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A glycotherapeutic approach to functionalize biomaterials-based systems

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Keywords: Lectins, biomaterials, glycans, therapeutics, drug delivery, cancer

Abstract:

In addition to being one of the primary building block materials of living cells, glycans also have a potential as the favorable candidates for therapeutic applications. In the mid-20th century, the progress of glycans in the drug discovery field became surpassed by protein and DNA-focused treatments. However, the emergence of new analytical tools and methods to synthesize structurally specific glycans has encouraged and motivated the scientific community towards the development of glycan-based therapeutics. This review discusses the reemergence of glycan-based agents in the last decade and the technical strategies that played a key role in bringing the glycan-based treatments into the limelight of modern medicine. The review also includes the application of native glycans as the therapeutic agents along with the chemically engineered cell surface glycans and proteins to meet the preclinical and clinical scenario. Glycan-based therapeutic materials hold a huge potential that can be harnessed to meet the clinical needs in medicine.

Introduction:

Glycans are integral and basic modules of life and are observed in various forms in nature such as secreted mucus components of varying chain lengths from monosaccharide to long polysaccharides and large polysaccharides [1]. These carbohydrate moieties are the most abundant class of organic moieties in the world and can be found on the cellular surface of every organism[2]. The molecular structure of monosaccharides was explained for the first time in the mid-1880s by Fischer. However, it was almost a century after Fisher's discovery; before experts started to realize the crucial parts of these moieties modulating the biological phenomenon [3]. This delay in exploring the functionality of glycans was mainly because of the complexity associated with the assembly and regulation of these biomacromolecules. Cellular processes such as metabolism and signal transduction govern the biosynthesis and functionality of glycans, as they are not openly encoded by the genome [4]. Moreover, glycans can be attached to a group of stereochemistry and regiochemistry-based linkages. This could lead to a wide range of structural diversity which can be further explained via the modifications found in the present functional group[5].

It is widely reported that glycans play a crucial part in numerous biophysical phenomena such as cellular adhesion, proliferation, migration, differentiation, organism development, immune response modulation and disease progression [6]. In spite of extensive research and several reports on the influence of glycans on the disease, medical chemists and clinicians hardly recognized the glycan molecules as a potent target moieties or drugs[7]. The prime research interests of sugar in biology were in general limited to its role as a source of energy for a long time. This unawareness is starting to fade away as advanced methodology regarding sugar synthesis[8], sequencing[9] and biological evaluation[10] became more refined and easily accessible. Over the last few decades, researchers have been studying glycans, either bound to proteins or in lipids (Figure 1) and have become aware of their essential roles in different biological activities such as cell-cell recognition[11], cell signaling[12], cellular differentiation[13] and immune response[14]. This diverse functional ability of carbohydrates is now being exploited for the use of carbohydrates in therapeutic approaches.

To gain a better understanding of glycans and the emerging glycotherapy, in this review we have summarized a detailed history of the emergence of glycans as therapeutics. To achieve high targetability of drug molecules, glycan-based delivery systems are often used to exploit

the interaction between glycans and lectin receptors. Thus, the knowledge of different lectin receptors and their location in our body is of vital importance for the design of glycotherapeutics. We have provided here a brief overview of a variety of lectin receptors present in our body, the cells/tissues they are expressed on and the glycans that can be recognized by them. Along with this, information about different glycosylation strategies that can be employed to achieve the desired glycoconjugation of the materials in focus mentioned in this review will help researchers for the design of glycotherapeutics for the desired targeting application. Lastly, we have provided a detailed summary of glycotherapeutics that have been designed in the last couple of decades for various delivery applications and as drug molecules. This review emphasizes and reevaluates various approaches available to engineer glycan's for biomedical application by closely analyzing the recent advances in the area of glyco-chemical biology and carbohydrate chemistry. Using available carbohydrate research tools, researchers are developing advanced glycan-based materials to improve human wellbeing and treat illness. The field of glycoengineering is considered relatively undeveloped by the scientific community, yet this largely uncharted area holds unlimited potential towards the development of new generation therapeutics.

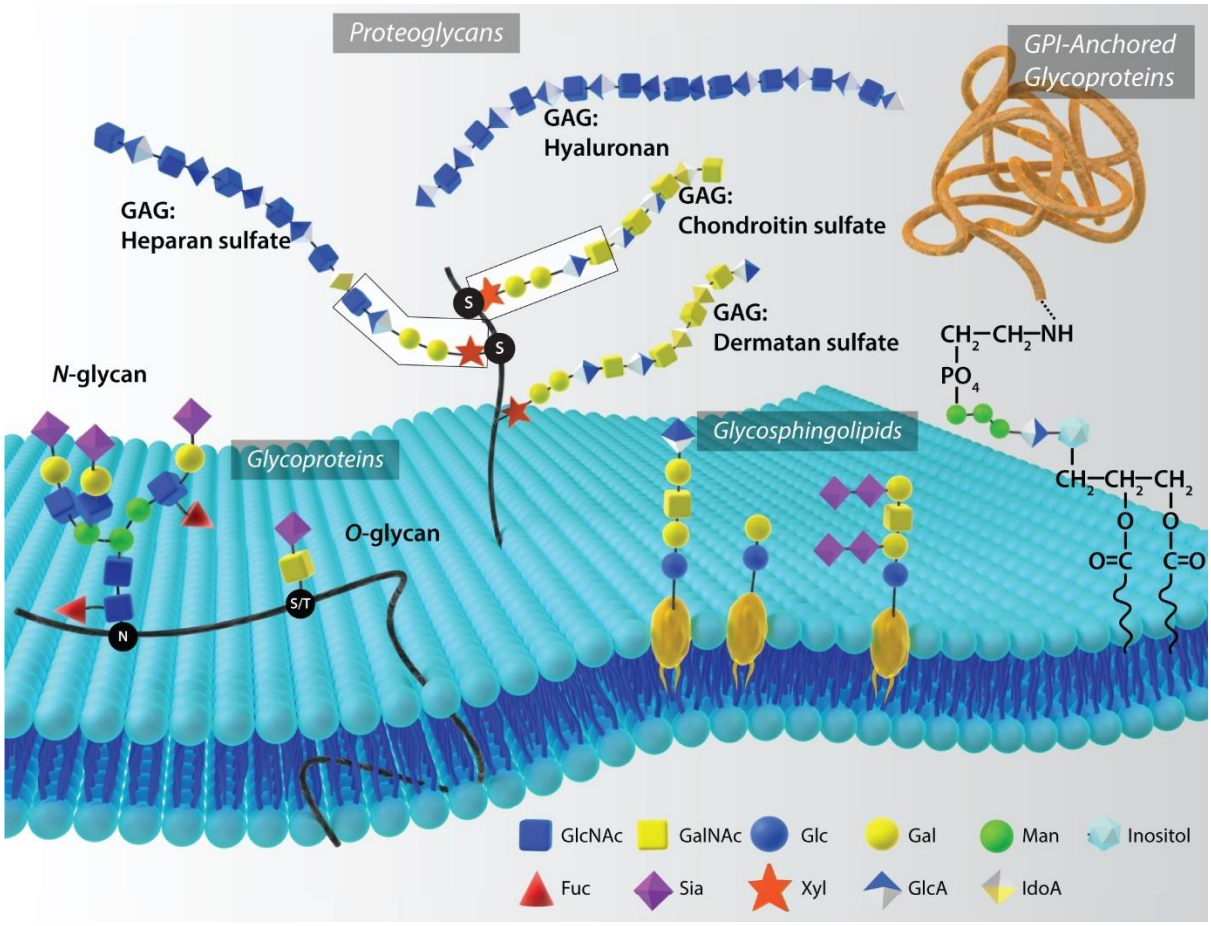


Figure 1: Cell surface glycans in mammals. The diagram depicts different types of glyco-protein and glyco-lipid linkages present on the exterior of the cell. Glycans can be bound to proteins as glycoproteins or proteoglycans. N- and O- linked glycans are very common in mammals. Glucosphingolipids (GSLs) are the major glycolipids in mammals found in the cell membrane of the organisms. Sugar symbols are according to the symbol nomenclature for glycans. Abbreviations in the key: GlcNAc, N-acetylglucosamine; GalNAc, N-acetylgalactosamine; Gal, galactose; Glc, glucose; Man, mannose; Fuc, fucose; Sia, sialic acid; Xyl, xylose; GlcA, glucuronic acid; IdoA,iduronic acid.

