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Lectin Antagonists in Infection, Immunity, and Inflammation

Joscha Meiers[1,2,3#], *Eike Siebs*[1,2,3#], *Eva Zahorska*[1,2,3#], *Alexander Titz*[1,2,3#*]

[1]Chemical Biology of Carbohydrates, Helmholtz Institute for Pharmaceutical Research Saarland (HIPS), Helmholtz Centre for Infection Research, D-66123 Saarbrücken, Germany
[2]Deutsches Zentrum für Infektionsforschung (DZIF), Standort Hannover-Braunschweig, Germany
[3]Department of Pharmacy, Saarland University, D-66123 Saarbrücken, Germany

# these authors contributed equally

* Corresponding author
Chemical Biology of Carbohydrates, Helmholtz Institute for Pharmaceutical Research Saarland
D-66123 Saarbrücken
Tel. +49 681 98806 2500
email: alexander.titz@helmholtz-hzi.de

Abstract

Lectins are proteins found in all domains of life with a plethora of biological functions, especially in the infection process, immune response, and inflammation. Targeting these carbohydrate-binding proteins is challenged by the fact that usually low affinity interactions between lectin and glycoconjugate are observed. Nature often circumvents this process through multivalent display of ligand and lectin. Consequently, the vast majority of synthetic antagonists are multivalently displayed native carbohydrates. At the cost of disadvantageous pharmacokinetic properties and possibly a reduced selectivity for the target lectin, the molecules usually possess very high affinities to the respective lectin through ligand epitope avidity. Recent developments include the advent of glycomimetic or allosteric small molecule inhibitors for this important protein class and their use in chemical biology and drug research. This evolution has culminated in the transition of the small molecule GMI-1070 into clinical phase III. In this opinion article, an overview of the most important developments of lectin antagonists in the last two decades with a focus on the last five years is given.

Introduction

Lectins are a highly diverse family of proteins found in all domains of life.[1,2] Various folds and classes have been identified and the common functional feature is their specificity for carbohydrate ligands. These glycan-binding proteins have many important roles in infection, cell recognition, communication and various intracellular processes, such as protein folding and protein targeting.

Numerous viral, bacterial, fungal, and parasitic pathogens employ lectins for initiation and maintenance of an infection by adhering to surface-exposed glycoconjugates of their host organisms.[3–5] On the other hand, the mammalian host has developed a plethora of lectin-containing pattern recognition receptors of the innate immune system recognising glycan structures on intruders.[6–8] In addition to recognising these non-self structures, other mammalian lectins bind to self-epitopes and thus mediate cell-recognition processes like inflammation and cancer metastasis.[9–11]

The natural ligands of lectins are mostly bacterial or fungal polysaccharides, bacterial lipopolysaccharide and peptidoglycan, or eukaryotic glycoconjugates of lipids or proteins.[1,12] Except for bacteria which can have a high diversity among their monosaccharides, generally a relatively small set of different monosaccharide subunits are shared between animals, plants, fungi, parasites, bacteria, and other organisms. These building blocks are assembled into more diverse oligosaccharides where a very high complexity can be achieved due to many possible stereo- and regioisomers. In many cases, this leads to organism-specific oligosaccharides, which can then be recognized by innate immunity as

non-self antigens and induce neutralization of the intruder[13], or on the other hand to allergic reactions as observed for insect glycans for example in bee venom.[14] The opposite phenomenon that pathogen and host have identical glycoconjugates is also observed. The latter has been termed molecular mimickry or glycomimickry, a stealth process of the pathogen believed to be an evolutionary adaptation for evasion of immune surveillance of the host.[15,16]

Despite the complexity of those oligosaccharide structures, lectins often recognize terminal monosaccharides or smaller oligosaccharides on a given glycoconjugate. Two common binding modes of carbohydrate ligands are shown in Figure 1A: (i) vicinal hydroxyl groups chelate a Ca^{2+} ion present in the binding site, or (ii) carbon-bond hydrogen atoms of the carbohydrate ring interact via CH-π stacking with aromatic amino acids in the binding site. Due to the recognition of rather small epitopes, common ligand specificity of different lectins with diverse functional roles often occurs. An example are the functionally different human DC-SIGN and the bacterial lectin LecB with shared specificity for Lewis blood group antigens.[17–19] A large data set for the glycan specificity of many lectins using microarrays is provided by the Consortium for Functional Glycomics (see http://www.functionalglycomics.org).

Specificity of the lectins can be further tuned by recognising functional groups attached to the essential carbohydrate, and for example lipids are recognised by a secondary site of the lectin Mincle,[20,21] *O*-methylation is required for recognition by the tectonins,[22,23] sulfates on nearby amino acids enhance binding of P-selectin to the Lewis-blood groups on glycoproteins[24] and phosphates are required for intracellular trafficking of proteins by the mannose-6-phosphate receptor.[25]

Lastly, the spatial presentation of ligand and/or lectin's carbohydrate binding sites (Figure 1B), as well as clustering of several lectin protomers into oligomeric bundles or membrane embedded protein complexes can contribute significantly to specificity by augmentation of apparent binding affinity through avidity.[7,26]

Carbohydrate specificity, requirements of additional functional groups and spatial presentation of binding sites are important aspects for the design and success of lectin-targeting probes in chemical biology and drug research. Therefore, the design of lectin antagonists usually follows various approaches from (i) competitive inhibition of a carbohydrate recognition site, (ii) targeting adjacent binding sites, (iii) allosteric inhibition, and (iv) multivalent competitive inhibition of two or more binding sites (Figure 1C).

