 6H, Gal-H4, -H5, -H6a, -H6b, 2 Cy-CH), 3.39-3.31 (m, 2H, Fuc-H4, Gal-H2), 3.22-3.18 (m, 1H, butyl-H3, major isomer), 3.13-3.10 (m, 1H, butyl-H3, minor isomer), 2.82-2.80 (m, 1H, butyl-H2, major isomer), 2.78-2.75 (m, 1H, butyl-H2, minor isomer), 2.49-2.35 (m, 3H, butyl-H4a, 2 OH), 1.96-1.86 (m, 2H, Cy-CH_2), 1.63-1.46 (m, 2H, Cy-CH_2), 1.37-1.28 (m, 1H, butyl-H4b), 1.24-1.08 (m, 4H, Cy-CH_2), 1.01-0.95 (m, 3H, Fuc-H6);

^{13}C-NMR (125.8 MHz, $CDCl_3$, selected data): δ 139.2, 139.1, 138.8, 138.7, 138.1, 137.9 (6C, Ar-C^i), 128.6-127.2 (30C, Ar-CH), 99.7 (Gal-C1), 94.7 (Fuc-C1), 82.6 (Gal-C2, maj.), 82.4 (Gal-C2, min.), 79.9 (Fuc-C3), […] 76.4 (Gal-C3, maj.), […], 75.4 (Gal-C3, min.), […], 71.3 (Gal-C5, maj.), 71.0 (Gal-C5, min.), 68.4 (Gal-C6, maj.), 68.1 (Gal-C6, min.), 65.9 (Fuc-C5), 61.7 (butyl-C1), 59.0 (butyl-C2, min.), 57.7 (butyl-C2, maj.), 52.5 (butyl-C3, min.), 52.3 (butyl-C3, maj.), 34.4 (butyl-C4), 30.0, 29.2, 23.2 (4C, Cy-CH_2), 16.7 (Fuc-C6);

ESI-MS (5.0 kV) calcd. for $C_{64}H_{74}O_{13}Na$ $[M+Na]^+$: 1073.5; found: 1073.5;

(*1R*,*2R*)-2-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-cyclohexyl 3-(*S*)-(1,4-*spiro*-2*R*-hydroxymethyl-3*S*-hydroxy-tetrahydrofuranyl)-2,4,6-tri-*O*-benz-

yl-β-D-xylopyranoside (53). Compound **52** (60 mg, 57.1 μmol) was dissolved in dry DMF (12 mL) and cooled to 0 °C under argon. NaH (60 mg, suspension in mineral oil, w = 50%) was added portionwise. After stirring for 5 h, the reaction was quenched with saturated aqueous Na_2CO_3 (10 mL), diluted with brine (40 mL) and extracted with CH_2Cl_2 (3 x 50 mL). The combined organic layers were dried over Na_2SO_4. After filtration, the volatiles were removed *in vacuo*. The residue was purified by column chromatography (SiO_2, petrol ether/EtOAc, gradient of 10-100%) to give a diastereomeric mixture of the oxetanes **54** (24.8 mg, 41%) and the title compound **53** (12.6 mg, 21%) as white foam.

R_f (petrol ether/EtOAc, 2/5) = 0.45;

^{1}H-NMR (500.1 MHz, $CDCl_3$): δ 7.33-7.09 (m, 30 H, Ar-H), 5.01 (A of AB, ^2J = 10.2 Hz, 1H, 1H of PhC*H*$_2$), 4.86 (A' of A'B', ^2J = 10.8 Hz, 1H, 1H of PhC*H*$_2$), 4.76 (d, ^3J = 3.0 Hz, 1H, Fuc-H1), 4.68-4.63 (m, 3H, 3H of PhC*H*$_2$), 4.59-4.49 (m, 4H, Fuc-H5, 3H of PhC*H*$_2$), 4.41 (B' of A'B', ^2J = 10.9 Hz, 1H, 1H of PhC*H*$_2$), 4.34 (d, ^3J = 8.0 Hz, 1H, Gal-H1), 4.29-4.27 (m, 2H, 2H of PhC*H*$_2$), 4.18 (A''' of A'''B''', ^2J = 11.5 Hz, 1H, 1H of PhC*H*$_2$), 4.07-4.03 (m, 1H, THF-H3), 4.00-3.96 (m, 1H, THF-H2), 3.87 (dd, ^3J = 3.1, ^3J = 10.2 Hz, 1H, Fuc-H2), 3.84 (dd, ^3J = 2.2, ^3J = 10.4 Hz, 1H, Fuc-H3), 3.78-3.74 (m, 1H, 1H of THF-C*H*$_2$OH), 3.71-3.68 (m, 2H, Gal-H2, 1H of THF-C*H*$_2$OH), 3.65-3.46 (4H, Gal-H6a, -H6b, 2H of Cy-C*H*), 3.41-3.37 (m, 2H, Gal-H4, -H5), 3.29 (br s, 1H, Fuc-H4), 2.11-2.07 (m, 1H, OH), 2.02 (d, ^2J = 14.1 Hz, 1H, THF-H4^{syn}), 1.97 (br s, 1H, OH), 1.94-1.81 (m, 2H, Cy-CH_2), 1.70 (dd, ^2J = 14.1, ^3J = 5.3 Hz, THF-H4^{anti}), 1.66-1.60 (m, 2H, Cy-CH_2), 1.33-1.11 (m, 4H, Cy-CH_2), 1.02 (d, ^3J = 6.5 Hz, 3H, Fuc-H6);

^{13}C-NMR (125.8 MHz, $CDCl_3$): δ 139.2, 138.9, 138.8, 137.8, 136.9 (6C, Ar-C^i), 128.6-127.2 (30C, 30 Ar-CH), 100.8 (Gal-C1), 94.7 (Fuc-C1), 87.1 (Gal-C3), 81.3 (Gal-C2), 79.9 (Fuc-C3), 79.3 (Gal-C4), 78.6 (2C, Fuc-C4, THF-C2), 78.3 (Cy-CH), 76.2 (Ph*C*H$_2$), 76.1 (Fuc-C2), 75.6 (Cy-CH), 75.5, 75.0, 73.5, 73.0 (4C, Ph*C*H$_2$), 72.7 (2C, THF-C3, Ph*C*H$_2$), 71.5 (Gal-C5), 68.3 (Gal-C6), 65.9 (Fuc-C5), 61.9 (THF-*C*H$_2$OH), 40.2 (THF-C4), 29.7, 29.5, 23.5 (4C, Cy-CH_2), 16.7 (Fuc-C6);

ESI-MS (3.5 kV) calcd. for $C_{64}H_{74}O_{13}Na$ $[M+Na]^+$: 1073.5; found: 1073.7;

$[\alpha]^{20}_D$ = -55.9 (*c* 0.60, $CHCl_3$);

Oxetanes 54:

R_f (petrol ether/EtOAc, 2/5) = 0.55, both diastereomers;

Major diastereomer, selected data
^{1}H-NMR (500.1 MHz, $CDCl_3$): δ 4.70 (m, 1H, oxetane-H2), 3.81 (m, 1H, C*H*(OH)CH_2OH), 3.51-3.26 (m, 2H, CH(OH)C*H*$_2$OH), 3.03 (dd, ^2J = 11.6, ^3J = 7.5 Hz, 1H, oxetane-H3a), 1.93 (dd, ^2J = 11.7, ^3J = 7.7 Hz, 1H, oxetane-H3b);

^{13}C-NMR (125.8 MHz, $CDCl_3$): δ 85.8 (Gal-C3 = oxetane-C4), 77.5 (oxetane-C2), 72.5 (*C*H(OH)CH_2OH), 61.9 (CH(OH)*C*H$_2$OH), 24.5 (oxetane-C3);

Minor diastereomer, selected data
^{1}H-NMR (500.1 MHz, $CDCl_3$): δ 4.57 (m, 1H, oxetane-H2), 3.70 (m, 1H, C*H*(OH)CH_2OH), 3.41-3.31 (m, 2H, CH(OH)C*H*$_2$OH), 2.63 (dd, ^2J = 10.8, ^3J = 7.9 Hz, 1H, oxetane-H3a), 2.07 (dd, ^2J = 10.9, ^3J = 7.0 Hz, 1H, oxetane-H3b);

^{13}C-NMR (125.8 MHz, $CDCl_3$): δ 86.7 (Gal-C3 = oxetane-C4), 77.5 (oxetane-C2), 73.4 (*C*H(OH)CH_2OH), 61.3 (CH(OH)*C*H$_2$OH), 26.4 (oxetane-C3);

ESI-MS (3.5 kV) calcd. for $C_{64}H_{74}O_{13}Na$ $[M+Na]^+$: 1073.5; found: 1073.7; both diastereomers;

(*1R,2R*)-2-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-cyclohexyl 3-(*S*)-(1,4-*spiro*-2*R*-(3,5-dinitrobenzoyl)oxy-3*S*-(3,5-dinitrobenzoyl)oxy-tetrahydro-furanyl)-2,4,6-tri-*O*-benzyl-β-D-xylopyranoside (55). Compound **53** (12.6 mg, 12.0 µmol) was dissolved in dry CH_2Cl_2 (2 mL) under argon. 3,5-Dinitrobenzoyl chloride (15.2 mg, 66.0 µmol) and DMAP (8.0 mg, 66.0 µmol) was added at r.t.. After stirring over night, the reaction was concentrated and the residue was purified by column chromatography (SiO_2, petrol ether/EtOAc, gradient of 4-30%) to give compound **55** (17.3 mg, quant.) as white foam.

R_f (petrol ether/EtOAc, 4/3) = 0.80;

^{1}H-NMR (500.1 MHz, $CDCl_3$): δ 8.97-8.95 (m, 1H, Ar-H), 8.91-8.88 (m, 3H, Ar-H), 8.72-8.71 (m, 2H, Ar-H), 7.34-6.75 (m, 30 H, Ar-H), 5.46 (dd, ^3J = 3.5, ^3J = 5.1 Hz, 1H, THF-H3), 4.90-4.72 (m, 5H, 2H of C*H₂*O(CO)R, 3H of PhC*H₂*), 4.67 (d, ^3J = 2.8 Hz, 1H, Fuc-H1), 4.62 (A of AB, ^2J = 12.3 Hz, 1H, 1H of PhC*H₂*), 4.57 (A' of A'B', ^2J = 11.5 Hz, 1H, 1H of PhC*H₂*), 4.53-4.43 (m, 4H, Fuc-H5, THF-H2, 2H of PhC*H₂*), 4.38 (A'' of A''B'', ^2J = 11.4 Hz, 1H, 1H of PhC*H₂*), 4.35-4.25 (m, 4H, Gal-H1, 3H of PhC*H₂*), 3.93 (A''' of A'''B''', ^2J = 11.3 Hz, 1H, 1H of PhC*H₂*), 3.78 (dd, ^3J = 3.0, ^3J = 10.2 Hz, 1H, Fuc-H2), 3.75 (dd, ^3J = 2.3, ^3J = 10.3 Hz, 1H, Fuc-H3), 3.72 (d, ^3J = 7.9 Hz, 1H, Gal-H2), 3.60-3.46 (5H, Gal-H4, -H5, -H6a, -H6b, 1H of Cy-C*H*), 3.36-3.31 (m, 1H, 1H of Cy-C*H*), 3.06 (br s, 1H, Fuc-H4), 2.67 (d, ^2J = 14.9 Hz, 1H, THF-$H4^{syn}$), 2.06 (dd, ^2J = 15.0, ^3J = 6.5 Hz, THF-$H4^{anti}$), 1.94-1.81 (m, 2H, Cy-CH_2), 1.66-1.60 (m, 2H, Cy-CH_2), 1.33-1.11 (m, 4H, Cy-CH_2), 0.93 (d, ^3J = 6.5 Hz, 3H, Fuc-H6);

^{13}C-NMR (125.8 MHz, $CDCl_3$): δ 162.1, 162.0 (2C, ArCOOR), 148.4, 148.2, 139.2, 138.9, 138.8, 138.2, 137.7, 132.8, 132.6 (12C, Ar-C^i), 129.2-126.0 (34C, Ar-CH), 122.5 (2C, Ar-CH), 100.9 (Gal-C1), 94.5 (Fuc-C1), 88.2 (Gal-C3), 79.9 (Fuc-C3), 78.7 (Fuc-C4), 78.3 (2C, Gal-C2, -C4), 77.7 (THF-C3), 77.1 (Cy-CH), 76.2 (THF-C2), 75.9 (Fuc-C2), 75.7 (Ph*C*H₂), 75.3 (Cy-CH), 75.0, 74.4, 73.6, 72.9, 72.5 (5C, Ph*C*H₂), 70.8 (Gal-C5), 67.8 (Gal-C6), 65.8 (Fuc-C5), 64.4 (THF-*C*H₂OR), 36.4 (THF-C4), 30.2, 29.7, 29.4, 23.4 (4C, Cy-CH_2), 16.5 (Fuc-C6);

ESI-MS (5.0 kV) calcd. for $C_{78}H_{78}N_4O_{23}Na$ $[M+Na]^+$: 1461.5; found: 1461.6;

$[\alpha]^{20}_D$ = -22.9 (*c* 0.80, $CHCl_3$);

Oxetanes 56. Compound **54** (24.8 mg, 23.6 µmol) was dissolved in dry CH_2Cl_2 (2 mL) under argon. 3,5-Dinitrobenzoyl chloride (41.0 mg, 176 µmol) and DMAP (31.7 mg, 260 µmol) was added at r.t.. After stirring for 3 h, the reaction was concentrated and the residue was purified by column chromatography (SiO_2, petrol ether/EtOAc, gradient of 4-30%) to give a

mixture of diastereomers **56** (23.7 mg, 70%) as white foam.