The historical background of the therapeutic application of glycans

Glycans have had a very profuse past in therapeutic applications similar to other biomolecules such as nucleic acid and protein. Yet due to the evolution of DNA technologies and the discovery of the genetic code, lipids and glycans became a less valued candidate for biological target or drug as protein and DNA are considered as two main components of life. However, this relatively short period of oversight has not reduced the significance of glycans for medicinal usage [15]. The increase in type II diabetes and obesity was a vivid example of the

biological role of glycans and lipids in understanding and the treatment of the burgeoning epidemic[16].

Karl Landsteiner revealed the different blood groups (A, B and O) in 1900 and he was presented with Nobel Prize in Medicine in 1930. This discovery allowed clinicians to match blood donors to and perform the first successful blood transfusion in 1907. However, for the next 50 years, the ABO constituents' structures were not explored. Several research groups have tried to identify the structure of ABO constituents but they were not successful until the discovery of the following: (a) plant lectins that attached with blood group-specific cells and (b) ovarian cyst, a source of abundant active compound that was discovered in the 1950s[17]. Kabat et al. found that the fucose, a monosaccharide which attached with galactose (Gal) and *N*-acetylgalactosamine (GalNAc) to create B and A antigen, was the key component of H antigen, respectively[17-18]. In the 1960s, the complete structure was discovered by the ingenious application of a selective alkylation process associated with acid/base and enzymatic hydrolysis for defining the components and monosaccharide linkages[17].

In 1916, McLean discovered heparin, a polysaccharide and with progress in the method of isolation from animals; it was being used clinically by the 1930s[19]. These advances in heparin's application for therapeutic purposes made a strong impact on the medical and clinical community. Up until the 1950s, the main source of commercial heparin was porcine mucosa. At that time, the industrial production of heparin was around 100 tons/year and it was solely dependent on extraction from an animal source. The researchers who were trying to investigate the molecular mechanism of heparin fueled the discovery of antithrombin III, which plays a crucial role as an inhibitor of the enzymatic activity of thrombin[20]. Before 1980, the chemical structure of heparin's disaccharide unit was not revealed and afterward, Lindahl et al. reported that it comprises sulfated glucosamine and iduronic acid, underlining the linkage of heparin to the glycosaminoglycan (GAG) family[20b]. Fascinatingly, in a human mast cell subset, the endogenous heparin was observed where it was acting as a controller for the elements of granules that were used for immune protection purposes[21]. These findings, as well as the clinical application, have converted heparin into a billion-dollar industry.

It was observed by Avery and Dochez that a "soluble-specific substance" isolated from *Pneumococcus* was interacting with antisera (type-specific) isolated from diseased individuals[22]. Afterward, Avery collaborated with Heidelberger, a pioneer in the antibody

domain, and found that the “soluble-specific substance” is actually a polysaccharide-based type-specific soluble material[23]. The findings of Avery and Heidelberger were not fully recognized by the scientific community up until 1930 when Francis and Tillett reported that this respective glycan acts as a major element in vaccine preparation towards *Pneumococcus*[24]. Historically, these capsular polysaccharide-based therapeutic products were used for various clinical needs. Twenty-three purified capsular polysaccharides isolated from *Streptococcus pneumonia* were used to develop the vaccine Pneumovax (PPV23)[25], whereas a few amounts of capsular polysaccharide isolated from other microbes were able to generate enough IgG/IgM mediated immune response during vaccination. These findings became a milestone and motivated several research groups to explore the other potent sugars for vaccine development.

Aminoglycosides are well-known small molecule glycans, produced by some specific gram-positive bacteria’s such as the *Micromonospora* and *Streptomyces* genus. Streptomycin, the first aminoglycoside, was discovered in 1943. It was the first antibiotic that effectively cured tuberculosis and found pragmatic clinical application[26]. Kanamycin, neomycin, and gentamycin are widely used antibiotics which also belong to the same antibiotic family. Aminoglycosides act as inhibitors for protein synthesis; however, the exact mode of action for all the aminoglycosides is not yet fully explored [27]. Unfortunately, the clinical application for aminoglycosides has decreased due to the rapid onset of bacterial resistance, but then again, the increase in multidrug-resistant bacterial strains has re-motivated the scientific community to engineer new drugs or a target compounds to block the multidrug-resistant bacterial strains[10].

Positron emission tomography (PET) which revolutionized the field of clinical oncology was first developed around the 1950s. It was the discovery of the chemical synthesis procedure of 2-fluorodeoxy-deoxy-glucose (FDG) and its application in bioimaging that fueled the invention of PET 20 years later[28]. Cells with a higher metabolic rate usually take ^{18}FDG more quickly than other cells with less metabolic demand and this led to the imaging of brain cells as well as to the detection of tumors[29]. Alternative direct imaging techniques for glycans and other biomacromolecules took several more decades to appear and this has been discussed briefly but not elaborated further in this review [10, 30].

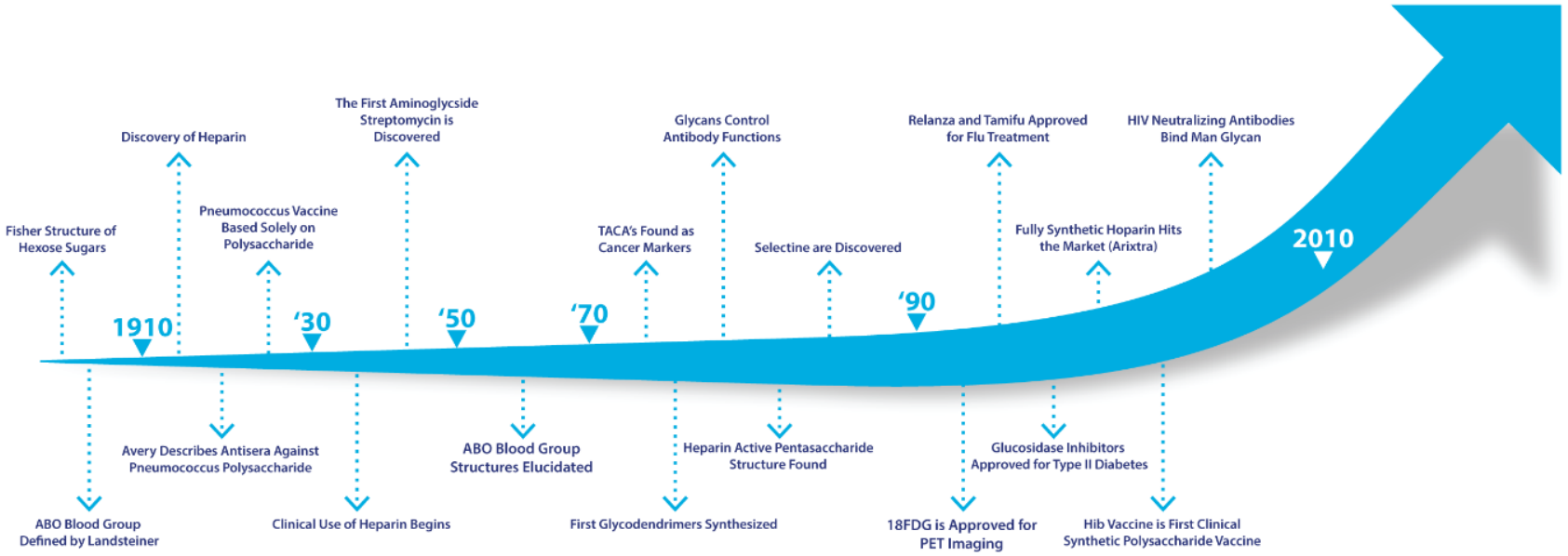


Figure 2: Timeline with the key milestones in the historical development of glycans in medicine. The beginning of the 20th century encompasses major breakthroughs in medicine with glycan-based therapies. However, later progress in the clinical developments of glycan-based therapeutics was hindered by the lack of structural understanding. In the later 20th century and early 21st century, the development of new techniques and the key findings of the specific glycan structure activity resulted in the emergence of a research field called glycobiology.