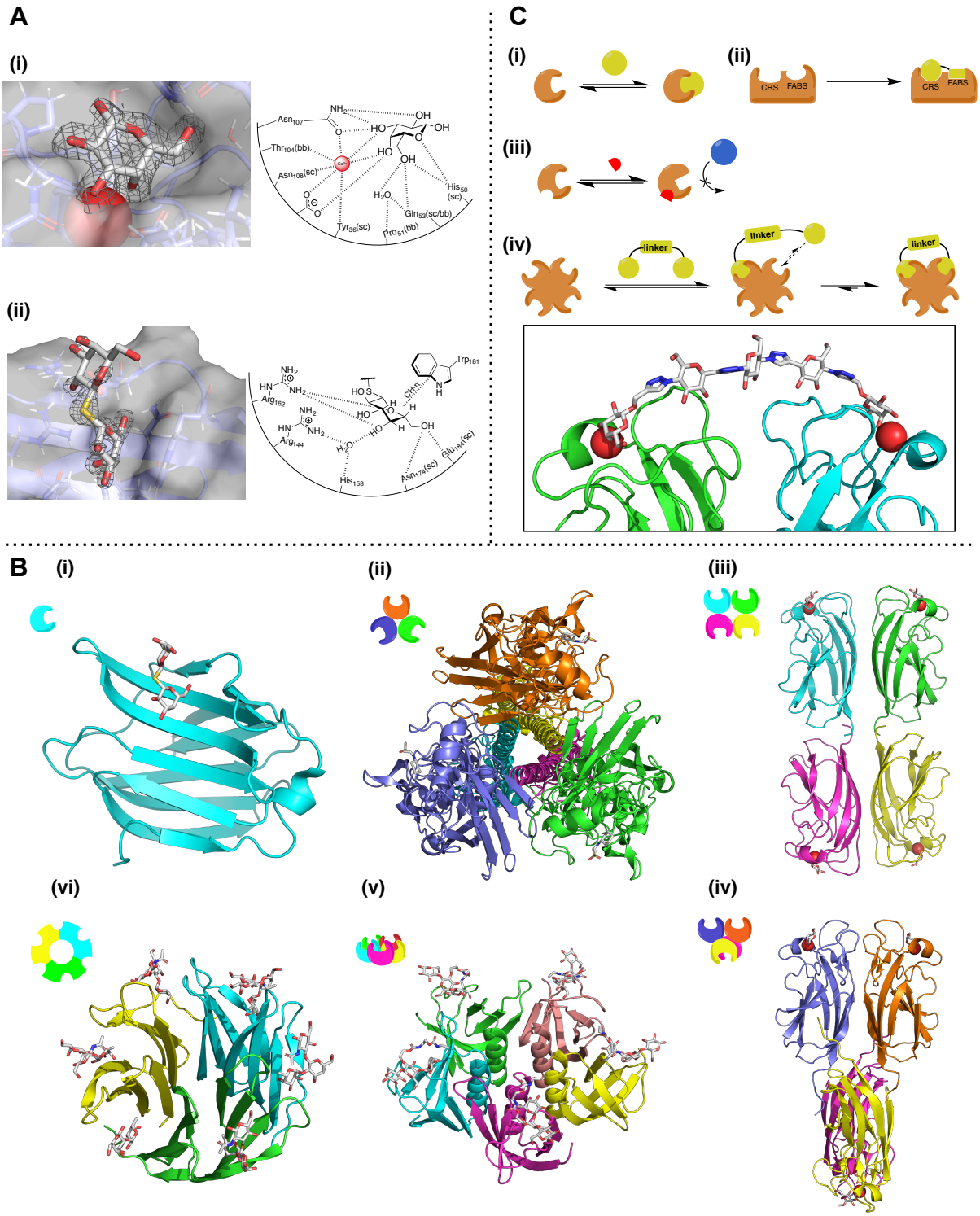

Figure 1: A: Schematic representation of two important recognition modes of carbohydrates by lectins: (i) calcium-ion mediated binding of the ligands, example β-galactoside and LecA (PDB: 1OKO) (ii) tryptophan-mediated stacking on hydrophobic faces of carbohydrates, example galactoside with galectin-3 (PDB: 4JC1). **B:** Various strategies for domain/binding site orientation: (i) monomeric in galectin-3 (4JC1), (ii) trimeric virus hemagglutinin (6CF5), (iii) tetrameric LecA (1OKO), (iv) tetrameric LecA ortholog PllA with altered domain orientation (5ODU), (v) pentameric Shiga-like toxin B subunit (1QNU), (vi) trimeric BambL containing 6 carbohydrate binding sites in and between subunits

(3ZW2). **C:** Schematic representation of different lectin inhibition approaches: (i) direct inhibition of carbohydrate binding sites, (ii) growing towards non-carbohydrate binding sites, (iii) allosteric inhibition (iv) multivalent inhibition which refers to clustered binding sites, either multivalent proteins or monovalent lectins clustering on cell membranes.

Consequently, lectins have developed into attractive targets for chemical biology and medicinal chemistry over the past two decades.[27,28] Very active areas of research are the targeting of (i) lectins of pathogenic origin to interfere with mechanisms of infection by viruses and bacteria, and to a smaller extent also fungi and parasites, (ii) the selectins as a family of three closely related proteins crucial for cell migration in inflammation and cancer, as well as (iii) immunotherapeutic or immunomodulatory approaches for the mammalian lectins langerin in vaccine delivery, DC-SIGN in HIV infection or the galectins in cancer and immune modulation. Lectins discussed in this opinion article are summarized in Table 1.

Table 1: Overview of bacterial, viral, and mammalian lectins discussed in this opinion article

	Origin	Binding specificity	Key roles	Status of development/ Indicator
Bacterial Lectins				
FimH	*E. coli*	Man	Adhesion, biofilm formation	Lead optimization (**1**, **2**)[29,30], EB8018 in Phase 1 clinical trials (www.clinicaltrials.gov, NCT03709628)
FmlH	*E. coli*	Gal, GalNAc	Adhesion, biofilm formation	Hit optimization (**3**)[31]
LecA	*P. aeruginosa*	Gal	Adhesion, biofilm formation	Exploratory studies First covalent lectin inhibitor (**5**)[32]
LecB	*P. aeruginosa*	Man, Fuc	Adhesion, biofilm formation	Lead optimization (**6**, **7**)[33,34]
Shiga toxins	*S. dysenteriae*, *E. coli*	Gal, Glc	Toxin	Lead optimization on hold, First peptide-based inhibitor [35]
Cholera toxin	*V. cholerae*	Gal, Fuc	Toxin	Hit optimization (**8**)[36]
Viral Lectins				
Hemagglutinin	Human influenza virus	Neu5Ac	Adhesion, cell entry	Hit optimization (**12**) [37–39] and exploratory studies (**10**, **11**) [40–42]
Hemagglutinin-neuraminidase	Human parainfluenza virus	Neu5Ac	Adhesion and detachment, cell entry	Hit optimization [43,44]
Capsid protein P domain	Norovirus	HBGAs	Adhesion, cell entry	Exploratory studies (**14**, citric acid) [45–47]
Mammalian Lectins				
Langerin	Langerhans cells	Man, Fuc, GlcNAc, sulfated Gal, Glc	Immune response	Exploratory studies First allosteric mammalian lectin inhibitor (**15**)[48]
DC-SIGN	Dendritic cells	Man, Fuc, GlcNAc	Immune response	Exploratory studies
Selectins	L-Selectins: leukocytes P-selectin: platelets and endothelial cells E-Selectins: endothelial cells	sLe^x E/P-selectins: Fuc, GlcNAc P/L-selectins: Man, Gal and Sulfation[49]	Cell adhesion	GMI-1070 (**20**) in Phase 3 clinical trials against vaso-occlusive anemia (www.clinicaltrials.gov, NCT02187003)
Mincle	Immune system	Glycolipids with terminal Glc or Man	Immune response	Exploratory studies

Galectin	Circulating proteins	Gal *e.g.*, N-acetyllactosamine	Regulate cell death	TD139 (**24**) in Phase 2 clinical trials against idiopathic pulmonary fibrosis (www.clinicaltrials.gov, NCT03832946)
Siglecs	Immune-cells	Neu5Ac	Cell-cell signaling, immune response and adhesion	Exploratory studies

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Bacterial Lectin Antagonists

Bacterial antibiotic resistance is increasing worldwide at an alarming rate. As one consequence, antivirulence drugs have gained considerable research interest as alternative treatment approach with the aim to avoid the rapid onset of resistance.[50] In this context, the inhibition of bacterial lectins to prevent infection and persistence is a newly exploited strategy.[3,27] Targeting lectins involved in the formation of bacterial biofilms are of particular interest since bacteria embedded in their self-produced biofilm matrix exhibit increased antimicrobial resistance compared to free floating planktonic bacteria. Biofilm-associated bacterial infections are responsible for a broad range of chronic/recurring diseases. [51]