R_f (petrol ether/EtOAc, 2/1) = 0.33, both diastereomers;

Major diastereomer, selected data
^{1}H-NMR (500.1 MHz, $CDCl_3$): δ 5.69 (td, ^3J = 6.8, ^3J = 2.5 Hz, C*H*(OR)CH_2OR), 4.82 (m, 1H, oxetane-H2), 4.42 (m, 1H, CH(OR)CH_2OR), 3.51-3.26 (m, 1H, CH(OR)CH_2OR), 2.98-2.93 (m, 1H, oxetane-H3a), 2.27-2.21 (m, 1H, oxetane-H3b);

Minor diastereomer, selected data
^{1}H-NMR (500.1 MHz, $CDCl_3$): δ 5.63 (td, ^3J = 6.4, ^3J = 3.1 Hz, C*H*(OR)CH_2OR), 4.93 (m, 1H, oxetane-H2), 4.49 (m, 1H, CH(OR)CH_2OR), 3.51-3.26 (m, 1H, CH(OR)CH_2OR), 2.98-2.93 (m, 1H, oxetane-H3a), 2.27-2.21 (m, 1H, oxetane-H3b);

ESI-MS (5.0 kV) calcd. for $C_{78}H_{78}N_4O_{23}Na$ $[M+Na]^+$: 1461.5; found: 1461.5; both diastereomers;

2.4 Pre-organization of the Lewis^x core of sLe^x as a result of a lipophilic stabilization

Lemieux[220] postulated an attractive interresidue interaction between lipophilic patches of oligosaccharides. To support this hypothesis with experimental evidence, sLe^x derivatives with and without a lipophilic substituent at the fucose 5-position were designed, synthesized, and biologically and biophysically evaluated and compared to the natural epitope sLe^x.

This part is in preparation and will be submitted to *Angewandte Chemie, International Edition* in due course.

Lipophilic interactions between the fucose α-face and galactose β-face: Nature stabilizes the internal conformation of the core of sialyl Lewis^x in the bioactive conformation

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1. Introduction

The selectins play a key role in the early inflammatory cascade. The recruitment and extravasation of leukocytes into inflamed tissue mediated by E-, P- and L-selectin, a class of closely related cell adhesion molecules, is an essential mechanism of the immune defense. The selectin-leukocyte interaction leads to the characteristic tethering and rolling process of the white blood cells on the vascular endothelium. Upon secondary activation firm adhesion and migration to the site of injury through the endothelial cells takes place.[1,2] However, excessive influx of leukocytes into tissue can cause acute or chronic reactions as observed in reperfusion injury, stroke or rheumatoid arthritis.[3,4] As a result, the antagonism of the selectins is generally considered to be a validated approach towards the treatment of inflammatory diseases.

Since all physiological ligands of the selectins bear the terminal tetrasaccharide epitope sialyl Lewis^x (**1**, sLe^x, **D**-Neu*N*Ac-α-2,3 → **D**-Gal-β-1,4 → (**L**-Fuc-α-1,3 →) **D**-Glc*N*Ac, *figure 1*),[5,6] this motif served as a lead structure in selectin antagonist research.[7] Numerous publications determined the essential pharmacophores for binding to E-selectin, which are four hydroxyl groups (fucose 3- and 4-OH, galactose 4- and 6-OH) and the carboxylic acid of neuraminic acid.[8,9]

The bioactive conformation of sLe^x was determined by trNOE NMR experiments[10,11] and was subsequently confirmed in complex with all three selectins by X-ray crystallography.[12] Since carbohydrate-protein interactions usually are of low affinity (sLe^x has an IC_{50} of ca. 1 mM, all IC_{50}s discussed here are relative to sLe^x, *i.e.* rIC_{50} of sLe^x = 1.0) the stabilization of the bioactive conformation of sLe^x and its mimetics was found essential for binding.[13-16] It was rationalized that a conformational change of a ligand which does not adopt the bioactive conformation in solution, would lead to high entropic costs upon binding. This ultimately results in a loss of detectable binding. For dynamic non-equilibrium processes, such as E-selectin mediated rolling of leukocytes on activated endothelium, entropy is expected to play a major role.

Therefore, special focus was given to the conformational stabilization of the Lewis^x core in sLe^x mimetics with modifications at the Glc*N*Ac residue. Glc*N*Ac was successfully replaced by 1,2-*trans*-diols.[17-20] But only when conformationally locked diols, *e.g.* (*1R*,*2R*)-cyclohexane-diol or (*3R*,*4R*)-tetrahydropyrane-diol, were used, the binding affinity was superior to sLe^x. When flexible diols, *e.g.* glycol or butane-2,3-diol were introduced as Glc*N*Ac replacements, binding was significantly reduced or vanished completely.

In 2001, we reported on the additional conformation-stabilizing effect of substituents at the Glc*N*Ac analogs, adjacent to the glycosidic linkage with fucose.[20] A sLe^x antagonist with Glc*N*Ac replaced by (*3R*,*4R*)-tetrahydropyrane-diol showed better affinity towards E-selectin when an equatorial methyl substituent was introduced at the position analogous to C-2 of Glc*N*Ac. Its IC_{50} dropped by a factor of 6 when compared to the unsubstituted analog in the static assay. This effect was even more pronounced in a dynamic assay (IC_{50} (dynamic) = 20-30 µM *vs.* IC_{50} (static) = 39 µM). Because the additional methyl group is not believed to have a direct contact with the protein surface (as derived from the X-ray structure of sLe^x in complex with E-selectin[12]), the increased affinity was assigned to lower entropic costs upon binding. This is solely resulting from a higher degree of pre-organization of the molecule in the bioactive conformation in solution.

In this communication we report on a lipophilic interaction between the fucose and the galactose residue in sLe^x as a second core conformation-stabilizing effect in sLe^x.

Because of the unfavorable pharmacokinetic properties of fucose, *i.e.* its lability towards enzymatic cleavage in the host, many reports addressed this issue with fucose replacements in sLe^x antagonists.[7,9] The hydroxyl groups at position 3 and 4 are essential and, therefore, analogs were based on modifications at positions 2 and 5. Of special interest here are the replacements of fucose with **D**-arabinopyranose and **L**-galactose (**2** and **3**, *figure 1*), which were introduced either by chemical synthesis or enzymatically using fucosyl-transferases.[21-24] Both **L**-galacto- and **D**-arabinopyranosides are considered stable towards hydrolysis in mammals.[22] In **L**-galactoside analogs

of sLe^x, the 6-position of fucose is hydroxylated and in **D**-arabinopyranoside analogs, the 6-position is absent, leaving two hydrogen atoms at the C-5. The **L**-galactoside derivative **3b** bearing a decapeptide as aglycon was reported to bind to E-selectin with an rIC_{50} of 1.25 when compared to the sLe^x derivative (**1b**, rIC_{50} = 1).[22] The arabinoside **2b** also showed binding to the protein with an rIC_{50} of 2.8. However, both analogs showed lower affinities towards E-selectin than the corresponding sLe^x **1b**.

Lemieux[25] reported on the hydrophobic properties of the α-face of **L**-fucosides. Therefore and concluded from the results of the Kunz group,[22] we hypothesized that the methyl group present in sLe^x stabilizes the internal conformation of the pharmacophores of the core in the bioactive conformation by a lipophilic interaction with the galactose β-face. Since this methyl group is not in direct contact with the protein[12,26] when bound, it does not contribute to binding enthalpy, but rather has a strong influence on the entropy of binding when compared to the **D**-arabino and to a lesser extent to the **L**-galacto counterparts **2** and **3**, respectively. In **3**, the hydroxymethyl substituent of **L**-galactose could still interact *via* its lipophilic CH_2 with the β-face of the **D**-galactose residue, whereas in **2**, this stabilizing force should be absent. As a result, the affinity of **2** to the receptor would be significantly reduced. This tendency in biological activity has indeed been reported by the Kunz group (**1b**, **2b** and **3b**, *figure 1*).[22]

Lipophilic interactions of carbohydrate α- or β- faces with binding partners have been reported in the literature and are of current interest.[27-30] Aromatic amino acid side chains are prevalent in the binding sites of carbohydrate recognizing proteins. In galactose-binding proteins, very often **D**-galactose is bound to the indole side chain of tryptophan, or to the phenyl ring of Tyr or Phe.[31] Recently, Screen[32] and co-workers have reported on the interaction between galactosides and toluene, based on the crystal structure[33,34] of a galactose-specific lectin in complex with methyl galactoside. In this model, the **D**-galactose β-face directly interacts with the plane of toluene, as observed in the crystal structure, where the same face is bound to a tyrosine side chain. Very recently, Terraneo *et al.* reported on intramolecular conformation-

stabilizing effects of arene rings with carbohydrate faces in glycoconjugates of phenyllactic acid, which lead to a significant influence on the overall molecular shape.[35]

1a, R = -O$(CH_2)_8CO_2Me$
1b, R = -NH-Asn-linked decapeptide
1c, R = -O$(CH_2)_2SiMe_3$

2a, R = -O$(CH_2)_8CO_2Me$
2b, R = -NH-Asn-linked decapeptide
2c, R = -O$(CH_2)_2SiMe_3$

3a, R = -O$(CH_2)_8CO_2Me$
3b, R = -NH-Asn-linked decapeptide

4, R = O$(CH_2)_2SiMe_3$

Figure 1: Sialyl Lewis^x (**1**) and derivatives with modified fucose: **D**-arabinoside **2**, **L**-galactoside **3** and 5*C*-phenyl arabinoside **4**.

To verify the hypothesis of a stabilization of the core as a result of a lipophilic interaction between fucose and galactose in sLe^x, we planned to modify the methyl group of fucose by introducing other lipophilic groups and compare the biological activity of these analogs to sLe^x (**1c**, *figure 1*) and an analog devoid of a lipophilic substituent (**2c**). In the early 1990s, Gesson and co-workers reported on the stereoselective synthesis of **L**-fucose and 5*C*-phenyl-**L**-arabinose from **D**-mannose using methyl or phenyl lithium respectively.[36] Attempts to obtain higher alkyl analogs *via* this organo-lithium route failed. In fact, those higher analogs could be obtained with Grignard reagents, but with inversed stereoselectivity resulting in **D**-altrose derivatives as major product.

For the synthesis of the fucose derivative **4** bearing a phenyl ring instead of a methyl group at the non-reducing end, we first synthesized building block **13**, following the strategy developed by Gesson (*scheme 1*).[36] As previously demonstrated by Mekki,[37] the addition of organolithium reagents (*e.g.* PhLi,

MeLi) to D-mannofuranose diacetonide **5** was selective according to the Felkin-Anh model. Allyl ether protection of the resulting hydroxyl functions in **6**, followed by hydrolysis of the two isopropylidene groups in **7** allowed the selective cleavage of the terminal diol **8** upon treatment with sodium periodate. Then, the formed aldose derivative underwent spontaneous cyclization to give the corresponding arabino furanose **9** as a mixture of anomers. Removal of the allyl protecting groups gave a mixture of furanose and pyranose forms from which the thermodynamically more stable six-membered ring isomer **10** was isolated after acetylation. Treatment of the peracetate **11** with ethanethiol in the presence of TMSOTf afforded the corresponding ethyl thioglycoside **12**, which was converted into building block **13** by conventional deacetylation and benzylation.

Scheme 1: (i) PhLi, Et_2O, -78 to -20 °C, 20 h, 76%, d.r. 4:1; (ii) AllBr, NaH, DMF, 0 °C-r.t., 1 h, 93%; (iii) $HOAc/H_2O$ (4:1), r.t., 10 h, quant.; (iv) $NaIO_4$, CH_2Cl_2, aq. $NaHCO_3$, 0 °C-r.t., 1 d, 77%; (v) Pd/C, CSA, dioxane/H_2O, 95 °C, 2 d; (vi) Ac_2O, DMAP, pyridine, r.t., 45 min, 69% (over 2 steps, α/β 1:2); (vii) EtSH, CH_2Cl_2, TMSOTf, 0 °C-r.t., 3 h, 65% (α/β 1:2); (viii) NaOMe, MeOH, r.t., 3 h; (ix) NaH, BnBr, DMF, 0 °C-r.t., 1 h, 88% (over 2 steps);

Initially, the synthesis of **4** was intended as an economically more favorable [2+2]-glycosylation approach (*scheme 2*). Therefore, Glc*N*Ac derivative **14**

was glycosylated with donor **13** using NIS/TfOH as promoter to give pseudo-disaccharide **15**. However, the subsequent [2+2]-glycosylation with disaccharide donor **17** did not give any detectable amounts of tetrasaccharide **18**, neither with NIS/TfOH nor with DMTST as promoter.

Scheme 2: (i) NIS, AgOTf, mol. Sieves, CH_2Cl_2, -78 °C to r.t., 5.5 h, 66% (α/β 5:1); (ii) BH_3•NMe_3, $AlCl_3$, H_2O, THF, r.t., 3.5 h, 62%; (iii) DMTST or DMTST/base or NIS/AgOTf, 0%;

Consequently, we proceeded with a [2+1+1]-strategy (*scheme 3*). Glc*N*Ac derivative **19** was readily obtained by chloroacetylation of its 3-OH precursor.[38] Selective reductive opening[39] of the benzylidene in presence water yielded acceptor **20**, which was glycosylated with donor **17**, to give trisaccharide **21** in good yield. The chloroacetyl group was then selectively removed using thiourea and the resulting trisaccharide acceptor **22** was subjected to glycosylation with phenyl-arabinoside donor **13**. The protected tetrasaccharide **18** was obtained in reasonable yield under Lemieux *in situ* anomerization conditions. Ester cleavage and careful hydrogenolytic deprotection gave **4** in good yield without affecting the hidden benzylic position of the ring oxygen in the phenylarabinose moiety.

For the synthesis of **1c** and **2c**, trisaccharide **23**[38] was treated with fucosyltransferase and GDP-Fuc or GDP-Ara to give selectively the desired 3-*O* fucosylated or arabinosylated compounds as reported earlier (*scheme 4*).[40,41]

The obtained derivatives of sLe^x were then tested in a static ELISA based assay for E-selectin binding,[42] and their affinity was measured relative to sLe^x (**1c**, rIC_{50} = 1). Both analogs showed a weaker affinity towards the receptor than sLe^x. Arabinoside **2c** lacking any lipophilic substituent was inactive up to 10 mM, whereas binding could be restored when a lipophilic phenyl group was introduced in **4** (rIC_{50} of 2.8).