Importance of lectin receptors in glycotherapy

The cell surface receptors that can bind with the carbohydrates are known as the membrane lectins. Various carbohydrates can be used as the ligands for these lectin membrane receptors to target therapeutic agents [31]. The lectin's interaction with the glycosylated composite could be evaluated by studying the binding of glycosylated materials with lectins viz., concanavalin A (Con A) and Ricinus communis agglutinin [32]. The interaction between endogenous ligands and different carbohydrates such as lactose, mannose, fructose, galactose, and fucose can be used to target sugar ligands [33]. It has been widely reported that the glycosylated drug carrier i.e. a drug carrier conjugated with sugar moieties as surface ligands can be recognized and later endocytosed by lectin receptors. LecB, a fucose specific lectin is usually linked to tissue attachment as well as to biofilm construction due to microbial contamination specifically from *Pseudomonas aeruginosa*. Kolomiets et al. have designed a glycopeptides dendrimer-based system using dendrimers of C-fucosyl peptide for preparing LecB inhibitor as an antimicrobial agent [34]. Enzyme-linked lectin assay (ELLA) showed a strong affinity of these fucose-terminal-glycopeptides dendrimers towards lectin. Octavalent and Tetravalent ligand showed significantly higher binding affinity than fucose. The concept of the uptaking of a glycosylated carrier via a lectin receptor has been investigated thoroughly for screening different molecules that have therapeutic potential. Likewise, it was observed that C-fucosyl conjugated glycopeptide dendrimers selectively bind with Ulex europaeus lectin. The modified dendrimers also showed a higher binding affinity for PA-IIL lectin derived from *P. aeruginosa* [35].

Table:1 A summary of lectin receptors

Types of lectin receptor	Glycans	Expressed on	Tissue/Organ	Ligand	Targets	Ref
Mannose receptor (lectin receptor C-type)	α-Man	Kuffer and endothelial cells (Non-parenchymal origin)	Liver	Glycoproteins with mannose moieties	Receptor driven rapid endocytosis	[36]
		Macrophages	Lungs, liver, spleen, bone marrow and brain	Glycoproteins with mannose residue	Macrophage internalization	[37]
		Dendritic cells	Blood, lymph node and spleen	Complex protein terminal with mannose residue	Delivery of targeted nucleic acid for pathogen recognition (vaccination)	[37b, 38]
Asialoglycoprotein receptors	β-Gal	Hepatic-parenchymal cells	Liver	Galactosyl residues	Therapeutic agent targeting to liver cells	[39]
Galactosyl receptor	β-Gal	Kupffer and liver endothelial cells	Liver	Compound with galactose moieties	Sulfated glycoprotein removal from blood	[40]
Fucose receptors DG-SIGN	α-Fuc	Kupffer cells	Liver	Compound with fucose moieties	Targeted therapeutic agent delivery to Kupffer cells	[41]
Fucose receptor	α-Fuc, α-Man	Conjunctival and corneal cells	Eye	N-acetyl-D-galactosamine and N-acetyl-D-glucosamine	Delivery of therapeutic molecules to ocular space	[42]
		Buccal cells, oral epithelium	Oral cavity	Significant lectin affinity, specially lectins originating from Arachis hypogaea and Pisum sativum	Delivery of selected therapeutic agent to Buccal cavity	[43]
DC-SIGN	Lewis x Gal-β1-4GlcNAc Fucα1-3	Malignant tumor cells	Tumor/Cancerous tissue	Sugars like mannose, lactose, galactose, fucose, SiaLe-X	Targeted delivery of antineoplastic agents into malignant tumor tissue	[33b, 44]
	Lewis y Fucα1-2Gal-β1-4GlcNAc Fucα1-3	Malignant tumor cells	Tumor/Cancerous tissue	Sugars like mannose, lactose, galactose, fucose, SiaLe-Y	Targeted delivery of antineoplastic agents into malignant tumor tissue	[45]
SIGN-R1 DC-SIGN	Lewis a Galβ1-3GlcNAc Fucα1-4	Malignant tumor cells	Tumor/Cancerous tissue	Sugars like mannose, lactose, galactose, fucose, SiaLe-A	Targeted delivery of antineoplastic agents into malignant tumor tissue	[46]
	H type Fucα1-2Gal-β1-3GlcNAc	Malignant tumor cells	Tumor/Cancerous tissue		Targeted delivery of antineoplastic agents into malignant tumor tissue	[47]

Table 1 shows a summary of lectin receptors expressed by a variety of cells. It is well known that a variety of receptors for sugar molecules are expressed in liver cells. Hepatocytes, the liver parenchymal cells can detect a galactose molecule or compound having residues of galactosyl using the asialoglycoprotein transmembrane receptors; whereas the non-parenchymal cells viz., (a) liver endothelial and (b) Kupffer cells possess a mannose receptor [36, 39a, 39b, 48]. The mannose receptor present on the membrane of macrophages and liver endothelial cells is responsible for the fast internalization via receptor-assisted endocytosis of glycoprotein having a mannose terminal. Magnusson et al. observed a very prompt receptor-mediated endocytosis of glycoprotein with a mannose terminal (ovalbumin) in sinusoidal endothelial rat liver cells. The authors claimed that the highest Ke was reported with respect to a receptor-mediated endocytosis system[36]. The plasma membrane of the macrophages expresses a variety of transmembrane receptors viz., Fc-receptors, mannose receptors, scavenger receptors, stearylamine receptors, and integrin, which play crucial roles in different biological events viz., (a) migration, (b) growth, (c) differentiation, (d) activation, (e) endocytosis and (f) antigen recognition in mononuclear phagocyte system. Receptors for mannose are abundant in Kupffer cells, alveolar macrophages, peritoneal macrophages, splenic macrophages and brain macrophages (microglia, astrocytes, and dendritic cells derived from monocyte)[37a, 49]. The mannose-transmembrane receptor present in dendritic cells and macrophages is a 17d kDa C-type lectin protein[37b, 50]. These mannose-based receptors fulfill a vital role in modulating the immune response of the host via a strong involvement in phagocytosis, antigen presentation and processing, intracellular signaling and cell migration [37c, 38a, 51]. More specifically, a high density of the mannose transmembrane receptors can be observed on the surface of dendritic cells [38b, 52]. Liver Kupffer cells also express galactosyl and fucose receptors, which can uptake particles with galactose moieties and fucosylated carriers, respectively[40-41]. Tumor neoplastic cells have a very high affinity towards sugar moieties like lactose, fructose, galactose, and mannose via lectin-like receptors comprising sugars such as Sialic Lewis-X [33b, 44]. Although the lectin receptors discussed here are the ones that are most commonly used in the therapeutic applications, additional receptors are also present in buccal cells, oral epithelium, conjunctiva and corneal epithelium [42-43].

Glyco-conjugation strategies

Researchers have used different glycoengineering approaches to functionalize biomaterials for various applications such as vaccine preparation, antibody effector and other immune functions, reducing the toxicity of biomaterial and host tissue response, etc. Conjugation of sugar on a biomaterial surface can be achieved by several means [53]. The chemoselective coupling reaction is usually performed to alter the sugar molecule for sugar conjugation. Properties of final sugar conjugates such as saccharide length, sugars' modification pattern (for example O-acetylation), type of carrier protein and saccharide/protein ratio, linkers and conjugation chemistry finally utilized for coupling reaction are significantly relevant for immunogenic point of view. The selection of appropriate natural or chemically modified sugar moiety and available functional groups in the biomaterial are the two fundamental pillars of this process. In some cases, chemical activation of sugar might be required for successful grafting. The activation of sugar can be achieved by using non-selective techniques (see ref 53 and references therein) such as targeting hydroxyl groups in a random manner with (a) cyanilating agents used to create cianide groups for chemical reaction with hydrazines or amines, or (b) carboimidazole used to add carboxyl moieties for further reaction with ethylenediamine [53-54]. This can also be obtained using more chemo-selective techniques such as periodate oxidation of *cis*-diols in the carbohydrate ring or at the sialic acid's glycerol moiety to produce aldehydes or sialic or uronic acid's carboxyl groups activation.

For reducing steric hindrance between saccharide and other macromolecules (e.g. protein), chemical handles can be inserted using a linker for conjugation. ε-amine of lysine residue is usually reacted with the oligosaccharides with end terminal aldehydes. Alternatively, ammonium salts can be used to perform reductive amination to derivatize with di-hydrazide spacers or to deliver an amine for a coupling reaction[53]. The chemically inserted amine can be openly reacted with proteins' carboxylic acid groups or can be coupled to the different linker with bifunctionality to integrate maleimide, squaric ester, alkyne or azide moieties, thiol to support conjugation. Synthetic sugar with reactive groups viz., alkenes or thiols, amine can be used for aldehyde generation.