The Gram-negative bacterium *Escherichia coli* is the prime pathogen in urinary tract infections (UTIs) and important for intestinal infections as a consequence of Crohn's disease (CD). *E. coli* can build various organelles called pili and fimbriae which are oligomeric cell appendices built up of several proteins. These organelles are often employed for bacterial adhesion. The pilus or fimbria lectins FimH and FmlH, localized on the top of the different organelles, play decisive roles in host colonization, invasion, and biofilm formation.[52] Thus, inhibition of these lectins to antagonize infections presents a viable therapeutic strategy.[53,54]

FimH is located on the tip of fimbriae and usually binds to mannosylated glycoconjugates in the bladder endothelium. Pathogenicity of *E. coli* clinical isolates expressing different *fimH* alleles varies, but the mannose binding pocket is invariant.[52,55,56] Hultgren's group demonstrated the activity of high affinity mannoside FimH inhibitor against different uropathogenic *E. coli* strains.[57] In recent years, several research groups have been developing FimH antagonists for treatment of urinary tract infections and gut inflammations associated with CD. X-ray crystallography guided drug design focused on optimization of interactions with the so-called tyrosine gate adjacent to the mannose binding site. Introduction of aryl and alkyl aglycons increased the binding affinity significantly compared to simple mannose.[58–60] Nanomolar binding affinities were achieved by introducing biaryl aglycons that are tightly coordinated by the tyrosine gate.[61–63] High affinity biaryl mannosides were further optimized to increase metabolic stability by replacing the labile O-glycosidic bond with carbon-based linkers to the aglycon.[29,64] Ester and phosphorylated prodrugs were successfully explored to improve oral bioavailability of both O- and C-mannosides.[29,65,66] Rational design and optimization of FimH antagonists are summarized in a recent review by Mydock-McGrane *et al*..[67] The promising preclinical candidate **1** (EC_{50} = 31 nM, Figure 2) is one example of a highly optimized FimH inhibitors with good metabolic stability and high efficacy in mouse models of acute and chronic UTI.[29] Recent optimization attempts yielded thiomannosides (*e.g.* **2**, EC_{50} = 0.31 μM, Figure 2) with improved metabolic stability compared to respective O-mannosides, ability to inhibit biofilm formation *in vitro*

and with a prophylactic effect in a mouse UTI model.[30] The first FimH antagonist entering clinical trials was EB8018 from Enterome (Paris, France) designed for the treatment of CD, but its structure has not been disclosed. In collaboration with Takeda, EB8018 has completed Phase Ia and the Phase Ib trial is ongoing in early 2019 (www.clinicaltrials.gov, NCT03709628). Furthermore, Fimbrion Therapeutics (St. Louis, MO) has announced the selection of a not further specified clinical candidate as antibiotic sparring molecule against UTIs in collaboration with GSK (www.fimbrion.com, press release Dec 06, 2018).

As a secondary target of uropathogenic *E. coli*, the FimH-like adhesin FmlH recognizes Gal(β1-3)GalNAc epitopes on bladder epithelium and enhances *E. coli* urinary tract colonization.[54] Recently, first structure-based inhibitor design approaches FmlH have been reported.[31,68] To date, the best FmlH inhibitor **3** (Figure 2) is based on N-acetyl galactosamine carrying a further substituted biphenyl aglycon and displays very high binding affinity (IC_{50} = 34 nM), good aqueous solubility and high metabolic stability. Unfortunately, **3** showed only low oral bioavailability in rats of less than 1% and further optimization is therefore mandatory.[31,68]

The opportunistic pathogen *Pseudomonas aeruginosa* has two soluble lectins, the extracellularly secreted proteins LecA (Figure 1) and LecB, both mediating bacterial virulence and being crucial components for biofilm formation.[69–71] Consequently, both proteins have been subject to intense research towards biofilm modulators and in drug discovery for antivirulence drugs.[27,28,72–74] LecA binds to various α-galactoside-terminating glycoconjugates with the glycosphingolipid Gb3 as proposed natural ligand.[75] This homotetrameric lectin was later shown to mediate bacterial uptake via Gb3 where it acts as a lipid zipper.[76,77] The affinity of LecA to galactose and simple glycosides thereof is rather weak in the 50-100 μM range. Consequently, development of LecA antagonists mainly focused on multivalent display of galactosides using many different linkers and maximizing the number of presented epitopes.[28,78] Very potent tetravalent galactoclusters with low nanomolar binding affinities towards LecA have been developed.[79–83] In contrast to the high target binding affinity, they showed only moderate inhibition of biofilm growth in the micromolar range *in vitro*.

The Pieters group has undertaken a different approach and focused on divalent galactosides oriented in a perfect manner to bridge two adjacent binding sites in the LecA tetramer. Several highly potent divalent inhibitors with the rigid spacers consisting of glucose and triazole groups were obtained, including the most potent LecA inhibitor reported so far with a K_d of 12 nM (**4**, Figure 2).[84,85] Again, recent optimization of these highly potent molecules on the target revealed a need for additional multimerization and rather high micromolar concentrations for biofilm blocking.[82,86]

Monovalent galactose-derived ligands with binding affinities in low micromolar range could be obtained after introduction of a β-aryl aglycon which establishes a π-stacking interaction with an imidazole-CH of His50 adjacent to the carbohydrate binding site (Figure 1A).[87–89] However, the specificity for further variations appears relaxed and changing substituents at the phenyl aglycon did not lead to significant potency improvements. As an alternative approach to the generally employed glycosides of unmodified galactose residues in LecA ligands, we have embarked on the modification of the galactose residue itself. A cysteine residue in the carbohydrate binding site of LecA was targeted with the aim to develop a covalent lectin inhibitor using a small electrophilic headgroup in a modified galactose.[32] Despite the fact that covalent inhibitors are widespread for many other protein classes, epoxide **5** (Figure 2) was established as the first-in-class covalent lectin inhibitor. Due to its moderate affinity towards LecA (IC_{50} = 64 μM), the molecule was converted into a tool compound after synthetic derivatization and conjugation to fluorescein enabling the visualization of *P. aeruginosa* biofilm aggregates by confocal fluorescence microscopy.[32]

The second *P. aeruginosa* lectin LecB also forms a homotetrameric quarternary structure, binds broadly to fucosides and mannosides and the highest affinity was determined for Lewis blood group antigens.[17,90] In contrast to LecA, the protein sequence of LecB varies among clinical isolates and two important types occurring in the clinical isolates PAO1 and PA14 have been identified as representative for all studied isolates.[18,91] Despite the observed amino acid sequence differences in LecB between strains, its carbohydrate binding specificity is conserved, underpinning the suitability of LecB as a drug target with conserved specificity among all isolates. Also for LecB, multivalent inhibitors have been the first choice for inhibition.[28,78] However, due to a sterically more distant and less favorable orientation of binding sites in LecB compared to LecA, the obtained multivalent ligands could not achieve a comparable boost in affinity. Nevertheless, two types of multivalent ligands carrying fucosides stand out of the very broad field: tetravalent glycopeptide dendrimer **6** (IC_{50} = 140 nM, Figure 2) was able to efficiently prevent biofilm formation of *P. aeruginosa* at a concentration of 20 μM *in vitro*;[33] furthermore, a calixarene carrying four fucose residues was tested in an infection model in mice.[79] This compound significantly reduced the number of bacteria colonizing lung and spleen, but was unable to inhibit bacterial biofilms *in vitro* at a concentration of 100 μM despite its high affinity at the target (K_d = 48 nM).