19 → (i) → **20**

(ii) with **17** → **21**, R=ClAc; (iii) → **22**, R=H

(iv) with **13** → **18**, R_1=Ac, R_2=Bz, R_3=Bn, R_4=Bn; (v)-(vi) → **4**, R_1,R_2,R_3=H, R_4=Na

Scheme 3: (i) $BH_3 \bullet NMe_3$, $AlCl_3$, H_2O, THF, r.t., 2 h, 92%; (ii) DMTST, mol. sieves, CH_2Cl_2, 0 °C, 2.5 d, 68%; (iii) $H_2NC(S)NH_2$, 2,6-di-*tert.*-butyl-pyridine, DMF, 70 °C, 22 h, 63%; (iv) 1. **13**, Br_2, CH_2Cl_2, 0 °C, 30 min 2. **22**, Et_4NBr, mol. sieves, DMF/CH_2Cl_2, r.t., 3 d, 37%; (v) H_2 (1 atm), Pd/C, dioxane/H_2O, r.t., 14 h; (vi) NaOMe, MeOH, r.t., 12 h, 59% (2 steps);

To assess the conformational behavior of the core structure of sLe^x and its analogs we performed NMR measurements (*figure 2*). The preferred conformation of **1c**, **2c** and **4** in aqueous solution was studied by jump-symmetrized ROESY[43] NMR experiments. The average distance between

H5^{ax} in the fucose residue of **1c** and in its analogs (**2c**, **4**) and H2 of galactose was determined by the intensity of their cross-peak from the nuclear Overhauser effect. In sLe^{x} (**1c**) and arabinoside **2c** the average distance between those two nuclei is very similar (**1c**: 2.54 Å, **2c**: 2.49 Å), whereas in phenyl-arabinoside **4** (2.75 Å) this average distance is 10% longer.

23

(i)

1c, R = CH_3
2c, R = H

Scheme 4: (i) FucT-III, for **1c**: GDP-Fuc (89%), for **2c**: GDP-Ara (81%);

To show that the reduced affinity of **2c** results from entropic costs of binding, the flexibility of the different fucose analogs will be studied by temperature dependent NMR. A correlation between chemical shifts and coupling constants in their fucose and arabinose residues with temperature should further support the hypothesis of a lipophilic stabilization in **1**, and its absence in **2**.

Considering the information gained from NMR experiments together with the biological activity of compounds **1c**, **2c** and **4**, we could show that there is a lipophilic core-stabilizing interaction between the fucose α-face with the galactose β-face in the sLe^{x} motif. Although the average distances of the core are similar in sLe^{x} (**1c**) and arabinoside analog **2c**, their bioactivity differs dramatically. Because the methyl group is not in contact with the protein and does therefore not contribute to the binding enthalpy, this strong decrease in activity is a direct result of the high degree of flexibility, and hence the unfavorable entropy of binding of the non-stabilized arabinose residue in **2c** when compared with **1c**.[44]

In case of phenyl-arabinoside **4**, a lipophilic group was introduced and biological activity was regained. Because of the additional phenyl group,

conformational stabilization *via* lipophilic interactions between the two saccharide residues was reinstalled although with slight distortion of the optimal geometry of sLe^x. This is probably the reason for the decrease in affinity of **4** when compared to sLe^x (**1c**).

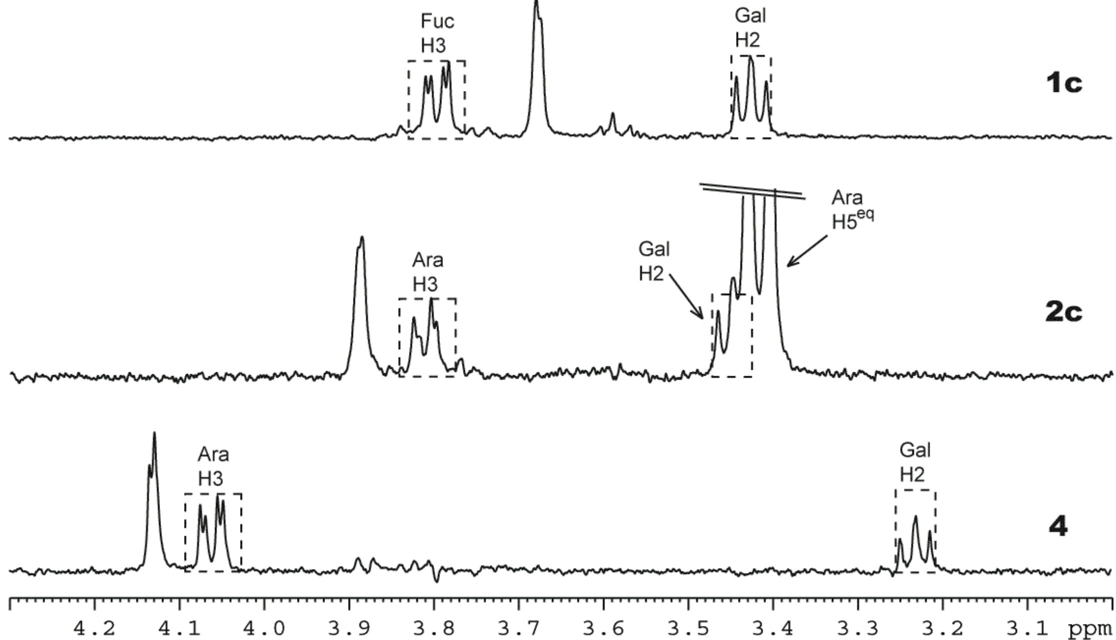

Figure 2: Selective ROESY experiments measuring the transfer of magnetization from the $H5^F$ to $H2^G$ in selectin ligands **1c**, **2c** and **4** at equal mixing times.

Nature has chosen sLe^x as epitope for binding to the selectins. Specificity for its receptor is gained through the large number of hydrogen bonds involved in the complex of sLe^x with the selectins.[12,45] Because carbohydrates have an intrinsically low affinity towards their protein targets, they are usually displayed in an oligovalent fashion by the interacting partner. When leukocytes bind to the selectins under shear stress conditions, the pre-organization of the pharmacophores in the bioactive conformation is of particular importance for this binding process. In this report, we could show that in addition to previously reported factors, this pre-organization has been well achieved in the natural epitope sLe^x *via* a lipophilic interresidue stabilizing interaction between fucose and galactose. The understanding of the effects contributing to the conformational pre-organization of the pharmacophores in

the bioactive arrangement is essential for the development of functional selectin ligands into drugs.

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Experimental

Conformational analysis by NMR

The samples for the ROESY analysis consisted of approx. 5 mg of either **1c, 2c** or **4**, solvated in 99.8% D_2O (Armar Chemicals) at a pH of approx. 7.0 (uncorrected for D_2O) and were measured without the addition of a buffer. Shigemi NMR tubes were used to reduce the sample volume (200 µL) needed for measurement. Measurements were performed at 25 °C using the same Bruker AVANCE 500 NMR spectrometer that was used to validate the compounds synthesized. Chemical shifts were referenced with respect to earlier work,[20] which assigned a chemical shift of 4.60 ppm to the $H5^F$ resonance of **1c**.

The doubly-selective homonuclear Hartmann-Hahn scheme[46] was used to selectively transfer magnetization from $H6^F$ to $H5^F$. This scheme allowed a highly selective transfer of magnetization from $H6^F$ to $H5^F$ through their scalar coupling. The selective excitation of $H5^F$ allowed an accurate quantification of this resonance by avoiding the excitation of residual H_2O, which has similar chemical shift. To remove any remaining magnetization from $H6^F$, a selective gradient echo at the frequency of $H5^F$ was applied. A 200 ms REBURP[47] 180° refocusing pulse was applied to the $H5^F$ resonance. The REBURP pulse was sandwiched by a pair of Gaussian shaped gradients of 1 ms each and an amplitude of 20 G/cm. This additional spectral filter ensured that the observed

ROESY[48] peaks were due to magnetization that originated from the H5^F resonance.[49]

The jump-symmetrized CW-ROESY variation of the ROESY sequence was used in all experiments to minimize TOCSY artefacts.[43] This sub-element of the pulse sequence was inserted following the selective gradient echo. During the ROESY period, the transmitter frequency was shifted up or downfield during the first or second half of the mixing-time, respectively. The high-field spin lock was applied at 4.9 ppm and the low-field at 0.9 ppm. The spin lock was a rectangular pulse of 2 kHz amplitude. For the compounds measured (**1c**, **2c** and **4**) 10 experiments were run to record a build-up curve of the ROE transfer. The 10 experiments were sampled with increasing durations of the spin lock, beginning after 50 ms, and repeated after each 50 ms increment, resulting in a 500 ms spin lock duration for the final experiment.

Following the application of the spin lock, the transmitter was returned to the center of the spectrum, at 2.9 ppm, and the FID measured using 4096 complex points to sample a bandwidth of 7 ppm. To achieve a high signal-to-noise ratio, 1024 scans were measured for each mixing time. Using a prescan delay of 3 s, on average the experiments lasted approximately 1.2 hours each. The NMR data were analyzed using XWINNMR version 3.0 operating on a Silicon Graphics O2. The spectra were apodized with an exponential decay function with 2 Hz line broadening. An additional advantage of the selective experiments was the lack of signal overlap which allowed the possibility of integrating the signals without interference from other resonances.

To determine the internuclear distances, the rotating-frame cross-relaxation rates were calculated from the build up curves. Traditionally, the cross-relaxation rate is determined from fitting the spectra to a bi-exponential function that depends upon both the cross- and auto-relaxation rates.[50-52] The extent to which accurate cross-relaxation rates can be determined by this manner depends upon how well the auto-relaxation rate can be defined. Alternatively, it is possible to remove the dependence on the auto-relaxation by dividing the target-peak by the source-peak for each value of the mixing

time.[53-55] The resulting function is a hyperbolic tangent, the argument of which is the product of the cross-relaxation rate and the mixing time. For the longest mixing times performed and highest rate of cross-relaxation expected for the compounds studied herein, the hyperbolic tangent function is indistinguishable from a linear function, hence offering the potential to apply linear regression to extract the cross-relaxation rate. The above procedure resulted in values that were well described by linear functions. Removal of auto-relaxation through the conversion of bi-exponential into hyperbolic tangent functions has as well been recently applied to determine accurate relaxation rates in cross-correlation measurements.[56-58]

General Methods

Nuclear magnetic resonance spectroscopy was performed on a Bruker Avance 500 UltraShield spectrometer at 500.13 MHz (^{1}H) or 125.76 MHz (^{13}C). Chemical shifts are given in ppm and were calibrated on residual solvent peaks[59] or to tetramethyl silane as internal standard. Multiplicities were specified as s (singlet), m (multiplet) or interpreted according to 1st order[60] and higher order where possible.

The signals were assigned with the help of DEPT-135, ^{1}H,^{1}H-COSY/TOCSY and ^{1}H,^{13}C-HSQC/HMBC experiments. Assignments are indicated according to IUPAC nomenclature. For complex molecules, the following prefixes for substructures are used: Ara (arabinose), Gal (galactose), Glc*N*Ac (*N*-acetyl glucosamine), PhAra (5C-phenylarabinose) and Sia (sialic acid, *N*-acetylneuraminic acid). C^{i} indicates the *ipso* substituted carbons of aromatic systems.

Optical rotations were measured on a Perkin Elmer 341 polarimeter in the indicated solvents in *p.a.* quality. ESI mass spectra were recorded on a Waters micromass ZQ instrument. High resolution mass spectra were obtained on a ESI Bruker Daltonics micrOTOF spectrometer equipped with a TOF hexapole detector. Melting points were determined with a capillary apparatus and are uncorrected.

TLC was performed using silica gel 60 coated glass plates containing fluorescence indicator (Merck KGaA, Darmstadt, Germany) using UV light (254 nm) and by charring either in aqueous $KMnO_4$ solution or in a molybdate solution (a 0.02 M solution of ammonium cerium sulfate dihydrate and ammonium molybdate tetrahydrate in aqueous 10% H_2SO_4) with heating to 140 °C for 5 min. Column chromatography was performed using silica gel 60 (0.040-0.063 mm) from Fluka. Solvents were purchased from Fluka and dried prior to use. CH_2Cl_2 was dried by filtration through basic aluminum oxide (Fluka) and Et_2O by distillation from sodium/benzophenone. DMF was dried over activated molecular sieves and MeOH by distillation from sodium methoxide.

Biological data were obtained using the published ELISA procedure with CGP69669A[61] as reference compound.[42]

(1*S*)-2,3-5,6-Di-O-acetonide-(1*S*)-1*C*-phenyl-D-mannitol (6). To a stirred, cooled (-78 °C) suspension of **5** (6.73 g, 25.9 mmol) in anhydrous Et_2O (25 mL) was added dropwise phenyllithium (68 mL, 129.4 mmol, ca. 1.9 M solution in Et_2O), the mixture was warmed to -41 °C whereupon a clear brown solution was obtained. The mixture was stirred over night and then quenched with saturated aqueous $NaHCO_3$ (10 mL) at -20 °C to give a yellow solution. After dilution with EtOAc (500 mL) the mixture was washed with half-saturated brine (2 x 150 mL). The combined aqueous layers were extracted with EtOAc (2 x 500 mL). The combined organic layers were dried (Na_2SO_4) and concentrated. Purification of the residue by column chromatography on silica (PhMe/EtOAc, 4/1) yielded the separate (1*R*) diastereomer and the title compound **6** (sum of diastereomers: 6.50 g, 76%, d.r. 1/4).

^{1}H-NMR (500.1 MHz, $CDCl_3$): δ 7.47-7.43 (m, 2H, Ar-H), 7.40-7.36 (m, 2H, Ar-H), 7.34-7.30 (m, 1H, *p*-Ar-H), 4.97 (d, ^3J = 3.8 Hz, 1H, H-1), 4.52 (dd, ^3J = 3.9, ^3J = 7.5 Hz, 1H, H-2), 4.39 (d, ^3J = 7.5 Hz, 1H, H-3), 4.11-4.01 (m, 3H, H-5, H-6a/b), 3.60 (d, ^3J = 7.2 Hz, 1H, H-4), 1.60, 1.41, 1.33, 1.27 (4s, 12H, CH_3);

^{13}C-NMR (125.8 MHz, $CDCl_3$): δ 140.7 (Ar-C^i), 128.9, 128.5, 127.3 (5C, Ar-

CH), 109.5, 108.8 (2C, ketal-C), 80.3 (C-3), 76.3 (C-5), 76.0 (C-2), 72.2 (C-1), 70.4 (C-4), 67.3 (C-6), 26.9, 26.7, 25.5, 24.9 (4C, CH_3);

elemental analysis calcd. for $C_{18}H_{26}O_6$: C, 63.89; H, 7.74;
found: C, 63.85; H, 7.62;

ESI-MS calcd. for $C_{18}H_{26}O_6Na$ $[M+Na]^+$: 361.2; found: 361.0;

$[\alpha]^{20}_D$ = -19.5 (*c* 1.0, $CHCl_3$);

(1*S*)-2,3-5,6-Di-*O*-acetonide-1,4-di-*O*-allyl-(1*S*)-1*C*-phenyl-D-mannitol (7). A stirred, cooled (0 °C) solution of **6** (3.28 g, 9.68 mmol) in DMF (27 mL) was treated with 5 portions of NaH (in total 2.00 g, 76 mmol, of a 50% dispersion in oil) and, after 5 min, allyl bromide (5.0 mL, 58.1 mmol) was added. Stirring was continued for 1 h at r.t., then the mixture was quenched with aqueous saturated $NaHCO_3$ at 0 °C. The residue was suspended in brine (500 mL) and extracted with CH_2Cl_2 (3 x 300 mL). The combined organic layers were dried (Na_2SO_4) and concentrated. Purification by column chromatography on silica (petrol ether/EtOAc, gradient of 0-10%) yielded **7** (3.77 g, 93%) as a colorless oil.