Broadly, sugar conjugation can be classified into two categories; random and site-selective conjugation. In random conjugation, one of the widely used technique is to graft sugar moieties onto the amine functional group present on biomaterial surface. This can be

achieved by coating the biomaterial with amino acids (glutamic, aspartic or lysine) (Figure 3) using covalent or non-covalent process. Because of the hydrophilic nature of the amine groups, it is abundantly exposed/available on the surface of the biomaterials making the process significantly smoother. In some cases, linkers with exposed functional groups such as alkynes, azides, maleimides and hydrazides, may be needed to conjugate natural or activated sugar onto the biomaterial surface[53]. We would like to refer the readers to the ref. 55 for a more detailed review on this topic[55]. Site-selective coupling is usually accomplished by targeting highly nucleophilic residues present on the material surface. To achieve such conjugations, materials can be functionalized with functional groups such as thiols, hydroxyls, amines, azides, amidines or their derivatives. These highly nucleophilic groups successively react with a variety of electrophilic reagents, including saccharide derivatives functionalized with thio, selenoether, maleimides or haloalkyl groups which results in the formation of glycoconjugates[56]. Chemo-selective ligation is forthcoming as a promising method for the site-selective conjugation of carbohydrates and highly functionalized substrates such as proteins. The method involves the reaction of two functionalized groups that have high selective affinity for each other under mild conditions. Researchers have achieved this by using a sulfhydryl group which has high nucleophilicity in contrast to other functionalized groups[57]. Anomeric oxygen of O-glycopeptide is replaced by sulfur or nitrogen, to form S- and N-linked glycoconjugate[58]. The modification is not only biocompatible but is also less prone to acid/base or enzyme-mediated hydrolysis. Thio-oligosaccharides thus formed, can be conjugated with different functional groups on proteins and other functionalized substrates to synthesize glycoconjugates.

In highly functionalized materials such as proteins, to carry out a selective reaction and obtain a homogeneous product, the cysteine residue can be attached to the electrophilic chemical handle, like a bromooxetane[59] or a bromoisobutylene[60] which protects the thiol group of the cysteine and undergoes the halogen displacement with a thiosugar. One of the ways to modify the sulfur group present on cysteine is by using the organoselenium compound – selanyl bromide - to form a stable intermediate under mild conditions[61]. The intermediate further reacts with thio-oligosaccharides to form glycoprotein with the disulphide bond. Similarly, bis-alkylation-elimination can be used to convert cysteine into dehydroalanine. In a wide array of protein modification reactions, dehydroalanine acts as a synthetic precursor. To

form glycoprotein, the cysteine converted into dehydroalanine can undergo a Michael addition reaction with thiosugar[62] or aza-Michael addition reaction with alkylamine linked carbohydrates[63]. The cysteine group is, therefore, an important site for the modification of proteins[53] and attachment of the cysteine-mimicking group on scaffolds will be useful for facilitating the conjugation of material with glycan moiety.

Disulfide bonds present in proteins help to stabilize the protein structure[53]. However, it is possible to cleave the disulphide bond temporarily to attach two cysteines by forming a short covalent bridge. This reaction can be used for glycoconjugation by functionalizing the bridge with carbohydrates[64]. Furthermore, Triazolinediones can be used to attach phenol functionalized substrates to sugar molecules[65]. Enzymatic modification of substrates is possible by incorporating a short amino acid tag either on the substrate or on the sugar to be conjugated[66].

Moreover, thiol click-chemistry reactions are nowadays being used for easy, metal-free modification of thiol groups. The avoidance of potentially toxic metal catalysts decreases the biotoxicity of the reaction products and the high heterolytic and homolytic reactivity of thiols towards many functional groups avoids the need for orthogonality (a requisite for efficient click-chemistry reaction) unnecessary. Thiol-ene coupling (TEC) reaction involves radical alkenes hydrothiolation in the presence of radical sources[67]. The sulfanyl radicals ($RS^{\cdot}$) are added to carbon-carbon double bonds in an anti-Markovnikov fashion. The reaction is mild and uses benign catalysts and solvents along with providing high reaction rates, high yield and complete regio-selectivity. Though TEC reaction undergoes faster addition reaction[68], it is usually reversible in nature and hence significant excess starting products are required to shift the equilibrium towards sulphide products[69]. To overcome this obstacle, thio-yne coupling (TYC) reaction is emerging as a promising click chemistry reaction for replacing/complementing existing thiol click chemistry reactions. Similar to TEC, TYC follows anti-Markovnikov addition but the stereoselectivity is lost in the process in the case of TYC. TYC reactions are efficient when carried out at equimolar concentrations of reagents as the intermediate vinyl radicals formed are irreversible in nature and are more reactive than alkyl radicals.

The thiol group is widely explored as a functional group for glycoconjugation of a substrate, especially in vaccines and antibodies; however, functional groups such as amine, azide and triazolinediones have also been exploited for the glycoconjugation of the materials[53].

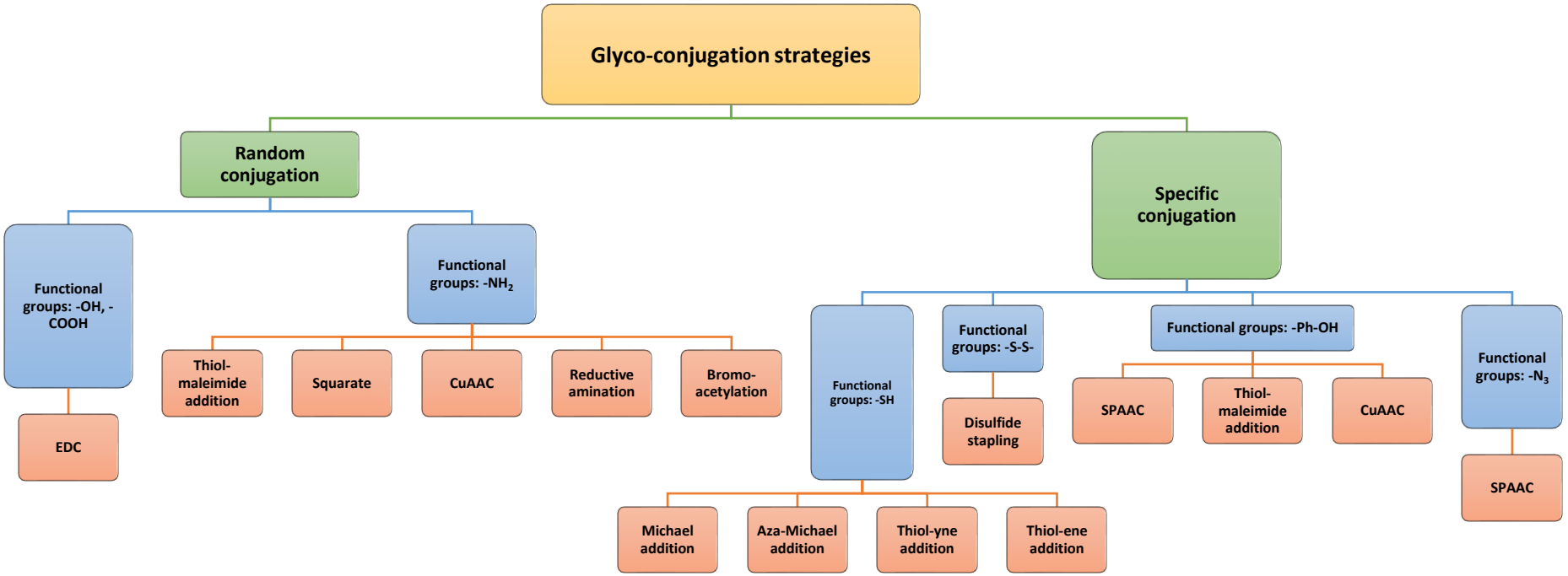


Figure 3: Glyco-conjugation strategies for functional materials. The figure illustrates different glycoengineering strategies for the functionalization of materials with suitable carbohydrate moiety. The selection of appropriate sugar molecule and functional group are essentials and can be decided based on the target tissue/cell and the biomaterial in consideration. Broadly, glyco-conjugation strategies can be divided into two groups – random and selective. The type of conjugation strategy can be selected based on the desired application of the final glycoconjugate.

Engineering of glycans for therapeutic application

In spite of the initial clinical success of sugar-based therapeutic agents in the early 20th century, the later progress of the carbohydrate engineering field was fraught with complications. Chemists failed to discover a proper synthesis technique to produce a bulk quantity of carbohydrates for biological analysis whereas the separation procedures for heterogeneous natural sugars using analytical chemistry tools were still at an early stage. A discovery relating to the specific glycan structures in the second part of the twentieth century led to the advent of glycobiology as an independent research field [3a, 3b]. Afterward, researchers started to use this information to engineer glycans for therapeutic applications.