To overcome the intrinsic disadvantages associated with large molecules and multidirectional valency in biofilm formation, we have used the small molecule LecB ligand mannose as a starting point for the rational design of monovalent biofilm targeting glycomimetics.[92] These compounds exhibited rather good target binding potency (K_d = 3 - 20 μM) and prevented bacterial adhesion to a glycosylated surface at 100 μM. Further optimization[93] and removal of the anomeric center [94] finally yielded C-glycosidic inhibitors of LecB (*e.g.*, **7**, Figure 2) with good target binding potency (K_d = 290 nM) and

very long receptor residence times ($t_{1/2}$ = 28 min).[34] Glycomimetic **7** showed approx. 85% inhibition of biofilm growth *in vitro* at 100 μM, which contrasts the lack of antibiofilm activity of the natural LecB binder methyl α-L-fucoside, despite its very high target binding affinity (K_d = 430 nM). Furthermore, glycomimetic **7** is orally bioavailable which is not possible for large multivalent molecules.

Shiga and cholera toxins are bacterial proteins responsible for severe symptoms in gastrointestinal infections. These so-called AB_5 toxins consist of one catalytic A-subunit and five lectin-like B-subunits (Figure 1B) which are responsible for the binding of the complex to the host cell surface in the gut. Inhibition of the B-subunits and thereby preventing adhesion is a potential treatment strategy.[95]

Shiga toxins (Stxs) are produced by *Shigella dysenteriae* and some enteropathogenic *E. coli* strains, *e.g.* enterohemorrhagic *E. coli* (EHEC). Kitov et. al designed the pentavalent ligand STARFISH to match the carbohydrate binding sites of the five B-subunits with subnanomolar inhibitory activity against Shiga-like toxins I and II (Stx1 and Stx2).[96] A modified version of STARFISH, called DAISY, improved the *in vivo* activity and provided full protection against the toxins when administered simultaneously in a mouse model despite its lower target binding potency.[97] However, further development of DAISY-based inhibitors appears halted (no further publications) since the compound proved ineffective in a treatment scenario, i.e. drug administration after infecting mice with the Shiga toxin producing strain *E. coli* O91:H21. Nishikawa et al. designed a series of carbosilane dendrimers called SUPERTWIG. The most potent compound of the series was able to completely neutralize Stxs in the blood stream and protect mice against a fatal dose of the Shiga toxin producing strain *E. coli* O157:H7 even when administered after establishment of infection.[98] The rather complex synthesis of multivalent-trisaccharide inhibitors is hindering further clinical development.

From a peptide library, the branched proline and arginine rich high molecular weight peptide Ac-PPP-tet was identified to bind to Stx2 B-subunit and inhibit Stx2 cytotoxicity.[35] This peptide affects the intracellular transport of Stx2 and protected mice from a fatal dose of *E. coli* O157:H7 even when administered after an established infection; this molecule further protected rabbit intestines *ex vivo* against the toxic effect of Stx2.[35,99] Recent efforts include the synthesis of sugar-amino acid hybrid polymers with highly clustered globotriaosyl residues that showed low micromolar affinities to both Stxs with the ability to neutralize the toxic effects on Vero cells.[100]

Vibrio cholerae produces cholera toxin where each B-subunit (CTB) has two binding sites – one primary binding site recognized by the ganglioside GM_1 and a secondary low affinity site recognized by fucosylated glycans.[101] A number of derivatives mimicking the terminal galactose from GM_1 has been screened and m-nitrophenyl α-D-galactoside and 3,5-disubstituted phenylgalactosides were identified as monovalent CTB inhibitors.[102,103] Numerous multivalent inhibitors targeting the primary site

with down to picomolar binding affinities (*e.g.* **8**, IC_{50} = 34 pM, Figure 2)[36] have been developed and were summarized in a recent review by Kumar and Turnbull.[104] Targeting the fucose binding site as new strategy was reported by Kohler and co-workers who reported inhibition of CTB binding to cell surfaces with 2'-fucosyllactose and a fucosylated polymer.[105]