^{1}H-NMR (500.1 MHz, $CDCl_3$): δ 7.39-7.30 (m, 5H, Ar-H), 6.01-5.85 (m, 2H, All-H), 5.44-5.39 (m, 1H, All-H), 5.29-5.21 (m, 1H, All-H), 5.20-5.12 (m, 2H, All-H), 4.76 (d, ^3J = 6.3 Hz, 1H, H-1), 4.51 (t, ^3J = 6.2 Hz, 1H, H-2), 4.38-4.23 (m, 2H, All-CH_2), 4.08 (dd, ^3J = 6.3, ^3J =7.9 Hz, 1H, H-4), 4.02-3.92 (m, 4H, H-3, H-6a/b, 1H of All-CH_2), 3.82-3.77 (m, 1H, 1H of All-CH_2), 3.54 (t, ^3J = 4.6 Hz, 1H, H-5), 1.60, 1.39, 1.38, 1.29 (4s, 12H, CH_3);

^{13}C-NMR (125.8 MHz, $CDCl_3$): δ 138.5 (Ar-C^i), 135.3, 135.1 (2C, All-C), 128.8 (2C, Ar-CH), 128.6 (*p*-Ar-CH), 128.4 (2C, Ar-CH), 117.1, 116.4 (2C, All-C), 109.5, 108.6 (2C, ketal-C), 80.5 (C-2), 79.0 (C-1), 78.9 (C-3), 78.0 (C-5), 77.9 (C-4), 72.8, 69.1 (2C, All-CH_2), 66.8 (C-6), 26.7, 26.4, 26.2, 25.1 (4C, CH_3);

HR-MS calcd. for $C_{24}H_{34}O_6Na$ $[M+Na]^+$: 441.2253; found: 441.2284;

$[\alpha]^{20}_D$ = +33.9 (*c* 1.0, $CHCl_3$);

(1*S*)-1,4-Di-*O*-allyl-(1*S*)-1*C*-phenyl-D-mannitol (8). A solution of **7** (3.63 g, 8.66 mmol) in AcOH/H_2O (200 mL, 4:1) was stirred at r.t. for 10 h. Concentration of the solution gave spectroscopically pure **8** (2.93 g, quant.). A small sample was purified on silica gel with Et_2O and then EtOAc to give **8** as a solid.

m.p. 63-64 °C (Et_2O/cyclohexane);

^{1}H-NMR (500.1 MHz, $CDCl_3$): δ 7.39-7.26 (m, 5H, Ar-H), 5.94-5.79 (m, 2H, All-H), 5.28-5.10 (m, 4H, All-H), 4.76 (d, ^3J = 1.7 Hz, 1H, H-1), 4.11-4.09 (m, 2H, All-CH_2), 4.05-4.00 (m, 1H, All-CH_2), 3.94-3.84 (m, 3H, H-3, H-4, 1H of All-CH_2), 3.81-3.74 (m, 2H, H-5, H-6a), 3.70-3.65 (m, 1H, H-6b), 3.59 (dd, ^3J = 2.0, ^3J = 8.9 Hz, 1H, H-2), 2.87 (br s, 4H, OH);

^{13}C-NMR (125.8 MHz, $CDCl_3$): δ 139.5 (Ar-C^i), 134.6 (2C, All-C), 128.7, 128.1, 127.4 (5C, Ar-CH), 117.8, 117.7 (2C, All-C), 79.2 (C-1), 77.5 (C-5), 75.7 (C-2), 73.4 (All-CH_2), 72.1 (C-4), 70.8 (C-3), 70.4 (All-CH_2), 63.9 (C-6);

elemental analysis calcd. for $C_{18}H_{26}O_6$: C 63.89, H 7.74;
found: C 63.65, H 7.80;

ESI-MS calcd. for $C_{18}H_{26}O_6Na$ $[M+Na]^+$: 361.2; found: 361.0;

$[\alpha]^{20}_D$ = +68.0 (*c* 1.0, $CHCl_3$);

2,5-Di-*O*-allyl-(5*S*)-5*C*-phenyl-α,β-D-arabinofuranose (9). To a vigorously stirred mixture of **8** (2.03 g, 8.66 mmol) and saturated aqueous $NaHCO_3$ (6 mL) in CH_2Cl_2 (80 mL) was added portionwise $NaIO_4$ (2.41 g, 11.3 mmol) at 0 °C. The suspension was stirred at r.t. for 22 h, then diluted with H_2O (50 mL). The phases were seperated and the aqueous layer was extracted with CH_2Cl_2 (2 x 100 mL). The combined organic layers were dried (Na_2SO_4) and concentrated. The residue was eluted from a column of silica gel (CH_2Cl_2/MeOH, gradient of 2-12%) to give **9** (2.05 g, 77%) as a mixture of anomers and recovered starting material (320 mg, 11%). A pure sample of **α-9** was obtained by crystallization; anomeric configurations were assigned based on the chemical shift of the anomeric carbon.[62]

m.p. 97-99 °C (EtOAc/cyclohexane);

$[\alpha]^{20}_{D}$ = +41.4 (*c* 0.9, $CHCl_3$);

^{1}H-NMR (500.1 MHz, $CDCl_3$): δ 7.38-7.29 (m, 5H, Ar-H), 5.95-5.81 (m, 2H, All-CH), 5.36-5.17 (m, 4H, All-CH_2), 5.31 (dd, ^3J = 4.4, ^3J = 8.3 Hz, 1H, H-1), 4.44 (d, ^3J = 5.4 Hz, 1H, H-5), 4.21 (dd, ^3J = 5.4, ^3J = 6.5 Hz, 1H, H-3) , 4.17-4.04 (m, 2H, All-CH_2), 3.92-3.77 (m, 4H, H-2, H-4, 2H of All-CH_2);

^{13}C-NMR (125.8 MHz, $CDCl_3$): δ 137.6 (Ar-C^i), 134.3, 134.2 (2C, All-CH), 128.9, 128.7, 128.0 (5C, Ar-CH), 118.3, 118.2 (2C, All-CH_2), 95.5 (C-1), 85.0 (C-4), 84.6 (C-2), 81.7 (C-5), 75.2 (C-3), 71.7, 70.0 (2C, All-CH_2);

elemental analysis calcd. for $C_{17}H_{22}O_5$: C 66.65; H 7.24;
found: C 66.81, H 7.28;

ESI-MS calcd. for $C_{17}H_{22}O_5Na$ $[M+Na]^+$: 329.1; found: 328.9;

NMR data for **β-9** (selected):
^{1}H-NMR (500.1 MHz, $CDCl_3$): δ 7.38-7.29 (m, 5H, Ar-H), 5.95-5.81 (m, 2H, All-CH), 5.43 (br s, 1H, H-1), 4.40 (d, ^3J = 8.3 Hz, 1H, H-5), 4.33 (dd, ^3J = 4.0, ^3J = 8.1 Hz, 1H, H-4), 4.06-4.02 (m, 2H, All-CH_2), 3.83-3.77 (m, 2H, H-2, H-3);

^{13}C-NMR (125.8 MHz, $CDCl_3$): δ 137.9 (Ar-C^i), 134.7, 134.2 (2C, All-CH), 129.0, 128.8, 128.2 (5C, Ar-CH), 117.6, 117.5 (2C, All-CH_2), 101.2 (C-1), 88.1 (2C, C-2, C-4), 82.4 (C-5), 76.5 (C-3), 70.9, 69.7 (2C, All-CH_2);

1,2,3,4-Tetra-*O*-acetyl-(5*S*)-5*C*-phenyl-α,β-D-arabinopyranose (11). A mixture of **9** (1.67 g, 5.45 mmol), palladium on activated carbon (1.5 g, Degussa catalyst E101 NE/W 10%) and camphorsulfonic acid (0.24 g, 1.05 mmol) in dioxane/H_2O (1:1, 38 mL) was degassed and saturated with nitrogen. The suspension was stirred at 95 °C for 45 h in a screw-capped vial, then cooled to r.t., diluted with Et_3N (0.5 mL) and filtered through a pad of celite. The solution was concentrated to give crude phenylarabinose **10**. A solution of the crude product and DMAP (40 mg) in pyridine (10 mL) and acetic anhydride (10 mL) was stirred at r.t. for 45 min. Concentration and

purification by column chromatography on silica (petrol ether/EtOAc, 5:2) gave **11** (1.49 g, 69%) as a mixture of anomers (α:β = 1:1.8).

NMR data for **α-11**:
^{1}H-NMR (500.1 MHz, $CDCl_3$): δ 7.38-7.26 (m, 5H, Ar-H), 6.57 (d, ^{3}J = 3.5 Hz, 1H, H-1), 5.59 (br s, 1H, H-4), 5.57-5.52 (m, 1H, H-3), 5.48-5.43 (m, 1H, H-2), 5.24 (br s, 1H, H-5), 2.17, 2.06, 2.01, 1.91 (4 s, 12H, 4 $COCH_3$);

^{13}C-NMR (125.8 MHz, $CDCl_3$): δ 170.4, 170.2, 170.0, 169.2 (4C, $CH_3\mathit{C}O$), 135.6 (Ar-C^{i}), 128.5, 128.4, 126.4 (5C, Ar-CH), 90.1 (C-1), 73.0 (C-5), 70.9 (C-4), 68.0 (C-3), 66.7 (C-2), 21.1, 20.9, 20.8, 20.6 (4C, $\mathit{C}H_3CO$);

NMR data for **β-11**:
^{1}H-NMR (500.1 MHz, $CDCl_3$): δ 7.38-7.26 (m, 5H, Ar-H), 5.86 (d, ^{3}J = 8.2 Hz, 1H, H-1), 5.57-5.52 (m, 1H, H-4), 5.48-5.43 (m, 1H, H-2), 5.28 (dd, ^{3}J = 3.2,^{3}J = 10.5 Hz, 1H, H-3), 4.92 (br s, 1H, H-5), 2.15, 2.08, 2.00, 1.93 (4 s, 12H, 4 CH_3CO);

^{13}C-NMR (125.8 MHz, $CDCl_3$): δ 170.3, 170.0, 169.7, 169.3 (4C, $CH_3\mathit{C}O$), 135.2 (Ar-C^{i}), 128.5, 128.4, 126.3 (5C, Ar-CH), 92.6 (C-1), 75.8 (C-5), 71.5 (C-3), 70.2 (C-4), 68.1 (C-2), 21.1, 20.9, 20.8, 20.6 (4C, $\mathit{C}H_3CO$);

ESI-MS calcd. for $C_{19}H_{22}O_9Na$ $[M+Na]^+$: 417.1; found: 416.9.

elemental analysis calcd. for $C_{19}H_{22}O_9$ (394.4) C 57.86, H 5.62;
found: C 58.05, H 5.73.

Ethyl 2,3,4-tri-*O*-acetyl-(5*S*)-5*C*-phenyl-1-thio-α,β-D-arabinopyranoside (12). To a stirred solution of **11** (1.58 g, 4.00 mmol) and ethanethiol (0.89 mL, 12.0 mmol) in anhydrous CH_2Cl_2 (20 mL) was added dropwise trimethylsilyl triflate (0.73 mL, 4.0 mmol) at 0 °C. The solution was stirred at r.t. for 3 h and then diluted with Et_3N (1 mL). The reaction was concentrated and the residue purified by column chromatography on silica (cyclohexane/EtOAc, 5/1) to give **α-12** (349 mg, 22%) as a syrup and **β-12** (682 mg, 43%) as a solid.

data for **α-12**:

^{1}H-NMR (500.1 MHz, $CDCl_3$): δ 7.35-7.29 (m, 5H, Ar-H), 5.92 (d, ^3J = 4.8 Hz, 1H, H-1), 5.58-5.56 (m, 1H, H-4), 5.48 (br s, 1H, H-5), 5.42-5.39 (m, 2H, H-2, H-3), 2.61-2.47 (m, 2H, SCH_2CH_3), 2.11, 2.00, 1.91 (3 s, 9H, 3 CH_3CO), 1.22 (t, ^3J = 7.4 Hz, 3H, SCH_2CH_3);

^{13}C-NMR (125.8 MHz, $CDCl_3$): δ 170.6, 170.2, 170.1 (3C, CH_3CO), 136.4 (Ar-C^i), 128.4, 128.2, 126.6 (5C, Ar-CH), 82.4 (C-1), 71.2 (C-4), 70.5 (C-5), 68.9 (C-2), 68.3 (C-3), 24.4 (SCH_2CH_3), 21.1, 20.9, 20.6 (3C, CH_3CO), 14.9 (SCH_2CH_3);

elemental analysis calcd. for $C_{19}H_{24}O_7S$ (396.5): C 57.56, H 6.10;
found: C 57.83, H 6.21;

$[\alpha]^{20}_D$ = -192.8 (*c* 1.0, $CHCl_3$);

data for **β-12**:
m.p. 124-125 °C (cyclohexane);

^{1}H-NMR (500.1 MHz, $CDCl_3$): δ 7.36-7.28 (m, 5H, Ar-H), 5.55 (dd, ^3J = 1.1, ^3J = 3.4 Hz, 1H, H-4), 5.37 (t, ^3J = 9.9 Hz, 1H, H-2), 5.25 (dd, ^3J = 3.3, ^3J = 10.2 Hz, 1H, H-3), 4.80 (br s, 1H, H-5), 4.65 (d, ^3J = 10.0 Hz, 1H, H-1), 2.88-2.71 (m, 2H, SCH_2CH_3), 2.11, 1.99, 1.92 (3 s, 9H, 3 CH_3CO), 1.32 (t, ^3J = 7.5 Hz, 3H, SCH_2CH_3);