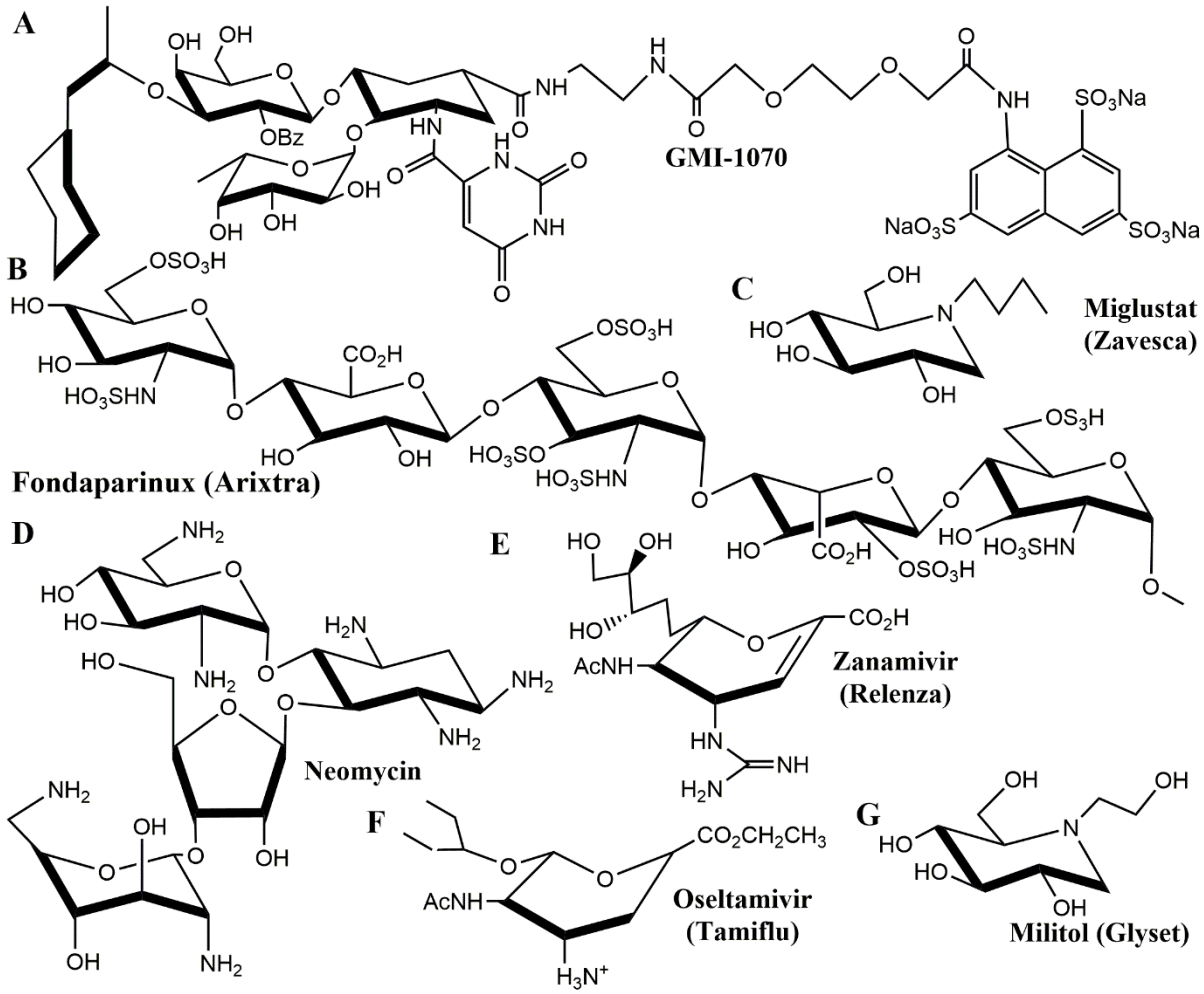


Figure 4: Chemical structure of commercially available / pre-clinical small molecule glycoengineered drugs that are derived from natural sources. (a) GMI-1070 used to treat sickle cell anemia, (b) Fondaparinux used as an anticoagulating agent, (c) Miglustat used to treat Gaucher disease, (d) Neomycin acts as an antibiotic and is used to prevent bacterial

infection, (e) Zanamivir used to prevent viral infection, (f) Oseltamivir used to prevent viral infection and (g) Militol used to treat diabetics.

Small molecule glycoengineered drugs

To the present time, most small-molecule glycoengineered drugs have been glycosidase inhibitors or lectins (Figure 4) [7, 70]. The most popular drugs to come from glycoengineered carbohydrate compounds have been anti-viral drugs such as oseltamivir (Tamiflu; Figure 4f [70a]) and zanamivir (Relenza, Figure 4e)[71]. The influenza virus's life cycle includes the attachment of hemagglutinin (HA) to the sialic acid containing polysaccharide present on the cell surface of the host. These polysaccharides assist viral neuraminidase (NA) by providing a substrate that ensures the maturation and release of the virus [72]. Oseltamivir and Zanamivir bind with NA in nanomolar affinity and prohibit the viral entry inside cells as well as viral budding [73]. Resistance towards Tamiflu has provided the motivation necessary to develop a novel drug molecule targeting the influenza virus. Incidentally, a difluorinated covalent NA inhibitor has lately been designed by Withers and coworkers[74].

For controlling the levels of blood sugar, another successful glycoengineered compounds are the diabetes mellitus type II drugs viz., (a) acarbose (Precose, Glucobay) and (b) miglitol (Glyset), which can inhibit amylases and glucosidases in the gut, respectively (Figure 4g). Actelion designs N-butyl-deoxynojirimycin-based drug (Miglustat, Zavesca, Figure 4c). It is mainly applied to cure the Gaucher disease. Butters and Dwek initially prepared Miglustat, which is an imino-based sugar. It was observed that the native deoxynojirimycin's N-alkylated analogs acted as efficient inhibitors for the glucosyltransferase produced from glucosylceramide[75]. It was found that miglustat treatment could increase the concentration of sphingolipid in Gaucher disease. Lectins are one of the factors that play a vital part in managing the bacterial attachment to the host cells and therefore several research groups have focused on the design and development of glycan-based compounds for inhibiting this interaction[7, 76]. After screening, the most effective compound was tested for its binding affinity towards virulence factors FimH of *Escherichia*. *coli* and PA-IIL & PA-IL of *Pseudomonas aeruginosa*. Both of these bacteria are categorized as adaptable microbes with a growing antibiotic-resistance nature.

In 1982, Thunberg et al. elucidated the structure of heparin, the most celebrated clinical GAG[77]. This enabled Linhardt et al. to synthesize a heparin derived pentasaccharide in a fully active form[78]. The reporting of this led to the design and chemical synthesis of the first defined small compound fondaparinux, heparin(ARIXTRA, Figure 4b), which came on the market in 2002 [79]. Fondaparinux has successfully reduced the risk of heparin-induced thrombocytopenia mainly because of its small size and longer half-life [80]. In 2011, Xu et al. reported the chemoenzymatic synthesis process for the heparin pentasaccharide [81]. The significance of preparing well-defined heparin for the therapeutic application was highlighted in 2008 because of the contamination due to the oversulfated version of chondroitin sulfate which resulted in a health catastrophe at an international level [82]. An analogous method was used by Copeland et al. to create 3-O-sulfated heparin octasaccharide which blocks the admission of Herpes simplex virus type 1 into the cell[83].

In the later 1980s, selectins' discovery was a prime milestone in the field of glycobiology. It stimulated several decades of novel therapeutic agents' progress. These play a vital role in controlling the movement of leukocytes to the inflammation site by binding with the carbohydrate structures of sialyl Lewis^a (sLe^a) and sialyl Lewis^x (sLe^x)[84]. Apart from trafficking leucocytes into the inflammation site[85], sLe^x also plays an important role in the migration of tumors [86]. Recently, it was reported that it also acts as a ligand for the binding of human sperm to the zona pellucida in the course of fertilization[87]. Most of the clinical trials for small molecule-based glycan structures like Cylexin (CY-1503) were stopped in spite of the early enthusiasm in the field of small molecule glycoengineering[88]. However, during clinical trials for vaso-occlusive crisis (sickle cell anemia, GMI-1070, Figure 4a), asthma treatment (Bimosiamose, TBC-1269), fucosylated mimics of the Lewis compounds showed favorable progress [89]. Recently, the Ernst research group has designed ligands attached with a sLe^x construct conjugated with a second site ligand after virtually screening with a nuclear magnetic resonance-guided fragment[90]. This method allowed the application of E-selectin inhibitors in nano-molar concentration, even though most of these antagonists are yet to be examined in an animal model.