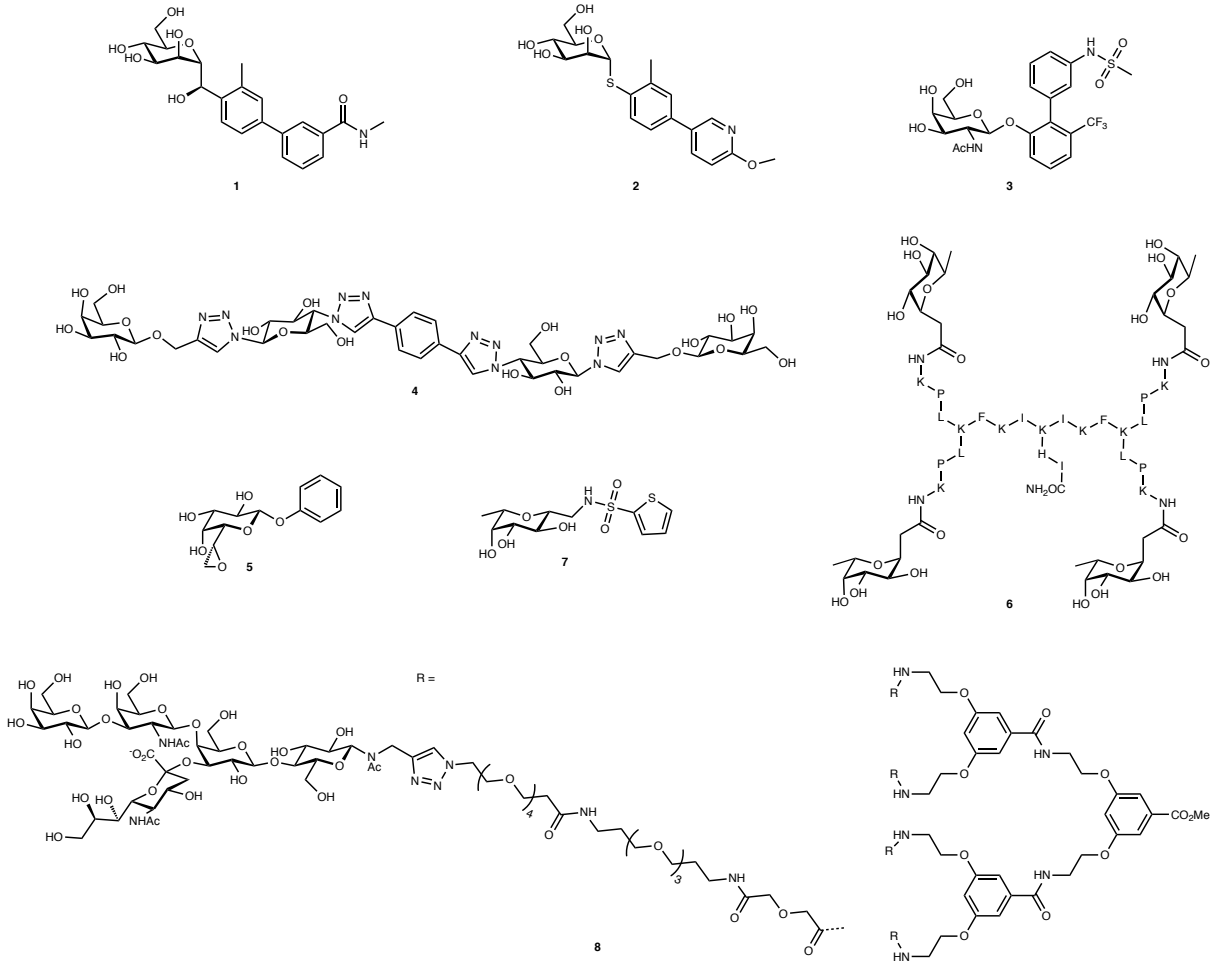

Figure 2: Inhibitors targeting lectins of pathogenic bacteria in *E. coli* (**1**-**3**), *P. aeruginosa* (**4**-**7**), and toxins of *V. cholerae* (**8**).

Viral Lectin Inhibitors

Viral infections are difficult to treat, control and prevent. Frequent antigen variation, for which the influenza virus is a perfect example, prevents efficient protection and virus clearance by the human immune system. In many viruses, lectin-carbohydrate interactions are crucial for an efficient infection of the host. Hemagglutinin is the sialic acid binding lectin on the surface of the influenza viral envelope and plays a key role in the host cell-virus interaction. Sialic acids are defined as a family of acidic sugars with a nine carbon atom backbone and the most abundant member found in vertebrates is *N*-

acetylneuraminic acid (**9**, Neu5Ac, Figure 3).[106] Because the binding interaction of one monomeric hemagglutinin to sialylated glycans is weak (K_d > 1 mM)[107], trimerization of hemagglutinin on the viral envelope and a high sialic acid density on the host cell lead to an increased avidity. This binding event then triggers the internalization of the virus by endocytosis.[108] Therefore, inhibition of the hemagglutinin-sialic acid interaction could yield prophylactic as well as therapeutic treatments of an influenza virus infection.

For this purpose, Strauch et al.[42] developed a trimeric influenza neutralizing protein, targeting the hemagglutinin receptor binding site. This protein was designed to mimic the key interactions of broadly neutralizing antibodies and its optimization led to a highly avid protein with a trimeric binding mode and nanomolar apparent K_d values. *In vivo*, using an H3 HK68 influenza infection mouse model, prophylactic and therapeutic treatment significantly protected mice from establishing disease and weight loss. Unfortunately, this designed protein does not show broad spectrum activity since it does not bind to the pathogenic 'bird flu' subtype H5N1. Limitations in high scale production and price, together with challenging pharmacokinetic properties will impact on its commercial use as an anti-influenza drug.

A recent review by Li, Ma and Wang describes a wide range of chemical scaffolds and strategies to inhibit the hemagglutinin - host cell interaction. Mostly, trimeric sialosides are presented as binders to the receptor binding site.[109]

2,3-Sialyllactose (2,3-SL) conjugated to three way junction (3WJ) DNA, with each DNA strand presenting one, three or five 2,3-SL molecules complementary to the hemagglutinin trimer geometry was reported by Yamabe et al..[40,41] Hemagglutinin inhibition revealed 3WJ DNA with three sialic acid residues per arm in compound **10** as best inhibitor with a K_i = 0.25 μM, which corresponds to an 80'000-fold increase compared to monomeric 2,3-SL and an 8-fold increase compared to 3WJ DNA with only one sialic acid per strand. Surprisingly, 3WJ DNA presenting 5 sialic acid per strand led to a reduction in activity (K_i^{HAI} > 4.0 μM) which probably originates from an altered orientation of the carbohydrate epitopes induced by steric hindrance. In contrast to the neuraminidase labile *O*-linked **10**, the more stable thio-linked sialic acid derivative **11** was synthesized as a follow up. For **11,** an increased stability towards influenza neuraminidase present on the viral envelope was observed, while its activity was retained. However, in presence of the full virus both derivatives, i.e. *O*- and *S*-glycoside, were stable under the conditions tested. Another approach using a macromolecular scaffold by Nagao et al. yielded a trimeric star-shaped glycopolymer presenting 6'-sialyllactose on each of the three arms, synthesized by reversible addition-fragmentation chain transfer polymerization.[110] The degree of polymerization dictated the length of each arm. Hemagglutinin inhibition clearly depended on the arm-length, resulting in a K_i = 21 μM for their best glycopolymer.

Conjugation of sialic acid or ascorbic acid derivatives onto pentacyclic triterpenes by Zhou and co-workers[37,38] was inspired by the broad antiviral activity of *Dipsacus asperoides* triterpenes and the corresponding synthetic leads.[39] In both cases, conjugation to betulinic acid as in **12** led to a strong reduction of infection by influenza A/WSM/33 in MDCK cells. Cytotoxicity of the triterpenes was also reduced by conjugation to sialic acid or ascorbic acid and a hemagglutination assay and SPR experiments with immobilized hemagglutinin suggested hemagglutinin as the putative target (K_d = 17 μM for the sialic acid conjugate, K_d = 8.0 μM for the ascorbic acid conjugate). Interestingly, the synthetic 2,3-di-*O*-benzyl ascorbic acid intermediate showed a higher affinity for hemagglutinin (K_d = 3.78 μM) and improved inhibition of viral plaque formation (IC_{50}'s of 8.7 μM vs. 41.3 μM).

Small molecules possess superior pharmacokinetic properties for drug development than the rather large structures described above. Kadam and Wilson[111] identified the common buffer molecule CHES (**13)** by X-ray crystallography in complex with hemagglutinin. The molecule's binding mode with hemagglutinin mimics the one of sialic acid and its sulfonic acid superimposes with the carboxylate of sialic acid in the complex. Furthermore, the cyclohexyl moiety of CHES forms a CH-π interaction with W153 of hemagglutinin which is normally established by the *N*-acetyl group of sialic acid. As binding of CHES, although in slightly different binding modes, was confirmed for H3- and H5-hemagglutinin, Kadam and Wilson proposed this non-carbohydrate molecule as a starting point for fragment growing to overcome its very low affinity (K_d > 20 mM) in the discovery of new types of hemagglutinin inhibitors.