^{13}C-NMR (125.8 MHz, $CDCl_3$): δ 170.4, 170.2, 170.0 (3C, CH_3CO), 136.2 (Ar-C^i), 128.4, 128.3, 126.3 (5C, Ar-CH), 83.9 (C-1), 78.8 (C-5), 72.6 (C-3), 70.7 (C-4), 67.4 (C-2), 24.3 (SCH_2CH_3), 21.1, 20.9, 20.7 (3C, CH_3CO), 15.2 (SCH_2CH_3);

elemental analysis calcd. for $C_{19}H_{24}O_7S$ (396.5): C 57.56, H 6.10;
found: C 57.68, H 6.12;

$[\alpha]^{20}_D$ = +15.0 (*c* 0.9, $CHCl_3$);

Ethyl 2,3,4-tri-*O*-benzyl-(5*S*)-5*C*-phenyl-1-thio-β-D-arabinopyranoside (β-13). A solution of **β-12** (600 mg, 1.52 mmol) in MeOH (16 mL) was treated

with freshly prepared NaOMe in MeOH (1 M, 1.0 mL). After 3 h at r.t., the solution was neutralized with Amberlite IR 120 and concentrated. A stirred, cooled (0 °C) solution of the crude product in DMF (8 mL) was treated with NaH (260 mg, 6.8 mmol of a 60% dispersion in oil) for 10 min and then benzyl bromide (0.65 mL, 5.4 mmol) was added dropwise. Stirring was continued for 1 h at r.t., then the mixture was diluted with MeOH (2 mL) and after 10 min concentrated in high vacuum. The residue was suspended in H_2O (20 mL) and extracted with CH_2Cl_2 (2 x 60 mL). The combined organic layers were dried (Na_2SO_4) and concentrated. The residue was eluted from a column of silica gel (cyclohexane/Et_2O, 10:1) to give **β-13** (720 mg, 88%) as a solid.

m.p. 114-116 °C (MeOH);

^{1}H-NMR (500.1 MHz, $CDCl_3$): δ 7.45-7.41 (m, 2H, Ar-H), 7.38-7.27 (m, 13H, Ar-H), 7.17-7.12 (m, 3H, Ar-H), 6.93-6.91 (m, 2H, Ar-H), 4.93, 4.84 (A, B of AB, ^2J = 10.3 Hz, 2H, $PhCH_2$), 4.73, 4.70 (A', B' of A'B', ^2J = 11.9 Hz, 2H, $PhCH_2$), 4.58 (d, ^3J = 9.6 Hz, 1H, H-1), 4.47 (s, 1H, H-5), 4.45, 4.18 (A'', B'' of A''B'', ^2J = 11.8 Hz, 2H, $PhCH_2$), 3.95 (t, ^3J = 9.4 Hz, 1H, H-2), 3.92 (d, ^3J = 2.4 Hz, 1H, H-4), 3.74 (dd, ^3J = 2.9, ^3J = 9.4 Hz, 1H, H-3), 2.90-2.70 (m, 2H, SCH_2CH_3), 1.32 (t, ^3J = 7.5 Hz, 3H, SCH_2CH_3);

^{13}C-NMR (125.8 MHz, $CDCl_3$): δ 138.4-138.2 (4C, Ar-C^i), 128.4-126.4 (20C, Ar-CH), 85.1 (C-1), 84.2 (C-3), 79.7 (C-5), 78.0 (C-4), 77.4 (C-2), 75.7, 74.3, 72.6 (3C, $PhCH_2$), 24.5 (SCH_2CH_3), 15.2 (SCH_2CH_3);

elemental analysis calcd. for $C_{34}H_{36}O_4S$ (540.7): C 75.52, H 6.71;
found: C 75.40, H 6.73;

ESI-MS calcd. for $C_{34}H_{36}O_4SNa$ $[M+Na]^+$: 563.2; found: 563.3;

$[\alpha]^{20}_D$ = +42.3 (*c* 1.0, $CHCl_3$);

2-Trimethylsilylethyl β-D-2-acetamido-4,6-*O*-benzylidene-3-*O*-[2,3,4-tri-*O*-benzyl-(5*S*)-5*C*-phenyl-α-L-arabinopyranosyl]-2-deoxy-gluco-pyranoside (15). A solution of donor **13** (121 mg, 0.22 mmol), Glc*N*Ac acceptor **14** (84 mg, 0.21 mmol) and freshly activated molecular sieves (4Å, 400 mg) in

dry CH_2Cl_2 (20 mL) was stirred under argon at r.t. over night. After cooling the mixture to -70 °C, NIS (126 mg, 0.56 mmol) and silver triflate (23 mg, 0.09 mmol) were added. The initially colorless reaction was protected from light and allowed to warm to r.t. during 5 h. The cherry-red suspension was quenched with aqueous satd. Na_2SO_3 (20 mL), the phases were separated and the aqueous layer was extracted with CH_2Cl_2 (2 x 20 mL). The combined organic layers were dried over Na_2SO_4 and concentrated to give the crude product as foam. Purification by column chromatography (PhMe/EtOAc, 5:1) gave **β-15** (20 mg, 11%) as a side product and **α-15** (98 mg, 55%).

^{1}H-NMR (500.1 MHz, $CDCl_3$): δ 7.39-6.78 (m, 25H, Ar-H), 5.60 (d, ^3J = 7.6 Hz, 1H, NH), 5.46 (s, 1H, PhC*H*(OR)$_2$), 5.36 (d, ^3J = 3.4 Hz, 1H, PhAra-H1), 5.11 (s, 1H, PhAra-H5), 4.94 (A of AB, ^2J = 11.2 Hz, 1H, PhC*H$_2$*), 4.76-4.70 (m, 3H, Glc*N*Ac-H1, 2H of PhC*H$_2$*), 4.68 (B of AB, ^2J = 11.3 Hz, 1H, PhC*H$_2$*), 4.41 (A' of A'B', ^2J = 11.2 Hz, 1H, PhC*H$_2$*), 4.31 (dd, ^2J = 10.4, ^3J = 5.0 Hz, 1H, Glc*N*Ac-H6a), 4.23 (t, ^3J = 9.6 Hz, 1H, Glc*N*Ac-H3), 4.19 (dd, ^3J = 3.5, ^3J = 10.1 Hz, 1H, PhAra-H2), 4.13 (dd, ^3J = 2.6, ^3J = 10.1 Hz, 1H, PhAra-H3), 3.99 (B' of A'B', ^2J = 11.2 Hz, 1H, PhC*H$_2$*), 3.92 (td, ^2J = 10.3, ^3J = 4.8 Hz, 1H, 1H of OC*H$_2$*CH$_2$), 3.90 (br s, 1H, PhAra-H4), 3.73 (t, ^3J = 10.3 Hz, 1H, Glc*N*Ac-H6b), 3.60 (t, ^3J = 9.3 Hz, 1H, Glc*N*Ac-H4), 3.57-3.47 (m, 2H, Glc*N*Ac-H2, 1H of OC*H$_2$*CH$_2$), 3.43 (td, ^3J = 5.0, ^3J = 9.7 Hz, 1H, Glc*N*Ac-H5), 1.67 (s, 3H, NHAc), 0.97-0.83 (m, 2H, OCH$_2$C*H$_2$*), 0.00 (s, 9H, Me$_3$Si);

^{13}C-NMR (125.8 MHz, $CDCl_3$): δ 170.7 (CH$_3$*C*O), 138.8, 138.7, 138.3, 137.3 (5C, Ar-C^i), 129.0-126.2 (25C, Ar-CH), 101.5 (Ph-*C*H(OR)$_2$), 100.8 (Glc*N*Ac-C1), 98.5 (PhAra-C1), 81.1 (Glc*N*Ac-C4), 79.6 (PhAra-C3), 79.1 (PhAra-C4), 77.4 (PhAra-C2), 75.6 (Glc*N*Ac-C3), 75.1, 74.5 (2C, Ph*C*H$_2$), 72.6 (PhAra-C5), 72.5 (Ph*C*H$_2$), 69.0 (Glc*N*Ac-C6), 67.5 (O*C*H$_2$CH$_2$), 66.4 (Glc*N*Ac-C5), 57.8 (Glc*N*Ac-C2), 23.5 (*C*H$_3$CO), 18.2 (OCH$_2$*C*H$_2$), -1.2 (Me$_3$Si);

HR-MS calcd. for $C_{52}H_{61}NO_{10}SiNa$ $[M+Na]^+$: 910.3962; found: 910.3950;

$[\alpha]^{20}_D$ = -69.5 (*c* 1.3, $CHCl_3$);

2-Trimethylsilylethyl β-D-2-acetamido-6-*O*-benzyl-3-*O*-[2,3,4-tri-*O*-benzyl-(5*S*)-5*C*-phenyl-α-L-arabinopyranosyl]-2-deoxy-glucopyranoside (16). Benzylidene **15** (84 mg, 0.10 mmol) was dissolved in dry THF (2 mL) under argon and BH_3•Me_3N (28 mg, 0.38 mmol) was added, followed by anhydrous $AlCl_3$ (76 mg, 0.57 mmol). Water (3.4 µL, 0.19 mmol) was added to the clear solution, whereupon the reaction mixture turned turbid. After stirring for 3.5 h at r.t., the mixture was quenched with water (1 mL) and aqueous HCl (0.5 M, 1 mL), followed by extraction of the product with CH_2Cl_2 (3 x 10 mL). The combined organic layers were washed with aqueous satd. $NaHCO_3$ (10 mL), dried over Na_2SO_4 and concentrated *in vacuo*. The crude product was purified by column chromatography on silica (PhMe/EtOAc, 5:1) to give **16** as white solid (53 mg, 62%).

^{1}H-NMR (500.1 MHz, $CDCl_3$): δ 7.40-6.85 (m, 25H, Ar-H), 5.44 (d, ^3J = 5.4 Hz, 1H, NH), 5.18 (d, ^3J = 2.8 Hz, 1H, PhAra-H1), 5.10 (s, 1H, PhAra-H5), 4.94 (d, ^3J = 8.0 Hz, 1H, Glc*N*Ac-H1), 4.90 (A of AB, ^2J = 11.6 Hz, 1H, PhCH_2), 4.76, 4.73 (A',B' of A'B', ^2J = 11.8 Hz, 2H, PhCH_2), 4.70 (B of AB, ^2J = 11.6 Hz, 1H, PhCH_2), 4.57, 4.55 (A'', B'' of A''B'', ^2J = 12.3 Hz, 2H, PhCH_2), 4.46 (A''' of A'''B''', ^2J = 11.3 Hz, 1H, PhCH_2), 4.18 (dd, ^3J = 3.4, ^3J = 10.1 Hz, 1H, PhAra-H2), 4.14 (dd, ^3J = 1.5, ^3J = 10.2 Hz, 1H, PhAra-H3), 4.08-4.05 (m, 2H, Glc*N*Ac-H3, 1H of PhCH_2), 3.98-3.93 (m, 2H, PhAra-H4, 1H of OCH_2CH$_2$), 3.76 (d, ^2J = 10.8 Hz, 1H, Glc*N*Ac-H6a), 3.61 (dd, ^2J = 10.8, ^3J = 5.3 Hz, 1H, Glc*N*Ac-H6b), 3.56-3.42 (m, 3H, Glc*N*Ac-H4, -H5, 1H of OCH_2CH$_2$), 3.27-3.22 (m, 1H, Glc*N*Ac-H2), 1.48 (s, 3H, NHAc), 0.98-0.84 (m, 2H, OCH$_2$CH_2), -0.02 (s, 9H, Me_3Si);

^{13}C-NMR (125.8 MHz, $CDCl_3$): δ 171.0 (CH$_3$*C*O), 138.6, 138.5, 138.3, 138.1, 137.8 (5C, Ar-C^i), 128.8-126.6 (25C, Ar-CH), 99.4 (Glc*N*Ac-C1), 97.6 (PhAra-C1), 82.5 (Glc*N*Ac-C3), 79.4 (PhAra-C3), 78.6 (PhAra-C4), 76.0 (PhAra-C2), 75.0 (Ph*C*H$_2$), 74.9 (Glc*N*Ac-C4), 74.6, 73.6 (2C, Ph*C*H$_2$), 73.3 (PhAra-C5), 72.7 (Ph*C*H$_2$), 69.8 (Glc*N*Ac-C5), 69.7 (Glc*N*Ac-C6), 67.1 (O*C*H$_2$CH$_2$), 56.5 (Glc*N*Ac-C2), 23.2 (*C*H$_3$CO), 18.2 (OCH$_2$*C*H$_2$), -1.2 (Me_3Si);

HR-MS calcd. for $C_{52}H_{63}NO_{10}SiNa$ $[M+Na]^+$: 912.4119; found: 912.4108;

$[\alpha]^{20}_{D}$ = -26.1 (*c* 0.52, $CHCl_3$);

2-Trimethylsilylethyl β-D-2-acetamido-3-*O*-chloroacetyl-6-*O*-benzyl-2-deoxy-glucopyranoside (20). Benzylidene **19** (29 mg, 0.06 mmol) was dissolved in dry THF (1.2 mL) under argon and BH_3•Me_3N (19 mg, 0.26 mmol) was added, followed by anhydrous $AlCl_3$ (52 mg, 0.39 mmol). Water (2 µL, 0.12 mmol) was added to the clear solution, whereupon the reaction mixture turned turbid. After stirring for 2 h at r.t., the mixture was quenched with water (1 mL) and aqueous HCl (0.5 M, 1 mL), followed by extraction of the product with EtOAc (3 x 1 mL). The combined organic layers were washed with brine (1 mL), dried over Na_2SO_4 and concentrated *in vacuo*. The crude product was purified by column chromatography on silica (PhMe/EtOAc, 1:1 to 2:3) to give **20** as white foam (27 mg, 92%).