N-acetylglucosamine prepared by O-GlcNAcylation has also drawn the interest of scientists owing to its improved regulation in obesity, cancer, Alzheimer's disease and diabetes [91]. The modification process of O-GlcNAcylation is usually controlled by O-GlcNAcase (OGA) and

diphosphate-N-acetyl-D-glucosamine: polypeptide transferase (OGT), which respectively remove and add the O-GlcNAc molecule from Thr and Ser residues. The David Vocadlo research group has a selective and low nano-molar inhibitor for OGA, thiamet-G[92]. They observed an increasing level of O-GlcNAcylation in the rodent brain after treatment with thiamet-G followed by a reduction in tau aggregation as well as neuronal cell loss in an Alzheimer's disease mouse model [92].

In the glycan chemical space, new approaches have been employed to fabricate carbohydrate scaffolds for screening the more potent lectin inhibitors involved in diseased condition [93]. Apart from the compounds mentioned above, many materials are glycosylated in nature which affects their specificity and efficacy for therapeutic application. The rich field constituted by these compounds is, however, outside the scope of this review [94].

Apart from the use of glycans to engineer small molecule drugs, glycosylated nanocarriers are being widely used to enhance the targeting abilities of pre-existing small molecule drugs which also results in an increase in circulation times of the drug *in vivo* and a reduction in the systemic side effects[95]. The glycosylated nanocarrier systems are discussed in detail in another section.

Table 2: Advances in small molecule glycan-based drugs and polyvalent glycan-based inhibitors

Small molecule glycan-based drugs									
Product name	**Acronym**	**Elimination half-life**	**Route of administration**	**Developed by**	**Mode of action**	**Target /species**	**Disease**	**Side effect/Tested on**	**Ref**
Oseltamivir	Tamiflu	1–3 hours, 6–10 hours (active metabolite)	Oral, inhalation	Gilead Sciences	Stop the viral entry inside cells and viral budding	Virus	Influenza (A&B)	Vomiting, diarrhea, headache, and trouble sleeping etc.	[70a]
Zanamivir	Relenza	2.5–5.1 hours	Oral, inhalation	Peter Colman and Joseph Varghese	Stop the viral entry inside cells and viral budding	Virus	Influenza (A&B)	Headache, dizziness, nausea, vomiting, diarrhea etc.	[71]
Acarbose	Precose, Glucobay	2 hours	Oral	Bayer AG	Inhibit amylases and glucosidases in the gut	NA	Diabetes mellitus type II	Flatulence and diarrhea	[96]
Miglitol	Glyset	2 hours	Oral	Bayer AG	Inhibit amylases and glucosidases in the gut	NA	Diabetes mellitus type II	Flatulence and diarrhea	[96]
N-butyl-deoxynojirimycin	Miglustat, Zavesca	6–7 hours	Oral	Butters and Dwek	Inhibit glucosyltransferase	NA	Gaucher disease	Diarrhea, stomach pain or bloating, gas, loss of appetite etc.	[75, 96]
Heparin/ fondaparinux	Arixtra	17-21 hours	Subcutaneous	Mclean	Anticoagulant	NA	Deep vein thrombosis	Vomiting,skin rash, headache etc.	[80] [77]
Rivipansel	GMI-1070	8 hours	Intravenously	Glycomimetics	Inhibits the interaction between leukocytes and the endothelium	NA	Sickle cell anemia	No adverse side effect	[89]
Bimosiamose	TBC-1269	3-4 hours	Inhalation	Encysive Pharmaceutical	Pan-selectin antagonist	NA	Asthma treatment	Mild discomfort, cramping etc.	[89, 96]
Neomycin	NA	2 to 3 hours	Oral, intravenous, topical	Selman Waksman	Protein synthesis Inhibitors	Bacteria	Antibiotic	Tinnitus, hearing loss, and vestibular problems	[96]

Kanamycin	NA	2 hours 30 minutes	Oral, intravenous, topical	Hamao Umezawa	Protein synthesis Inhibitors	Bacteria	Antibiotic	Loss of hearing, toxicity to kidneys, etc.	[96]
Gentamicin	NA	2 hours	Oral, intravenous, topical	Wagman et al.	Protein synthesis Inhibitors	Bacteria	Antibiotic	Low blood cell counts, Neuromuscular problems, Nerve damage, Kidney damage, Ear disorders	[96]
Polyvalent glycan-based inhibitors									
Oligovalent dendrimer	STARFISH	NA	NA	Kitov et al.	Inhibition towards Shiga like toxin, enterotoxin and cholera toxin	Bacteria	Cholera and *E. Coli* infection	E.Coli	[96-97]
Dendrimers with the sialic acid containing Pentasaccharide	GM1	NA	NA	Pukin et al., Sisu et al.	High *in vitro* binding affinity towards toxins as well as inhibition of cellular toxicity	Bacteria	Cholera and *E. Coli* infection	Endothelial cells	[98]
Sulfated lactose PEO star dendrimer	NA	NA	NA	Rele et al.	Bind with selectins with high affinity and can reduce acute inflammation	Inflammatory cell	Inflammatory diseases	Mouse	[99]
Glyconanoparticles conjugated with sle^x	NA	NA	NA	Van Kasteren et al.	Sle^x to bind with multimeric selectin	*In vivo* imaging	Cerebral inflammation, multiple sclerosis	Wistar rats	[100]
Oligomannose Dendron	NA	NA	NA	Wang et al.	Blocking the active site of HIV by mannose dendrimers conjugated dendrimer	Virus	HIV	Dendritic cells	[101]
Qβ glycodendronan particle	NA	NA	NA	Rendle Et al.	Prevent the Ebola infection of dendritic cells by blocking the binding of viral component to DC-SIGN	Virus	Ebola	Dendritic cells	[102]
Lipid nanoparticles	NA	NA	NA	O'Reilly and Paulson	Targeted cancer therapy using for new	Tumor cells	Cancer	Mice	[103]

					sialoside-based ligand for targeting Siglecs				
Glycopolymer-based system	NA	NA	NA	Kitov et al.	Upgraded STARFISH inhibitor by synthesizing a glycopolymer-based system comprise of Pk glycan conjugated (S)-polybait	Bacteria	Cholera and *E. Coli* infection	Transgenic mice	[104]
Glycomethanethiosulphonates (glyco-MTS) ligation technology	MTS	NA	NA	Davis et al.	Site-dependently attach a sulfated tyrosine and sle^x into the ssβg, bacterial enzyme	Bacteria	Inflammatory diseases	Rat	[105]
GAG-based glycopolymer	NA	NA	NA	Hsieh-Wilson group	Chemically prepared ROMP-CS mimics can prevent the outgrowth of neurite	NA	Wound healing, Neuro-inflammation	Mouse	[106]
Synthetic 4- and 6-position of the Galactosamine within CS	Anti-CS-E	NA	NA	Brown et al.,	Antibody against CS-E showed axon regeneration	NA	Neuro degenerative disease	Mouse	[107]

Polyvalent glycoengineered molecules and inhibitors

The major difficulty when targeting monovalent glycans (small molecules) is that their binding affinity towards lectin is quite low and so they cannot be designated as appropriate drug candidates. Keeping this drawback in mind, researchers from polymer and carbohydrate chemistry backgrounds have designed multivalent glycoconjugates, taking advantage of the popular "cluster glycoside effect" to improve lectin avidity[108]. There are essentially three types of macromolecular glycosylated structures viz., (a) glycopolymers, (b) glyconanoparticles, and (c) glycodendrimers[108a, 109]. While most of these synthesized materials are still in the development stage with respect to biological evaluation, they symbolize the next-generation movement of glycotherapeutics technologies. Arguably, glycodendrimer is the most clinically favorable scaffold [109a, 110]. A dendrimer is a global moiety having a single molecular weight. They typically comprise a central zone, branched layers and several end groups with different functionalization[111]. One of the very first glycodendrimer synthesis was reported by Yuan et al. [112]. However, STARFISH was the first therapeutically significant development of an oligovalent dendron-like complex having analogs of Gb3 trisaccharide (Pk) covalently linked to the pentavalent glucose moiety (Figure 5b). It is well known that Gb3 carbohydrate is a ligand for the *E. coli*-based Shiga-like contaminants. The *in vitro* inhibition towards Shiga-like toxin captured via the oligovalent display of Pk in STARFISH was $1–10^6$ times more than all well-known ligands with a univalent nature [97]. Researchers have tried similar mechanisms to deactivate heat-labile enterotoxin and cholera toxin [96]. Dendrimers with a sialic acid moiety conjugated with oligosaccharide-GM1 were synthesized and significantly high *in vitro* binding affinity towards toxins as well as inhibition of cellular toxicity was observed [98].