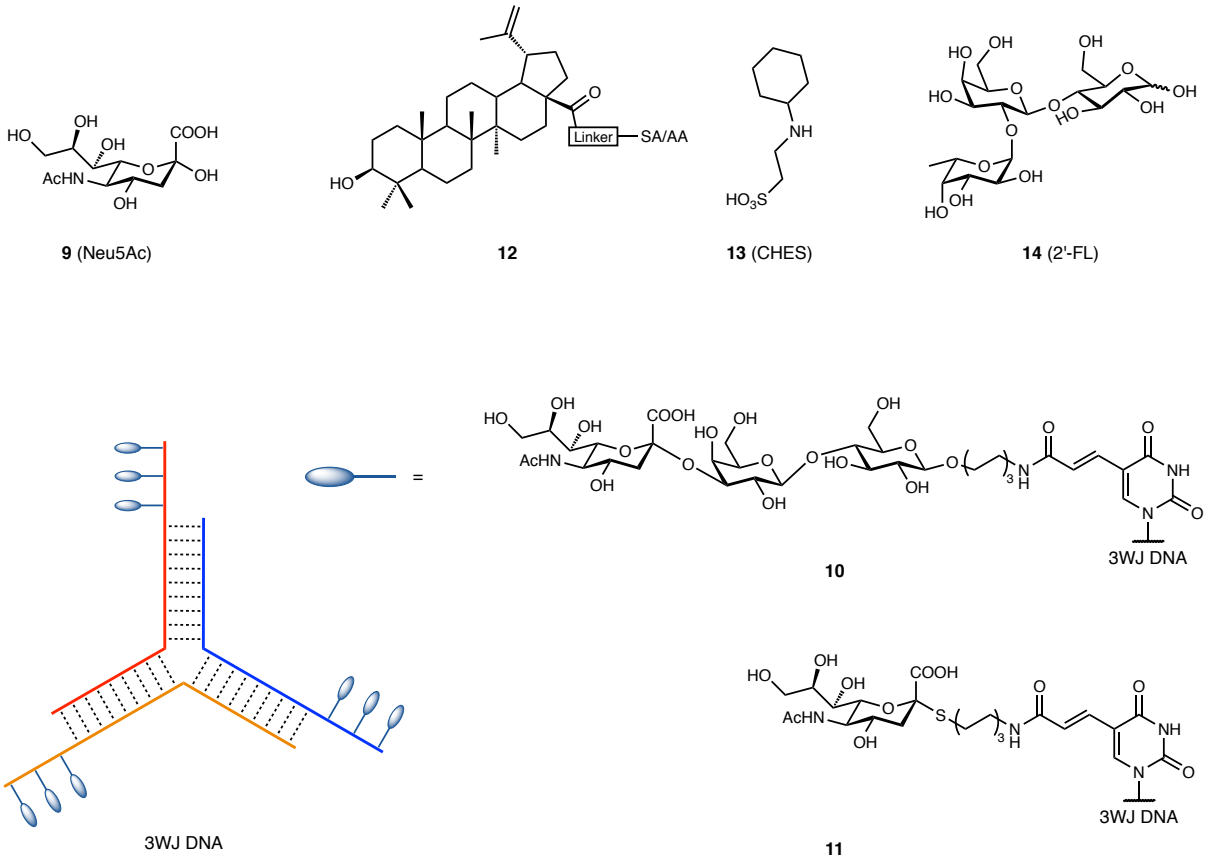

Figure 3: Inhibitors of influenza hemagglutinin: NeuNAc (9), macromolecular sialylated three way junctioned DNA **10** and **11 and** small molecules **12**-**13**; or. Norovirus spike protein can be blocked using the trisaccharide 2'-fucosyl lactose **14**.

The human parainfluenza virus causes respiratory tract diseases in children and elderly patients. In contrast to other influenza viruses, its multifunctional hemagglutinin-neuraminidase protein possesses both receptor-binding (hemagglutinin-function) and receptor-processing (neuraminidase-function) functionalities in one binding site.[112] Usually, lectins are defined as carbohydrate binding proteins without catalytic activity. However, this multi-functionality makes this parainfluenza virus protein an interesting topic for this review. Von Itzstein and co-workers synthesized a set of enzymatic intermediate-like *N*-acylated Neu-2-en and substrate-like *N*-acylated 2,3-difluoro-Neu derivatives to block both functionalities with a single molecule.[43,44] Especially the *N*-isobutyramido Neu-2-en derivatives showed potent hemagglutinin inhibition (IC_{50} = 1.15 μM) as well as inhibition of neuraminidase activity and virus growth.

Norovirus, a worldwide cause of mild to severe acute gastroenteritis, can lead to life-threatening infections for pediatric and geriatric patients and outbreaks, especially in day care centers or nursing homes, which are particularly problematic. To date, therapy of norovirus infections is only supportive

and limited to reversal of dehydration and loss of electrolytes.[113] Thus, to control and prevent outbreaks, new drugs are needed. The human norovirus capsid protein P domain interacts with human blood group antigens (HBGA) and plays an important role in infection.[114] This virus-host interaction can be blocked by human milk oligosaccharides such as 2'-fucosyl lactose (**14**, 2'-FL) as shown by Hansman and co-workers.[45,46] The very high concentrations of 2'-FL needed to inhibit the interaction of virus like particles with HBGA *in vitro* (IC_{50} = 13 - 50 mM), could be achieved because of the low toxicity of 2'-FL, its metabolic stability and low gastrointestinal absorption.[115] Indeed, 2'-FL is a major constituent of human milk with a concentration in the mM range and has been postulated to prevent infections in breast-fed newborns.[116] Another commonly used and safe food supplement, citrate, was shown to bind norovirus in a HBGA-like manner.[47]

Mammalian Lectin Antagonists

There are numerous mammalian lectins and the three important classes, siglecs, galectins and the C-type lectins, are currently addressed in chemical biology and medicinal chemistry. Sialic acid-binding immonoglubin-like lectins, siglecs, are cell-surface receptors, mainly expressed by cells of the immune system. They are involved in various processes ranging from self-/non-self discrimination to regulating inflammation caused by damage- or pathogen-associated molecular patterns (DAMP/PAMP).[117,118] Galectins, a family of soluble secreted lectins with 14 members, generally bind to β-galactosides.[119] Their functions are diverse and comprise mediation of cell-cell interactions, cell-matrix adhesion and transmembrane signaling.[120–122] C-type lectins are the largest and most diverse lectin family which share a conserved protein fold. The name giving Ca^{2+}-ion present in all carbohydrate recognizing family members directly mediates the binding to the glycan ligand.[7] Only a few examples exist for which Ca^{2+} is dispensable for carbohydrate recognition with dectin-1 being the most prominent example. The C-type lectin receptor family in mammals contains 17 members and many are part of innate immunity.[123,124]

Langerin, DC-SIGN

All cells of the innate immune system express a variety of pattern recognition receptors (PRR) such as toll-like receptors, NOD-like receptors and C-type lectin receptors, which allow the orchestration of an appropriate biological response to an incoming microbial threat. These PRRs are specialized to recognize PAMPs such as bacterial cell wall structures, fungal polysaccharides, the viral envelope and foreign RNA/DNA.[7,8] The signaling cascades initiated by these recognition events as well as the antigen uptake and processing pathways eventually lead to activation of cells of the adaptive immune system and hence are central elements bridging these two arms of immunity. For example, PAMPs

recognized and processed by dendritic cells can lead to differentiation of $CD4^+$-cells into T-helper cells.[123,126] Important C-type lectin receptors are langerin, DC-SIGN and dectin-1.[123]