^{1}H-NMR (500.1 MHz, $CDCl_3$): δ 7.35-7.27 (m, 5H, Ar-H), 5.55 (d, ^3J = 8.9 Hz, 1H, NH), 5.16 (t, ^3J = 9.8 Hz, 1H, H-3), 4.61-4.52 (m, 3H, H-1, 2H of PhCH_2), 4.09 (s, 2H, $ClCH_2$), 3.96-3.89 (m, 1H, OC$H_2$$CH_2$), 3.89-3.82 (m, 1H, H-2), 3.81-3.72 (m, 3H, H-4, H-6a,b), 3.57-3.48 (m, 2H, H-5, 1H of OC$H_2$$CH_2$), 3.05 (br s, 1H, OH), 1.92 (s, 3H, NHAc), 0.96-0.79 (m, 2H, OCH_2CH_2), -0.02 (s, 9H, Me_3Si);

^{13}C-NMR (125.8 MHz, $CDCl_3$): δ 170.6 (CH_3*C*O), 168.4 ($ClCH_2$*C*O), 137.6 (Ar-C^i), 128.8, 128.2, 128.0 (5C, Ar-CH), 100.3 (C-1), 77.4 (C-3), 74.0 (Ph*C*H_2), 73.6 (C-5), 71.3 (C-6), 70.7 (C-4), 67.3 (O*C*$H_2$$CH_2$), 54.5 (C-2), 41.1 (Cl*C*H_2), 23.6 (*C*H_3CO), 18.2 (OCH_2*C*H_2), -1.2 (Me_3Si);

HR-MS calcd. for $C_{22}H_{34}ClNO_7SiNa$ $[M+Na]^+$: 510.1691; found: 510.1678;

$[\alpha]^{20}_{D}$ = -45.5 (*c* 1.0, $CHCl_3$);

2-Trimethylsilylethyl (benzyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosidonate)-(2→3)-(2,4-di-*O*-acetyl-6-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-2-acetamido-6-*O*-benzyl-3-*O*-chloroacetyl-2-deoxy-β-D-glucopyranoside (21). To a solution of thioglycoside **17** (96 mg, 0.10 mmol) and acceptor **20** (97 mg, 0.20 mmol) in dry CH_2Cl_2 (4 mL) was added freshly activated molecular sieves (4Å,

400 mg). In a separate flask, DMTST (77 mg, 0.30 mmol) was dissolved in dry CH_2Cl_2 (1 mL) and freshly activated molecular sieves (4Å, 350 mg) were added. Both mixtures were stirred under argon for 6 h at r.t., then the DMTST mixture was transferred to the cooled (0 °C) mixture of donor and acceptor. After stirring for 2.5 days at 0 °C, the reaction was diluted with CH_2Cl_2 and filtered over celite. The celite was washed with CH_2Cl_2 (2 x 20 mL) and the solution was dried over Na_2SO_4 and concentrated to give the crude product as foam. Purification by column chromatography (CH_2Cl_2/acetone, gradient of 10:1 to 4:1) gave **21** (95 mg, 68%) as foam.

^{1}H-NMR (500.1 MHz, CD_2Cl_2): δ 8.09-8.05 (m, 2H, Bz-H), 7.60-7.57 (m, 1H, Bz-H), 7.50-7.46 (m, 2H, Bz-H), 7.38-7.30 (m, 9H, Ar-H), 7.28-7.23 (m, 1H, Ar-H), 5.77 (d, ^3J = 9.2 Hz, 1H, Glc*N*Ac-NH), 5.57-5.52 (m, 1H, Sia-H8), 5.33 (dd, 1H, ^3J = 2.6, ^3J =10.3 Hz, Sia-H7), 5.30 (A of AB, ^2J = 14.4 Hz, 1H, PhC*H₂*), 5.23 (d, ^3J = 10.0 Hz, 1H, Sia-NH), 5.17 (dd, ^3J = 8.8, ^3J = 10.5 Hz, 1H, Glc*N*Ac-H3), 5.14 (d, ^3J = 3.2 Hz, 1H, Gal-H4), 4.97 (B of AB, ^2J = 12.4 Hz, 1H, PhCH_2), 4.92-4.86 (m, 2H, Gal-H2, Sia-H4), 4.81 (d, ^3J = 8.1 Hz, 1H, Gal-H1), 4.70-4.66 (m, 2H, Gal-H3, PhCH_2), 4.54 (B' of A'B', ^2J = 12.0 Hz, 1H, PhCH_2), 4.49 (d, ^3J = 8.0 Hz, 1H, Glc*N*Ac-H1), 4.34 (dd, ^2J = 11.1, ^3J = 6.9 Hz, 1H, Gal-H6a), 4.28-4.19 (m, 3H, Gal-H6b, Sia-H9a, 1H of ClCH_2), 4.14 (d, 1H, ^2J = 14.9 Hz, ClCH_2), 4.05-3.92 (m, 5H, Gal-H5, Glc*N*Ac-H4, Sia-H5, -H9b, 1H of OCH_2CH_2), 3.89 ('q', ^3J = 8.9 Hz, 1H, Glc*N*Ac-H2), 3.84-3.80 (m, 1H, Glc*N*Ac-H6a), 3.74 (dd, ^2J = 10.8, ^3J = 4.5 Hz, 1H, Glc*N*Ac-H6b), 3.60-3.51 (m, 3H, Glc*N*Ac-H5, Sia-H6, 1H of OCH_2CH_2), 2.62 (dd, ^2J = 13.0, ^3J = 4.7 Hz, 1H, Sia-H3^{eq}), 2.19, 2.09, 2.08, 2.02, 1.96, 1.94, 1.91, 1.80 (8 s, 24H, 8 Ac), 1.64 ('t', ^2J = ^3J = 12.4 Hz, 1H, Sia-H3^{ax}), 0.99-0.83 (m, 2H, OCH_2CH_2), 0.01 (s, 9H, Me_3Si);

^{13}C-NMR (125.8 MHz, CD_2Cl_2): δ 171.0, 170.9, 170.7, 170.7, 170.6, 170.4, 170.0, 170.0 (8C, CH_3*C*O), 167.9 (Sia-C1), 167.8 (ClCH$_2$*C*O), 166.1 (ArCO), 138.9 (Glc*N*Ac-6-OBn-Ar-C^i), 135.3 (Sia-1O-Bn-Ar-C^i), 133.9 (Bz, Ar-C^i), 130.2, 129.1, 129.0, 128.8, 128.0 (15C, Ar-CH), 100.9 (Gal-C1), 100.8 (Glc*N*Ac-C1), 97.3 (Sia-C2), 75.9 (Glc*N*Ac-C3), 75.3 (Glc*N*Ac-C4), 75.0 (Glc*N*Ac-C5), 73.7 (Ph*C*H$_2$), 72.6 (Sia-C6), 71.9 (Gal-C3), 71.1 (Gal-C5), 70.8

(Gal-C2), 69.7 (Sia-C4), 68.9 (Ph*C*H_2), 68.7 (Glc*N*Ac-C6), 68.1 (Sia-C8), 67.8 (Gal-C4), 67.7 (Sia-C7), 67.5 (O*C*H_2CH_2Si), 62.9 (Sia-C9), 61.8 (Gal-C6), 54.3 (Glc*N*Ac-C2), 49.2 (Sia-C5), 41.6 (Cl*C*H_2), 37.9 (Sia-C3), 23.7, 23.5, 21.7, 21.5, 21.2, 21.1, 21.0, 21.0 (8C, *C*H_3CO), 18.4 (OCH_2*C*H_2), -1.2 (Me_3Si);

HR-MS calcd. for $C_{65}H_{83}ClN_2O_{27}SiNa$ $[M+Na]^+$: 1409.4539; found: 1409.4558;

$[\alpha]^{20}_D$ = +0.5 (*c* 0.5, $CHCl_3$);

2-Trimethylsilylethyl (benzyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosidonate)-(2→3)-(2,4-di-*O*-acetyl-6-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-2-acetamido-6-*O*-benzyl-2-deoxy-β-D-glucopyranoside (22). To a solution of chloroacetyl protected **21** (77 mg, 55 µmol) in dry DMF/2,6-di-*tert.* butyl pyridine (11:1, 1.5 mL) was added thiourea (21 mg, 277 µmol). The colorless solution was stirred for 22 h at 70 °C under argon. Then, the volatiles were removed and the residue was purified by column chromatography (CH_2Cl_2/acetone, gradient of 7:1 to 2:1) to give **22** (46 mg, 63%) as foam.

^{1}H-NMR (500.1MHz, CD_2Cl_2): δ 8.13-8.07 (m, 2H, Bz-H), 7.62-7.56 (m, 1H, Bz-H), 7.51-7.47 (m, 2H, Bz-H), 7.44-7.27 (m, 10H, Ar-H), 5.62 (d, ^3J = 8.5 Hz, 1H, Glc*N*Ac-NH), 5.54-5.50 (m, 1H, Sia-H8), 5.38 (A of AB, ^2J = 12.1 Hz, 1H, PhC*H_2*), 5.32-5.28 (m, 2H, Sia-H7, Sia-NH), 5.12 (d, ^3J = 2.9 Hz, 1H, Gal-H4), 5.05-5.00 (m, 2H, Gal-H2, 1H of PhC*H_2*), 4.91 (dt, ^3J = 4.6, ^3J = 11.5 Hz, 1H, Sia-H4), 4.81 (d, ^3J = 8.0 Hz, 1H, Gal-H1), 4.75 (dd, ^3J = 3.3, ^3J = 10.1 Hz, 1H, Gal-H3), 4.66, 4.55 (A',B' of A'B', ^2J = 11.9 Hz, 2H, PhC*H_2*), 4.47 (d, ^3J = 8.0 Hz, 1H, Glc*N*Ac-H1), 4.43 (dd, ^2J = 11.3, ^3J = 4.3 Hz, 1H, Gal-H6a), 4.27-4.10 (m, 3H, Gal-H5, -H6b, Sia-H9a), 4.01 ('q', ^3J = 10.4 Hz, 1H, Sia-H5), 3.95 (dt, ^2J = 10.1, ^3J = 4.7 Hz, 1H, 1H of OC*H_2*CH_2), 3.89 (dd, ^2J = 12.3, ^3J = 6.2 Hz, 1H, Sia-H9b), 3.80-3.50 (m, 8H, Glc*N*Ac-H2, H-3, -H4, -H5, -H6a/b, Sia-H6, 1H of OC*H_2*CH_2), 2.63 (dd, ^2J = 12.6, ^3J = 4.5 Hz, 1H, Sia-$H3^{eq}$), 2.21, 2.13, 2.09, 2.03, 1.96, 1.94, 1.90, 1.80 (8 s, 24H, 8 Ac), 1.63 ('t', ^2J = ^3J = 12.3 Hz, 1H, Sia-$H3^{ax}$), 0.99-0.83 (m, 2H, OCH_2C*H_2*), -0.01 (s, 9H, Me_3Si);

^{13}C-NMR (125.8 MHz, CD_2Cl_2): δ 171.1, 170.9, 170.8, 170.7, 170.7, 170.6, 170.5, 170.1 (8C, CH_3*C*O), 167.9 (Sia-C1), 166.5 (Ar*C*O), 139.1 (Glc*N*Ac-6-OBn-Ar-C^i), 135.3 (Sia-1O-Bn-Ar-C^i), 133.9 (Bz, Ar-C^i), 130.4, 130.1, 129.3, 129.1, 128.8, 128.0 (15C, Ar-CH), 102.1 (Gal-C1), 100.9 (Glc*N*Ac-C1), 97.4 (Sia-C2), 82.6 (Glc*N*Ac-C3), 74.7 (Glc*N*Ac-C4), 73.8 (Ph*C*H_2), 73.5 (Glc*N*Ac-C5), 72.8 (Sia-C6), 71.9 (Gal-C3), 71.8 (Gal-C5), 70.5 (Gal-C2), 69.8 (Sia-C4), 69.5 (Glc*N*Ac-C6), 69.0 (Ph*C*H_2), 68.4 (Sia-C8), 68.3 (Gal-C4), 67.9 (Sia-C7), 67.4 (O*C*H_2CH_2), 63.1 (Sia-C9), 63.0 (Gal-C6), 56.2 (Glc*N*Ac-C2), 49.2 (Sia-C5), 38.1 (Sia-C3), 23.9, 23.5, 21.8, 21.5, 21.2, 21.1, 21.1, 21.0 (8C, *C*H_3CO), 18.5 (OCH_2*C*H_2), -1.2 (Me_3Si);

HR-MS calcd. for $C_{63}H_{82}N_2O_{26}SiNa$ $[M+Na]^+$: 1333.4823; found: 1333.4813;

$[\alpha]^{20}_D$ = +18.9 (*c* 2.0, $CHCl_3$);

2-Trimethylsilylethyl (benzyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosidonate)-(2→3)-(2,4-di-*O*-acetyl-6-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-[(2,3,4-tri-*O*-benzyl-(5*S*)-5*C*-phenyl-α-L-arabinopyranosyl-(1→3)]-2-acetamido-6-*O*-benzyl-2-deoxy-β-D-glucopyranoside (18). To a solution of thioglycoside **13** (109 mg, 0.20 mmol) in dry CH_2Cl_2 (0.8 mL) was added Br_2 (11.7 µL, 0.23 mmol) at 0 °C. After stirring the mixture for 30 min at 0 °C, excess Br_2 was quenched with cyclohexene (2 drops). This solution was then transferred to a suspension of acceptor **22** (152 mg, 0.116 mmol), Et_4NBr (65 mg, 0.31 mmol) and activated molecular sieves (4Å, 2.0 g) in dry CH_2Cl_2 (1.2 mL) and DMF (0.9 mL). The reaction was stirred under argon at r.t. for 3 d. The mixture was filtered over celite and washed with dry CH_2Cl_2 (60 mL). The organic phase was washed with aqueous satd. $NaHCO_3$ (10 mL) and with brine (5 mL), and then dried over Na_2SO_4. Removal of the volatiles and purification by column chromatography (SiO_2, EtOAc) gave recovered acceptor **22** (84 mg, 55%) and **18** as off-white solid (77 mg, 37%).