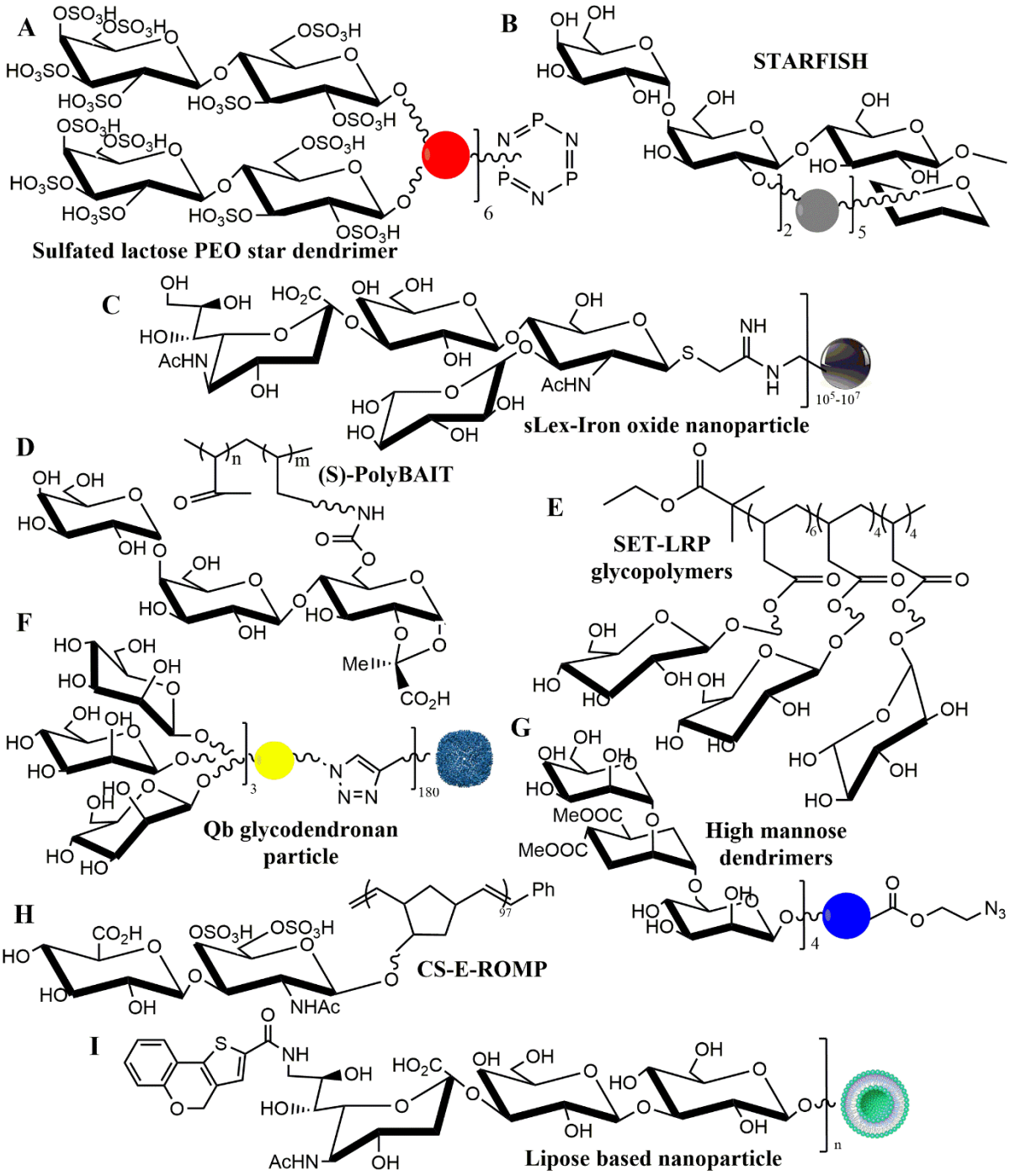


Figure 5. Chemical structure of polyvalent glycoengineered molecules and inhibitors for targeting lectins. (a) Sulfated-lactose based PEO star dendrimer system is used to inhibit selectins and reduce acute inflammation, (b) STARFISH used as an efficient inhibitor for E.coli based Shiga-like toxins, (c) sLe^x-Iron oxide nanoparticle used to image selectins, (d) (S)-poly-BAIT, a cyclic pyruvate ketal capable of binding with Shiga-like toxins for clearance process, (e) SET-LRP based glycopolymer system with measured optimization towards binding of DG-SIGN molecule, (f) Qβ glycodendronan particles prevent the Ebola infection of dendritic cells

by blocking the binding of viral component to DC-SIGN, (g) High mannose-based dendrimer blocks the active site of HIV using mannose residue, (h) Antibody against chondroitin sulfate-E showed neuronal healing properties in terms of axon regeneration, (i) Lipid-based nanoparticle for targeted cancer therapy using new sialoside-based ligand for targeting Siglecs.

Scientists have used dendrimer-based systems to image or block sugar-binding proteins in mammals. Chaikof and associates showed that the highly sulfated lactose ligands associated poly(ethylene oxide) (PEO) star dendrimer can bind with selectins with high affinity and can reduce acute inflammation in a mouse model [99] (Figure 5a). Glyconanoparticles consisting of chemically crosslinked amine-functionalized iron oxide were conjugated with sLe^x to bind with multimeric selectin (Figure 5c). Iron oxide is a well-known contrast agent for MRI and the feature of the engineered particle to specifically direct selectin proteins have allowed imaging of the inflammation of the brain in stroke and multiple sclerosis in animal models[100]. CD209, the dendritic cell-specific intracellular adhesion molecule grabbing non-integrin (DG-SIGN), is a standard target for dendrimer-based materials because of its role in the efficient binding of a wide variety of pathogens. It is a C-type lectin-based transmembrane receptor present on both dendritic cells and macrophages. To spread and evade the immune system, it can effectively bind with Ebola, Mycobacterium tuberculosis and HIV i.e. an escape mechanism for the pathogen[102]. During 2008, Wang et al. fabricated an oligomannose dendron-based system that can effectively inhibit the binding of a recombinant dimeric version of DG-SIGN with respect to IC50 value and gp120, the mannosylated envelop protein of HIV to anti-HIV antibodies [101a]. Following this, many other groups have also shown successful inhibition of HIV trans-infection by blocking the active site of HIV by mannose dendrimer-conjugated nanoparticles [101b]. Recent reports have also shown that a high concentration of mannose dendrimer can efficiently inhibit the HIV infection which is mediated by DG-SIGN at the cellular level as well as in human uterine cervix explant models (Figure 5g)[113].

Rendle et al. showed a highly valent “glycodendriprotein”-based system by conjugating glycodendrimers on the multiple sites of a protein[114]. This strategy was used to attach mannose dendrimers onto the icosahedrons of Qβ. The consequential construct of “glycodendronanoparticles” is observed as the utmost highly valent glycodendrimeric system regardless of its diameter of 32 nm with respect to 1,620 carbohydrate units (Figure 5f).

Ribeiro-Viana et. al. showed that these supervalent components were able to effectively remove the infection of Ebola through blocking the mechanism of viral binding to DG-SIGN[115]. García-Vallejo et al. prepared a tetrasaccharide Leb (DC-SIGN ligand) conjugated poly(amidoamine) (PAMAM) dendrimer-based system to increase the vaccine's delivery to the dendritic cells (DCs)[116]. Singh et al. showed that these dendrimers formed stronger DC activation as well as producing subsequent stimulation of T cells over that of previous conjugates of Lewis polysaccharide to the OVA peptide alone when they bind with the OVA protein as an antigen [117].

Sialic acid-binding immunoglobulin-like lectins (Siglecs) are another category of transmembrane cell surface receptors present in hematopoietic cells and is considered as a promising candidate for cancer therapy and other drug delivery applications [103a, 103b]. Paulson and coworkers have created a library of diversified sialoside for identifying new sugar-based ligand for targeting Siglecs[103c, 103d]. For directing lipid nanoparticles and toxic virus-like particles towards the particular lymphomas and leukocytes, they used these specific ligands based on their Siglac expression[118] (Figure 5I). In the areas of targeted cancer therapy and targeted gene delivery, these methodologies provide support and new targets for current efforts.