The homotrimeric protein langerin is expressed on Langerhans cells in epithelial and mucosal tissues and binds to D-mannose, L-fucose, and D-GlcNAc as well as sulfated D-galactose. Langerin mediates the uptake of *Yersinia pestis* and influenza A virus amongst others in host infection.[7,8] Capitalizing on these carbohydrate-mediated antigen uptake and processing pathways, langerin has also been described as an attractive target for targeted drug-delivery approaches to Langerhans cells.[129,130] This raised the interest in specific langerin ligands and for example Rademacher *et al*. reported the discovery of thiazolopyrimidines as murine langerin antagonists, revealing the first allosteric inhibition of a mammalian lectin.[48] Optimization of the initial hit **15** (Figure 4) was found beneficial at position 6 and led to up to 10-fold lower K_d and IC_{50}-values (K_d (**15**) = 0.7 mM; IC_{50} = 0.6 mM). Overall, a large series of langerin inhibitors was presented with IC_{50} values ranging in the two digit micromolar range. Furthermore, it is well known that langerin has high affinity for sulfated poly- or large oligosaccharides, *e.g*. heparin (K_d = ~2.4 nM). As the binding affinity is electrostatically driven, no binding was detected with pH values below 4 or at high salt concentrations above 0.5 M.[131] A screening for langerin binding molecules revealed a sulfonamide of glucosamine as weakly binding langerin ligand.[132,133] Based on this screening hit the modified phospholipids **16** and **17** were synthesized with the aim to produce glycomimetic modified liposomes for langerin targeting. These were tested against $Langerin^+$, $DC\text{-}SIGN^+$ or $Dectin\text{-}1^+$ Raji cells. Liposomes consisting of mannosylated phospholipid **17** bound specifically to $DC\text{-}SIGN^+$ cells and those consisting of sulfonamide **16** specifically to $Langerin^+$ cells. Intracellular trafficking of the langerin targeting liposomes consisting of **16** was then observed in $Langerin^+$ COS-7 cells by confocal microscopy.

Tetrameric DC-SIGN is expressed by myeloid dendritic cells and macrophages. Since DC-SIGN shares the same EPN amino acid motif with langerin, both proteins recognize similar monosaccharide ligands. While langerin was reported to be protective against HIV infections[134], DC-SIGN promotes viral dissemination via a process called trans-infection. Targeting DC-SIGN is therefore of interest to stop the transmission of HIV.[135]

One common approach to increase affinity for DC-SIGN is the multivalent presentation of monosaccharide ligands. Following such an avidity-driven strategy, a dodecavalent fuco-dendrimer with a 420-fold potency increase compared to fucose was reported.[136] However, unspecific binding to langerin due to its similar binding specificity imposes a selectivity issue. GlcNAc is recognized by both C-type lectins but sulfation of position 6 and replacement of the *N*-acetyl group by a *N*-sulfate led to a favored recognition of the negatively charged compound **18** by langerin.[125] The development of positively charged amino species in the pseudo-1,2-mannobioside **19** favored the selectivity towards

DC-SIGN (IC_{50} = 254 μM; langerin (IC_{50} > 4400 μM).[125] Pseudo-1,2-mannobiosides were shown to bind to the carbohydrate recognition domain in DC-SIGN using X-ray crystallography.[137] As an alternative approach to generate specificity, a recent report highlighted the presence of five secondary binding sites on DC-SIGN. These sites recognize drug-like compounds unrelated to carbohydrates, and hence constitute a potential starting point for future development.[138]

Dectin-1, a mammalian lectin of the innate immune system, recognizes β-glucans found on fungal cell walls and is able to function as a PRR in fungal-infection.[124] Liposomes carrying the currently used antifungal drug amphotericin B intercalated into the lipid membrane reduce the antifungal's toxicity compared to detergent-solubilized drugs. Coating of these liposomes with dectin-1 for the specific targeting towards fungal cells showed a 200-fold higher affinity to those cells then untargeted liposomes.[139] These dectin-modified delivery vehicles also reduced growth and viability of the mold *Aspergillus fumigatus* with higher efficiency and thus provide a new opportunity to fight those resistant and difficult to treat infections.

Selectins

Selectins are a subfamily of the C-type lectins consisting of three single-chain transmembrane glycoproteins, which are found on endothelial cells (E-selectin or CD62E), leukocytes (L-selectin or CD62L) and platelets (P-selectin or CD62P). They are involved in constitutive lymphocyte homing, chronic and acute inflammation processes and their minimal common binding epitope is the blood group antigen sialyl Lewis X (sLe^x).[140]

Based on the bioactive conformation of the tetrasaccharide sLe^x for E-selectin, this carbohydrate lead was successively optimized in a series of papers from Ernst and co-workers.[141–145] NMR screening of fragments allowed the identification of a second site binder and upon merging with the first site sLe^x mimic, 30 nM lectin antagonists were obtained from a 1 mM lead.[146] Subsequent addressing of the additional sulfate-binding domain in P-/L-selectins led to the successful pan-selectin antagonist Rivipansel (GMI-1070, **20**) out of the development program by Ernst, Magnani et al. that started in the mid-1990s, despite the common fashion to drop selectin research in pharmaceutical industry in the early 2000s.[147] Since June 2015, Rivipansel is in clinical phase III studies against vaso-occlusive anemia in hospitalized subjects with sickle cell disease (trial end date: June 2019, clinicaltrials.gov Identifier: NCT02187003).

Mincle

Mincle has been identified as a C-type lectin receptor of the innate immune system with glycolipid binding specificity that plays an important role in infection by mycobacteria. Mincle binds the

mycobacterial glycolipid trehalose dimycolate[20,21] and has recently been addressed by a number of groups describing synthetic molecules based on the bacterial glycolipid.[148–151]

Galectins

Galectin-3, the best described member of the galectin family, is involved in many biological processes, *inter alia*, cell growth, cell adhesion and apoptosis. Consequently, it plays an important role in many diseases, among them are cancer, inflammation, fibrosis, heart disease and stroke.[152–154] For that reason, galectin-3 became an important drug target, recently reviewed by Marino, Rabinovich and co-workers.[11]

Symmetric C3-aryltriazolyl-substituted thiodigalactosides have shown high affinities for galectin-3 down to K_d = 1-2 nM. However, most of the compounds also bound to galectin-1 raising concerns about the specificity (*e.g.*: **21**, K_d (galectin-1) = 69 nM; K_d (galectin-3) = 2.3 nM). After combining C3 aryltriazolyl groups with O3-coumaryl groups into asymmetrical thiodigalactosides the selectivity towards galectin-3 increased: specificity of compound **22** towards galectin-3 was achieved with a high affinity (K_d (galectin-1) = 340 nM; K_d (galectin-3) = 7.5 nM).[155] Dicoumaryl digalactoside **23** (K_d (galectin-1) = 16 μM; K_d (galectin-3) = 91 nM) was then analyzed *in vivo* in mice against bleomycin-induced lung fibrosis. At a dose of 3.5 mg/kg of digalactoside **23** the fibrosis score could be reduced but no effect on the inflammatory score was observed.[156] TD139 (**24**) is a derivative of **21** with a single fluorine atom in *meta*-position of the phenyl rings which is in clinical trials phase II as a galectin-3 inhibitor in idiopathic pulmonary fibrosis since February 2019 using the pulmonary route of administration (www.clinicaltrials.gov, NCT03832946).[157,158] Oral administration of these disaccharides is impeded by their poor membrane permeability. Currently, various research groups are optimizing this property and a new galectin-inhibitor class with only one sugar residue and low nanomolar affinity was discovered, *e.g.*, **25,** K_d = 37 nM.[159]