^{1}H-NMR (500.1 MHz, CD_2Cl_2): δ 8.07-8.01 (m, 2H, Bz-H), 7.53-7.50 (m, 1H, Bz-H), 7.46-7.15 (m, 30H, Ar-H), 6.94-6.89 (m, 2H, PhAra-Ar-H), 6.17 (d, ^3J = 9.3 Hz, 1H, Glc*N*Ac-NH), 5.63-5.60 (m, 1H, Sia-H8), 5.49 (d, ^3J = 3.3 Hz, 1H,

PhAra-H1), 5.32-5.28 (m, 2H, Sia-H7, 1H of PhCH_2), 5.15 (d, ^3J = 3.1 Hz, 1H, Gal-H4), 5.03 (d, ^3J = 10.2 Hz, 1H, Sia-NH), 4.98-4.84 (m, 6H, Gal-H2, PhAra-H5, Sia-H4, 3H of PhCH_2), 4.75-4.71 (m, 4H, Gal-H1, -H3, 2H of PhCH_2), 4.54 (A of AB, ^2J = 11.8 Hz, 1H, 1H of PhCH_2), 4.50-4.47 (m, 2H, Gal-H5, Glc*N*Ac-H1), 4.39 (B of AB, ^2J = 11.8 Hz, 1H, 1H of PhCH_2), 4.33 (dd, ^2J = 12.3, ^3J = 2.0 Hz, 1H, Sia-H9a), 4.14 (dd, ^3J = 3.3, ^3J = 10.2 Hz, 1H, PhAra-H2), 4.11-4.07 (m, 2H, Gal-H6a, Glc*N*Ac-H2), 4.05-3.86 (m, 11H, Gal-H6b, Glc*N*Ac-H3, -H4, -H5, -H6b, PhAra-H3, Sia-H5, -H9b, 1H of OCH_2CH_2, 2H of PhCH_2), 3.84 (br s, 1H, PhAra-H4), 3.81-3.78 (m, 1H, Glc*N*Ac-H6b), 3.57 (dd, ^3J = 2.0, ^3J = 10.8 Hz, 1H, Sia-H6), 3.48 (dt, ^2J = 10.7, ^3J = 6.2 Hz, 1H, 1H of OCH_2CH_2TMS), 2.61 (dd, ^2J = 12.5, ^3J = 4.4 Hz, 1H, Sia-$H3^{eq}$), 2.23, 2.12, 2.02, 2.01, 1.96, 1.94, 1.93, 1.80 (8 s, 24H, 8 Ac), 1.68 ('t', ^2J = ^3J = 12.3 Hz, 1H, Sia-$H3^{ax}$), 1.00-0.81 (m, 2H, OCH_2CH_2), -0.02 (s, 9H, Me_3Si);

^{13}C-NMR (125.8 MHz, CD_2Cl_2): δ 171.2, 171.0, 170.9, 170.8, 170.5, 170.5, 170.2, 170.1, (8C, CH_3CO), 167.9 (Sia-C1), 165.9 (Ar*C*O), 139.8, 139.5, 139.0, 139.0, 138.9 (5C, Ph-C^i), 135.4 (Sia-1O-Bn-Ar-C^i), 133.8 (Bz, Ar-C^i), 130.2-127.8 (35C, Ar-CH), 100.5 (Glc*N*Ac-C1), 99.4 (Gal-C1), 97.3 (Sia-C2), 96.4 (PhAra-C1), 79.9 (PhAra-C4), 79.1 (PhAra-C3), 76.8 (PhAra-C2), 75.4 (2C, Gal-C5, PhAra-C5), 75.1 (Glc*N*Ac-C3), 74.9 (Glc*N*Ac-C5), 73.6 (Glc*N*Ac-C2), 73.4, 73.1, 72.8 (4C, PhCH_2), 72.5 (Sia-C6), 71.4 (Gal-C3), 71.1 (Glc*N*Ac-C4), 70.8 (Gal-C2), 70.5 (Glc*N*Ac-C6), 69.8 (Sia-C4), 68.9 (PhCH_2), 68.1 (Sia-C8), 67.8 (2C, Gal-C4, Sia-C7), 67.1 (OCH_2CH_2Si), 63.3 (Sia-C9), 61.5 (Gal-C6), 49.2 (Sia-C5), 37.9 (Sia-C3), 23.6, 23.5, 21.8, 21.5, 21.2, 21.1, 21.1, 20.9 (8C, CH_3CO), 18.4 (OCH_2CH_2Si), -1.2 (Me_3Si);

HR-MS calcd. for $C_{95}H_{112}N_2O_{30}SiNa_2$ $[M+2Na]^{2+}$: 917.8449; found: 917.8443;

$[\alpha]^{20}_D$ = -35.7 (*c* 1.0, $CHCl_3$);

2-Trimethylsilylethyl (sodium 5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosidonate)-(2→3)-β-D-galactopyranosyl-(1→4)-[(5*S*)-5*C*-phenyl-α-L-arabinopyranosyl-(1→3)]-2-acetamido-2-deoxy-β-D-glucopyranoside (4). To a solution of **18** (8.2 mg, 4.58 µmol) in dry MeOH (1.5 mL) was added Pd/C (Degussa E101 NE/W, 10 mg) and the mixture was

stirred at r.t. under hydrogen (1 atm) over night. Fresh catalyst was added (20 mg) and the mixture was vigorously stirred under hydrogen for 2 h, then the mixture was filtered over celite, and concentrated *in vacuo*. The residue was dissolved in dry MeOH (2 mL), and NaOMe in MeOH (0.5 M, 15 µL) was added dropwise at r.t.. The reaction was stirred under argon for 20 h, concentrated and purified by chromatography on RP18 cartridges (H_2O/MeOH, gradient of 5-50%). Compound **4** (2.7 mg, 59%) was obtained as a white foam after lyophilization.

^{1}H-NMR (500.1 MHz, D_2O): δ 7.53-7.34 (m, 5H, Ar-H), 5.70 (s, 1H, PhAra-H5), 5.38 (d, ^3J = 3.8 Hz, 1H, PhAra-H1), 4.53 (d, ^3J = 7.9 Hz, 1H, Glc*N*Ac-H1), 4.46 (d, ^3J = 7.9 Hz, 1H, Gal-H1), 4.23 (br s, 1H, PhAra-H4), 4.15 (dd, ^3J = 3.3, ^3J = 10.3 Hz, 1H, PhAra-H3), 4.10-3.50 (m, 21H, Gal-H3, -H4, -H5, -H6a,b, Glc*N*Ac-H2, -H3, -H4, -H5, -H6a,b, PhAra-H2, Sia-H4, -H5, -H6, -H7, -H8, -H9a,b, 2H of OCH_2CH_2), 3.33-3.30 (m, 1H, Gal-H2), 2.76 (dd, ^2J = 12.4, ^3J = 4.9 Hz, 1H, Sia-H3^{eq}), 2.03, 2.02 (2 s, 6H, 2 Ac), 1.77 ('t', ^2J = ^3J = 12.2 Hz, 1H, Sia-H3^{ax}), 1.00-0.81 (m, 2H, OCH_2CH_2), -0.02 (s, 9H, Me_3Si);

^{13}C-NMR (125.8 MHz, D_2O): δ 175.7, 174.7, 174.6 (3C, 2 CH_3CO, Sia-C1), 138.6 (Ar-C^i), 129.1-126.8 (5C, Ar-CH), 102.8 (Gal-C1), 100.8 (Glc*N*Ac-C1), 100.3 (Sia-C2), 98.8 (PhAra-C1), 76.5 (CH), 75.9 (CH), 75.7 (CH), 74.8 (CH), 74.7 (CH), 73.6 (CH), 72.9 (PhAra-C4), 72.5 (CH), 71.9 (PhAra-C5), 69.9 (PhAra-C3), 69.8 (Gal-C2), 69.0 (OCH_2CH_2Si), 68.9, 68.7, 68.5, 68.2 (4 CH), 63.2 (Sia-C9), 62.1 (Glc*N*Ac-C6), 60.3 (Gal-C6), 52.3 (Sia-C5), 40.4 (Sia-C3), 23.0, 22.7 (2C, CH_3CO), 17.8 (OCH_2CH_2Si), -1.8 (Me_3Si);

HR-MS calcd. for $C_{41}H_{65}N_2O_{23}SiNa_2$ $[M+Na]^+$: 1027.3543; found: 1027.3553;

$[\alpha]^{20}_D$ = -26.8 (*c* 0.2, H_2O);

2-Trimethylsilylethyl (sodium 5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosidonate)-(2→3)-β-D-galactopyranosyl-(1→4)-[α-L-fucopyranosyl-(1→3)]-2-acetamido-2-deoxy-β-D-glucopyranoside (1c). **1c** was synthesized according to the reported procedure[21] from **23** in 89% yield.

The NMR data correspond to the values given by Baisch and co-workers[21] for the Lemieux-spacer analog of **1c**.

HR-MS calcd. for $C_{36}H_{63}N_2O_{23}SiNa_2$ [M+Na]$^+$: 965.3386; found: 965.3378;

2-Trimethylsilylethyl (sodium 5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosidonate)-(2→3)-β-D-galactopyranosyl-(1→4)-[α-D-arabinopyranosyl-(1→3)]-2-acetamido-2-deoxy-β-D-glucopyranoside (2c). 2c was synthesized according to the reported procedure[21] from **23** in 81% yield.

The NMR data correspond to the values given by Baisch and co-workers[21] for the Lemieux-spacer analog of **2c**.

HR-MS calcd. for $C_{35}H_{61}N_2O_{23}SiNa_2$ [M+Na]$^+$: 951.3230; found: 951.3231;

$[\alpha]^{20}_{D}$ = -48.6 (*c* 0.32, MeOH);

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3. Summary and Outlook

The selectins play an essential role in the inflammatory cascade. The initial tethering and rolling of the leukocytes on the vascular endothelium, mediated by the selectins, is a critical mechanism of the host immune defense. Excessive efflux of leukocytes from the blood stream into inflamed tissue can, however, lead to severe pathological disorders (*e.g.* rheumatoid arthritis, stroke or reperfusion injury). Therefore, blocking of the selectins is a valuable pharmaceutical approach to reduce inflammatory disorders.

The tetrasaccharide sialyl Lewis^x (sLe^x) is the natural binding epitope common to all selectin ligands, and consequently served as a lead compound in selectin antagonist research. As observed for many carbohydrate-protein interactions, the affinity of sLe^x towards the selectins is in the low millimolar range. Because binding of the selectins to their ligands occurs under shear stress conditions, the stabilization of the pharmacophores in sLe^x mimetics in the bioactive conformation is a prerequisite for binding.

More than 600 publications report on the synthesis and evaluation of potential selectin antagonizing substances.[221] Furthermore, large research programs at major pharmaceutical companies, *e.g.* Novartis, Höchst and Glaxo, were run to develop selectin antagonizing drugs in the 1990s. However, to date there is no drug on the market with selectin blocking properties.

The major drawback of most of the developed compounds was the lack of functionality. Although being ligands with high affinity, a lack of competitive inhibition under dynamic natural shear stress conditions was often observed. In this PhD thesis, both, the importance of pre-organization of the acid orientation in the bioactive conformation in sLe^x mimetics, but also the correct orientation of the core have been extensively studied.

The stabilization of the acid orientation was studied by a non-covalent approach, using (*R*)- and (*S*)-adamantyllactic acid as replacements for *N*-acetylneuraminic acid in sLe^x (**(*R*)/(*S*)-7**, *chapter 2.1*). It could be shown, that a (*S*)-configuration of the C-2 of adamantyllactic acid was essential for

binding. The corresponding (*R*)-diastereomer was deficient in detectable binding affinity towards E-selectin, resulting from the pre-organization of the acid orientation outside the bioactive conformation. These observations are in agreement with the literature on lactate derivatives.[167,168] The (*S*)-lactate literature examples display increasing binding affinity with growing steric bulk of their substituents. The adamantyl group in ***(S)*-7** was chosen as a more bulky substituent to strengthen the binding affinity of these mimetics. However, when ***(S)*-7** was tested in the biological assay, the predicted increase in affinity was not observed. This behavior might originate from unfavorable solvent interactions of the lipophilic adamantyl group, when the *in silico* binding mode is taken into account.

In a second approach to increase binding affinity of the ligands, a large unexplored area of the protein surface (*figure 2*, *chapter 2.2*) adjacent to the binding locus of the carboxylic acid in sLe^x mimetics was targeted. So far, this has not been exploited successfully for increasing binding affinity *via* additional pharmacophores targeting these amino acids. To date, there is one report in the literature, where various substituents at the lactate were introduced *via* amine, amide and sulfonamide linkages.[222] The various rather drug-unlike substituents studied were, however, not successful in increasing the binding affinity of the cyclohexyllactate CGP69669A. Since amine and amide linkages are prone to influence charge and orientation of the proximate acid *via* salt bridges or hydrogen bonding, a non-participating linking motif was foreseen. Consequently, a '*click chemistry*' library bearing druglike heterocycles or aromatic rings, based on a pre-organized (*S*)-triazololactate was investigated (*chapter 2.2*). The affinities of the library members investigated were not superior to CGP69669A or the molecules from the literature report.[222] Therefore, the idea emerged that the additional pharmacophores do interact either with the protein surface or, like aromatic amino acid side chains in galactose binding proteins, intramolecularly with the α-face of galactose. Both of these cases however, would lead to a mis-orientation of the carboxylic acid and therefore lower the binding affinity of the corresponding library members.

Encouraged by these results (*chapters 2.1* and *2.2*), a project was started where the acid orientation is first covalently locked in the bio-active orientation (*chapter 2.3*). Then, additional pharmacophores could be introduced *via* a proximate handle to target the protein surface as discussed before. The synthesis of this *spiro*-cyclic compound **17** involved various stereoselective transformations, being C-C bond formation or selective double bond oxidation. These transformations were first studied on part of the final molecule to reduce complexity and consumption of valuable starting material. Finally, a spiro-precursor of **17** could be synthesized, but according to Murphy's law with opposite stereochemistry. However, the concept of the synthesis was shown feasible and further studies should allow rapid access to the final covalently stabilized acid in **17**.

Besides the analysis of the orientation of the carboxylic acid, also the core conformation of sLe^x and mimetics thereof was studied in this work. Literature examples showed the importance of the core stabilization when different linkers were used to connect fucose and galactose, which bear the essential pharmacophores of the core.[57] For example, it was found that an additional methyl group in the linker region, which is not in contact with the protein but rather stabilizes the bioactive conformation, led to a six-fold increase in binding affinity when compared to the unsubstituted analog.[223] By varying the substitution of the fucose C-5 position in the present work, a lipophilic interresidue interaction between fucose and galactose was found to be responsible for stabilization of the core conformation (*chapter 2.4*). Molecules without substituent at C-5 of fucose (**2c**, *chapter 2.4*) were not binding to E-selectin, whereas molecules with lipophilic substituents were binding to the protein (**1c**, **4**, *chapter 2.4*). It could be shown, that the methyl group chosen by nature in sLe^x possesses an optimal size for stabilizing the bioactive conformation.

In general, the shallow binding site of sLe^x mimetics in E-selectin is a tough target for small molecule medicinal chemistry. Nature uses multivalent display of sLe^x on the surface proteins of leukocytes to gain significant binding. In order to further improve low molecular weight compounds as selectin

antagonists, experimental high resolution structures (*e.g.* X-ray crystallography or NMR) of sLe^x mimetics in complex with the selectins are indispensable. Studies regarding this issue are ongoing at the IMP and will give an excellent basis for further structure based medicinal chemistry in the selectin field.

4. Formula Index

The molecules synthesized in this work are shown on the following pages.
The formulas are numbered as they appear in the corresponding sections.

Chapter 2.1

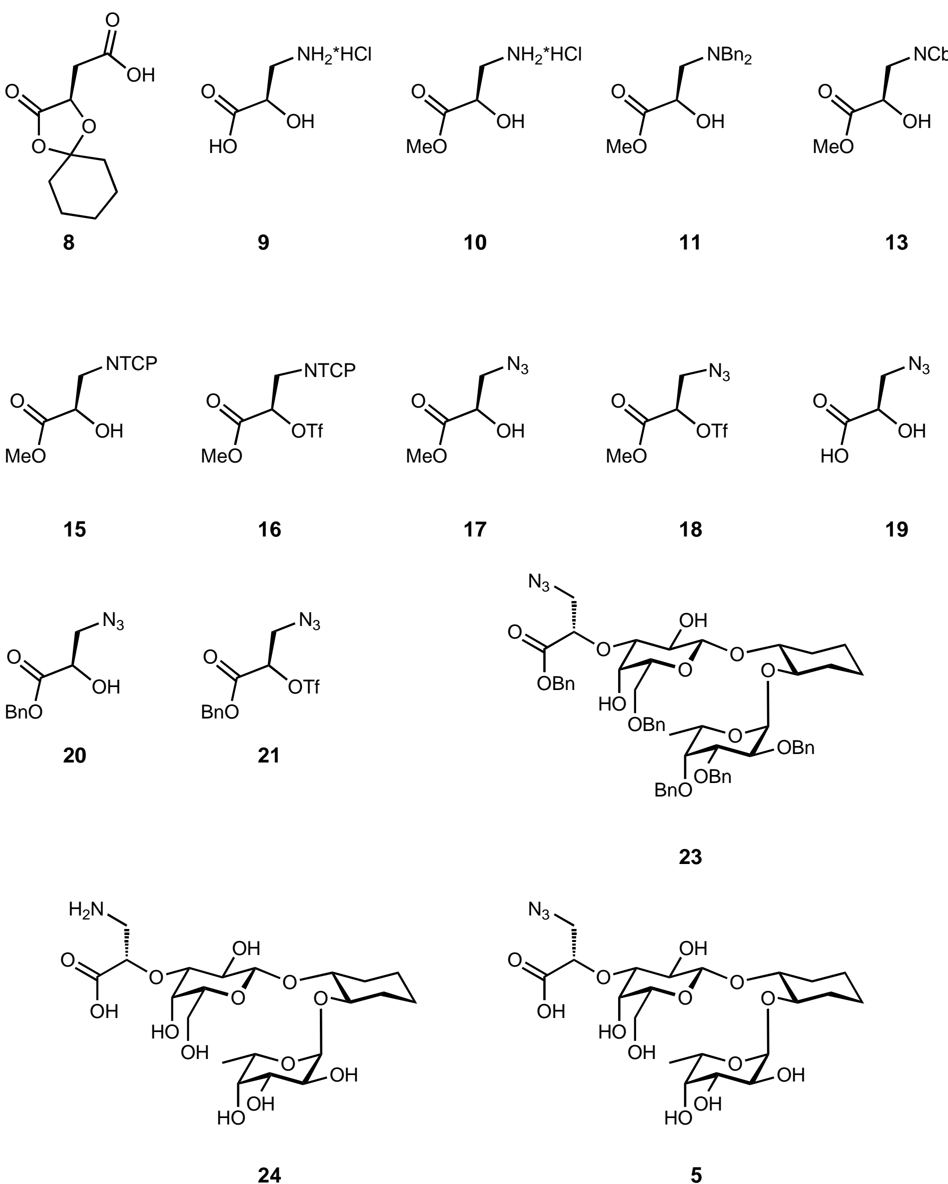
11 **rac-12** ***(S)*-13** ***(R)*-13** ***(S)*-14** ***(R)*-14**

16 **17** **10** **20**

21 **(*S*)-22** **24**

(*S*)-7 **(*R*)-7**

Chapter 2.2



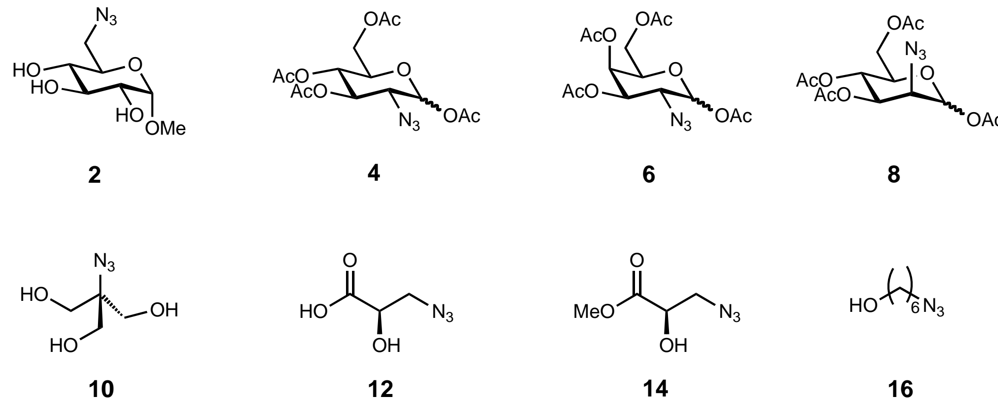
6a **6b**

6c **6d**

6e **6f**

25 **6g**

Chapter 2.2b



Chapter 2.3

26 27 28 29

30 31 32 33

34 35 36

37 38 41

42 43

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53

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55

56

Chapter 2.4

1c **6**

2c **7**

4 **8**

9 **11** **12** **13**

15 **16**

20

21

22

18

5. Curriculum Vitae

Alexander Titz

Place of birth	Erlenbach am Main, Germany
Nationality	German

Education

03.04.2008	PhD, *summa cum laude*.
10.2004 - 01.2008	PhD thesis, University of Basel, Basel, Switzerland.
01.03.2004	Graduation, Degree: Diplom-Ingenieur Chemie (Darmstadt Technical University, Darmstadt, Germany), Grade 1.1.
10.2003 - 02.2004	Diploma thesis, Novartis Pharma AG, Basel, Switzerland, Grade 1.0.
10.2001 - 07.2003	Main studies in chemistry, Darmstadt Technical University, Darmstadt, Germany.
10.2000 - 08.2001	Extracurricular training, University of Bordeaux I, Bordeaux, France.
10.1998 - 09.2000	Basic studies in chemistry, Darmstadt Technical University, Darmstadt, Germany.

Professional Experience

Since 01.02.2008	Postdoctoral research in glycobiology with Prof. M. Aebi, Insitute of Microbiology, ETH Zurich, Zurich, Switzerland.
10.2004 - 01.2008	PhD thesis under guidance of Prof. Dr. B. Ernst, Institute of Molecular Pharmacy, University of Basel, Basel, Switzerland: “E-selectin and the natural tetrasaccharide ligand sialyl Lewis^{x}: the importance of pharmacophore pre-organization in glycomimetics”.
08.2004 - 09.2004	Setup of an organic chemistry lab and synthesis of a fluorescent dye, Prof. Dr. H.-U. Göringer, Institute of Genetics, Darmstadt Technical University, Darmstadt, Germany.
09.2003 - 02.2004	Diploma thesis at Novartis Pharma AG, Basel, Switzerland under guidance of Dr. M.J.J. Blommers and Prof. Dr. B. Schmidt: “Synthesis

	and application of paramagnetic tools for structure elucidation by biomolecular NMR".
09.2002 - 10.2002	Traineeship under guidance of Prof. Dr. B. Schmidt, Clemens-Schöpf Institute, Darmstadt Technical University, Darmstadt, Germany: "Synthesis of Cu-(II)-spinlabeled SH2 domain phosphotyrosine kinase inhibitors".
10.2000 - 08.2001	Extracurricular traineeship under guidance of Dr. D. Deffieux and Prof. Dr. S. Quideau, Natural Products Laboratory, University of Bordeaux I, Bordeaux France: "An approach to electrochemical preparation of orthoquinone monoketals and synthesis of their precursors".

Teaching Experience

Since 02.2008	Instructor for numerous students in microbiology practical course, ETH Zurich.
10.2007 - 01.2008	Instruction and guidance of a diploma student in medicinal chemistry, University of Basel.
2005 - 2007	Instructor for numerous 3rd year pharmacy students in solid-phase peptide synthesis, University of Basel.
05.2005 - 09.2005	Instruction and guidance of a diploma student in medicinal chemistry, University of Basel.
01.2005 - 05.2005	Instructor for 30 3rd year pharmacy students in basic molecular biology techniques, University of Basel.
04.2002 - 07.2002	Instructor to 30 2nd year chemistry students in organic chemistry practical course, DaMocles project, Prof. Dr. W.-D. Fessner, Darmstadt Technical University.

Awards/Fellowships

03.04.2008	PhD, *summa cum laude*.
12.2007	Postdoctoral research grant (1 year), Roche Research Foundation, Basel, CH.
08.2007	"Novartis Biotechnology Leadership Camp", Seminar for 40 young european scientists organized and funded by Novartis Pharma AG, Basel, Switzerland.
10.2006	SCS Award for the best poster presentation in medicinal chemistry, Fall Meeting of the Swiss Chemical Society, Zurich, Switzerland.
12.2004	"Marketing für Naturwissenschaftler", Schloss Montabaur, Germany, Seminar for 20 young german scientists organized and funded by Roche Diagnostics GmbH, Mannheim, Germany.
10.2000 - 08.2001	ERASMUS fellowship of the European Union.

Publications

- Titz, A.; Papandreou, G.; Cutting, B.; Wagner, B.; Dondoni, A.; Marra, A.; Schwardt, O.; Magnani, J.; Ernst, B. Lipophilic interactions between the fucose α-face and galactose β-face: Nature stabilizes the internal conformation of the core of sialyl Lewis^x in the bioactive conformation. *Angew. Chem., Int. Ed.* ***in preparation***.

- Titz, A.; Patton, J.; Radic, Z.; Schwardt, O.; Magnani, J.; Ernst, B. Probing the carbohydrate recognition domain of E-selectin by a click-chemistry approach: the importance of the acid orientation in sLe^x mimetics for binding *Bioorg. Med. Chem.* ***in preparation***.

- Blommers, M.J.J.; Titz, A. *et al.* Paramagnetic spinlabeled E2 protein guided binding site identification and docking of the ligand by paramagnetic NMR restraints *J. Am. Chem. Soc.* ***in preparation***.

- Titz, A.; Patton, J.; Alker, A.; Porro, M.; Schwardt, O.; Hennig, M.; Francotte, E.; Magnani, J.; Ernst, B. Is adamantane a suitable substituent to pre-organize the acid orientation in E-selectin antagonists? *Bioorg. Med. Chem.* ***2008***, *16*, 1046.

- Titz, A.; Ernst, B. Mimetics of Sialyl Lewis^x: The Pre-Organization of the Carboxylic Acid is Essential for Binding to Selectins. *CHIMIA* **2007**, *61*, 194.

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- Titz, A.; Radic, Z.; Schwardt, O.; Ernst, B. A safe and convenient method for the preparation of triflyl azide, and its use in diazo transfer reactions to primary amines *Tetrahedron Lett.* **2006**, *47*, 2383.

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- Schmidt, B.; Jiricek, J.; Titz, A.; Ye, G.; Parang, K. Copper dipicolinates as peptidomimetic ligands for the src SH2 domain *Bioorg. Med. Chem. Lett.* **2004**, *14*, 4203.

Conference contributions

Oral presentation

- Titz, A.; Wagner, B.; Ernst, B. The pre-organization of the trisaccharide core of sialyl Lewis^x is essential for binding to E-selectin*. Fall Meeting of the Swiss Chemical Society* September 12th **2007**, Lausanne, Switzerland.

- Titz, A.; Wagner, B.; Ernst, B. The pre-organization of the trisaccharide core of sialyl Lewis^x is essential for binding to E-selectin. OP35, *13th*

European Carbohydrate Symposium August **2005**, Bratislava, Slovak Republic.

Posters

- Titz, A.; Alker, A.; Porro, M.; Schwardt, O.; Hennig, M.; Francotte, E.; Ernst, B. The pre-organization of the carboxylic acid of sialyl Lewis^x mimetics is essential for binding to E-selectin. *BioValley Science Day* October 23rd **2007**, Basel, Switzerland.

- Titz, A.; Patton, J.; Radic, Z.; Magnani, J.; Ernst, B. The pre-organization of the carboxylic acid of sialyl Lewis^x mimetics is essential for binding to E-selectin (Part II). *14th European Carbohydrate Symposium* September **2007** Lübeck, Germany.

- Titz, A.; Alker, A.; Porro, M.; Schwardt, O.; Hennig, M.; Francotte, E.; Ernst, B. Pre-organization of the carboxylic acid of sialyl Lewis^x mimetics is essential for binding to E-selectin. *Benzon Symposium No.54: Glycosylation-Opportunities in Drug Development* June **2007**, Copenhagen, Denmark.

- Titz, A.; Alker, A.; Hennig, M.; Francotte, E.; Ernst, B. The pre-organization of the carboxylic acid of sialyl Lewis^x mimetics is essential for binding to E-selectin. *Fall Meeting of the Swiss Chemical Society* October 13th **2006**, Zurich, Switzerland.

- Deffieux, D.; Farbre, I.; Titz, A.; Quideau, S. Electrochemically induced spirolactonization of 2-methoxyphenoxy-carboxylic acids into quinone ketals*. 221st ACS National Meeting Spring* **2001**, San Diego, USA.

Basel, 03.04.2008

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