Siglecs

A number of siglecs have attracted the attention in the past decades and several antibodies targeting siglecs are approved drugs or in clinical trials.[160,161] Many publications report the development of antagonists for siglec-4, also called myelin-associated glycoprotein (MAG).[162–164] This protein is important for glial scar formation after central nervous system lesions and inhibition of MAG is considered one therapeutic approach to prevent scar formation and enable axonal regeneration.[165,166]

Siglec-2 (CD22) is a target receptor in anti-cancer therapy of lymphoma, leukemia as well as in the treatment of autoimmune diseases such as lupus and rheumatoid arthritis.[167] Biphenylcarboxamidated sialic acid derivative **26** (IC_{50} = 2 nM) was developed with an over 500.000-fold stronger binding affinity compared to the minimal siglec ligand αMe-Neu5Ac (**27**, IC_{50} = 1.5 mM) against siglec-2.[168] Despite

the fact that this protein is a monomeric protein, di- or trivalent N-glycans show a very high affinity in the low nM/ high pM range. The group by Paulson *et al*. suggest that this high affinity in their assays originates from simultaneous binding to several CD22 lectins clustering on the cell surface within 30-50 Å to each other.[169]

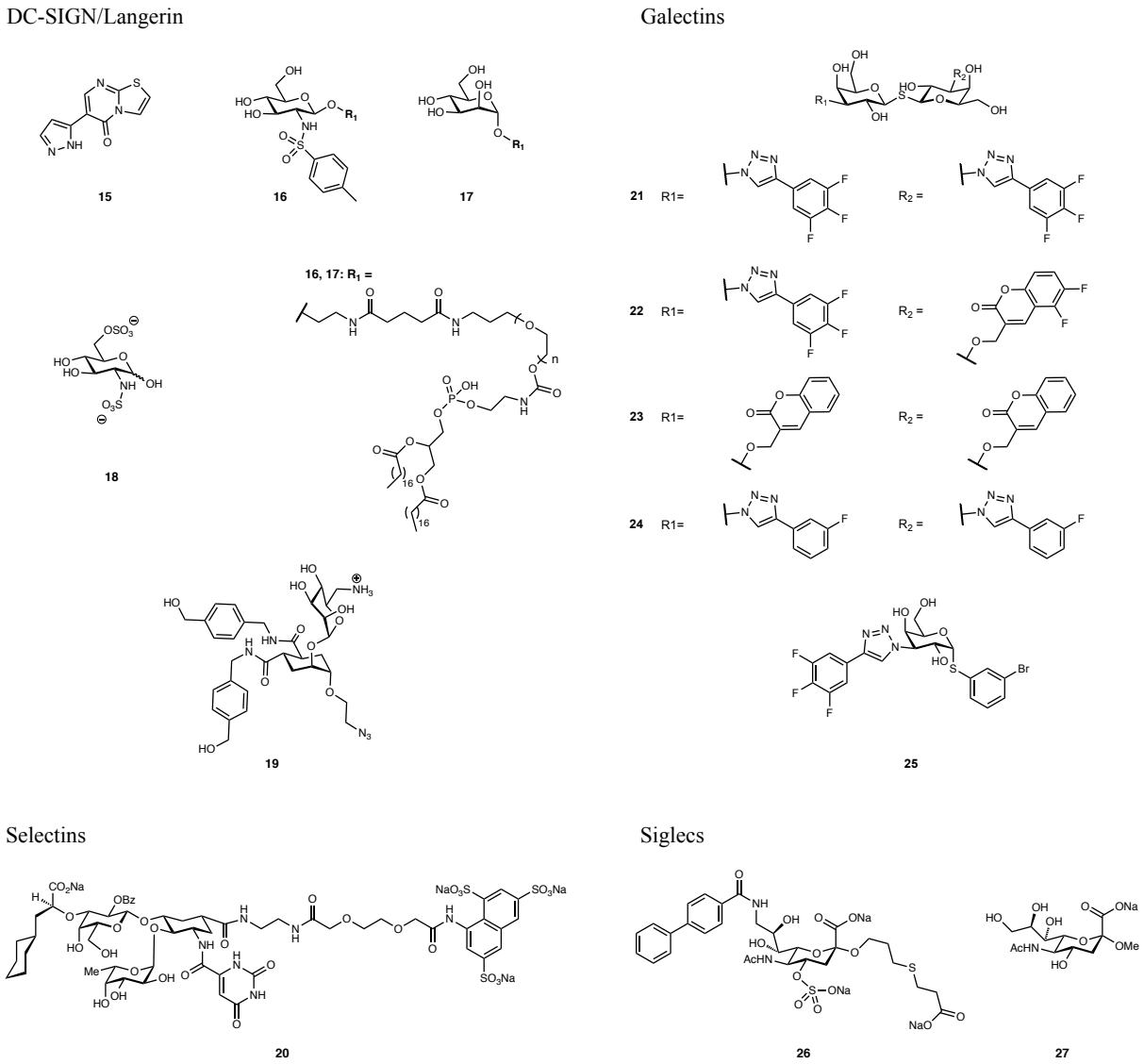

Figure 4: Allosteric (**15**) and carbohydrate-binding site directed (**16**-**27**) mammalian lectin antagonists.

Conclusions

Lectins are a large family of proteins that are present in each domain of life. These carbohydrate-binding proteins possess numerous functions, both intracellularly and outside the cell. Research towards lectin antagonists has developed rapidly over the past two decades focusing on lectins from selected fields, mainly related to immunity and infection involving mammalian lectins and those from pathogenic

bacteria and viruses. The largest block of literature focusses on the assembly of native carbohydrates onto a plethora of different multivalent scaffolds. With some important exceptions discussed here, these publications usually center around the chemical synthesis and compounds are only evaluated in a target binding assay and not employed further for questions of chemical biology and drug research.

However, in the last decade, a number of strategies towards glycomimetic lectin antagonists has been published that led to drug-like structures which proved equally useful in chemical biology research and early preclinical drug discovery. Antibacterial glycomimetic drugs applied alone or in combination with conventional antibiotics will provide new effective therapies for multiresistant bacterial infections. And due to an increasing resistance towards established drugs and the absence of effective drugs against several, so far untreated viruses, viral lectins have become attractive targets in recent years and further research will likely yield new tools for chemical biology and drug therapy. Despite the intrinsic difficulty of developing probes/therapeutics for these low affinity carbohydrate-protein interactions, the field is developing rapidly and the first lectin antagonist currently in phase III clinical trials is GMI-1070 (**20**, Figure 4).

Many new lectins are being uncovered every year providing a large playground for new lectin antagonists for chemical biology and potentially as therapeutic targets. Lectins from other organisms, such as fungi or bacteria that are not pathogenic to humans are active areas of research. It will be interesting to probe for example fungal lectins[22,23,170,171] with a distinct specificity for methylated glycans or those of bacteria[172–174] that live in symbiosis with nematodes and kill invaded insects. Furthermore, a large number of bacterial adhesins in pathogenic bacteria are being uncovered, *e.g.* the *Burkholderia* lectins[175–178] or carbohydrate binding adhesins from *Salmonella enterica*[179], and thus, there is a bright future for the chemical biology of lectin antagonists ahead.

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