The research group of Kiessling has contributed significantly to the field of glycopolymers, reporting that the molecular structure of multivalent ligands is a prime feature that governs the activity. However, the additionally designed construct can be needed in some cases for enhancing the specificity and avidity[119]. Based on this concept, the Bundle research group upgraded their previously reported STARFISH inhibitor by synthesizing a glycopolymer-based system that comprises Pk glycan conjugated (S)-PolyBAIT, a cyclic pyruvate ketal (CP) (Figure 5d)[104]. CP is a well-known ligand of the serum amyloid P component (SAP), capable of targeting the attached binders for the clearance process. It also plays a critical part in clearing the Shiga-like toxin. This polymer is injected intravenously in mice and later a fatal amount of Shiga-like toxin I was injected subcutaneously and zero percent mortality was observed. Fascinatingly, mice that were injected intravenously with a copolymer having randomly distributed CP and Pk showed severe Shigatoxemia signs. These reports show the growing importance of conjugating ligand on polymeric constructs for trafficking necessary proteins to improve drug efficacy and toxin clearance.

Reporting the further importance of attaching ligand onto glycan scaffold, Davis and coworkers prepared a synthetic ligand mimicking the P-selectin-glycoprotein-ligand-1. They used a combination of copper-catalyzed azide-alkyne cycloaddition and ligation method of glycomethanethiosulphonates to site-dependently attach a sulfated tyrosine and sLe^x into the SSβG, bacterial enzyme [105a]. These two components play an important role in site-specific binding to P-selectin. In a chronically inflamed rat cortex model, this modified SSβG with lacZ-type galactosidase property can be utilized to stain the P-selectin[105b].

The Hsieh-Wilson group has established the synthetic preparation of GAG glycopolymer mimics using ROMP because isolation of a homogeneous version of native GAGs from natural sources is quite challenging. Previous reports have shown that the chondroitin sulfate's (CS) sulfation pattern plays a crucial role in controlling the healing mechanism in the nervous system. However, it is restricted by the process of critically controlled sulfation patterns of CS[120]. The Hsieh-Wilson group reported that the chemically synthesized ROMP-CS mimics can inhibit the outgrowth of neurite [106] and, in the follow-up article, they identified the reason for this activity as 4- & 6-position sulfation of galactosamine unit in CS (Figure 5h) [107]. Chemical synthesis of GAGs has allowed this research group to produce a CS-E epitope-specific antibody that could stimulate the revival process of axon in a mice model with a glial wound in the optic neuron.

The space of glycopolymer is currently driven by the application of recently developed methods of controlled radical polymerization, allowing the generation of highly defined polymer structures [121]. However, most of these materials were not tested properly for their biological efficacy. The Haddleton research group has recently shown a technique to prepare a linear copolymer using single-electron transfer living radical polymerization (SET-LRP), where the sugar position can be controlled for the first time (Figure 5e)[122]. They have reported the synthesis of multi-block glycopolymers with the monomer of acrylate containing glucose, fucose and mannose residues and showed that the only structure of mannose monomer clustered in a certain way can bind to DG-SIGN.

Glycan-based vaccines

Glycan-based vaccines played a very crucial role in bringing carbohydrate chemistry into the limelight of relevant clinical platforms [8b, 123]. Numerous currently available vaccines are

based on carbohydrates viz., (a) Prevnar (*Streptococcus pneumonia*), (b) Menactra (*Neisseria meningitides*), (c) Hib (Haemophilus influenzae type b), and (d) TYPHIM Vi (*Salmonella typhi*)[123a, 124]; though these products are first isolated from natural sources and then nonspecifically conjugated with carrier proteins. In this review, we are focusing on the recent advances in glycoengineered synthetic vaccines, which have therapeutic potential that is more distinct. Development in the area of protein conjugation chemistry has significantly influenced the methods available to a glycan-based vaccine that can selectively conjugate with protein carriers[56, 125]. Detailed progress of glycan-based vaccines for bacteria[126], cancer[30b, 127] and HIV[128] is reviewed by several research groups.

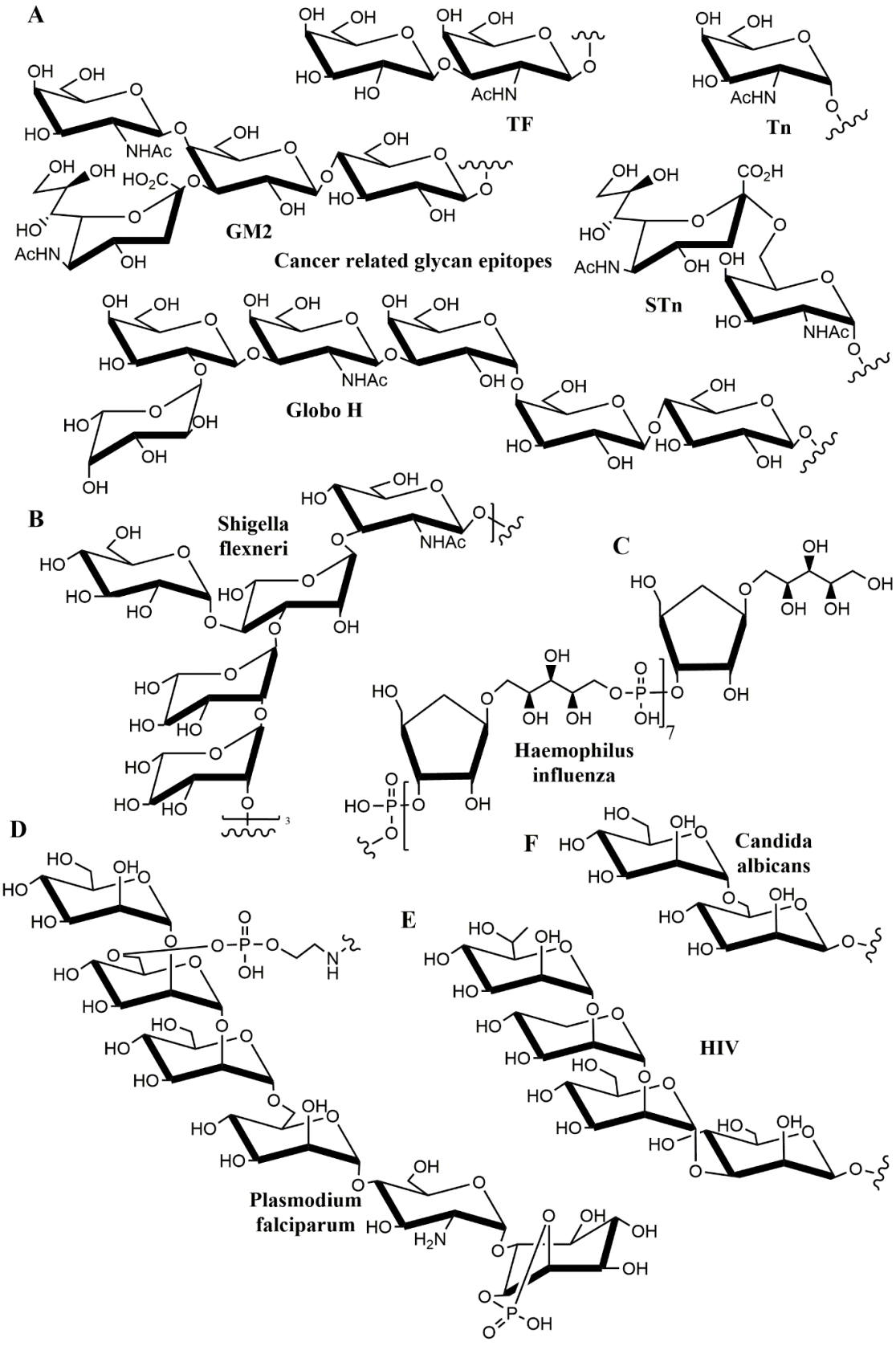


Figure 6: Chemical structure of commercially available and pre-clinical glycan-based vaccines. (a) Vaccine against cancer-related polysaccharide epitopes, (b) Vaccine against Shigella

flexneri, (c) Vaccine against *Haemophilius influenza*, (d) Vaccine against *Plasmodium falciparum,* (e) Vaccine against HIV and (f) Vaccine against *Candida albicans*

The Cuban Hib vaccine is by far the most successful and the first clinically approved candidate that is a completely synthetic glycan-based vaccine inspired by capsular polysaccharide antigen's structure of Hib (figure 6c)[129]. Pentasaccharide-tetanus toxin (TT) is produced in larger quantity in the GMP facility so that it can be fused to the current routine system of vaccination in Cuba. Seeberger et al. used a similar tactic to prepare an antimalarial vaccine using an automated chemical synthesis method of the glycosylphosphatidylinositol (GPI), isolated from *Plasmodium falciparum* (Malaria) (Figure 6d)[130]. In the mice model, Tamborrini et al. observed that the treated animal showed improved survival as well as protection against various disease symptoms observed during malaria parasite infection such as pulmonary edema, cerebral syndrome, malarial acidosis, and death. They also productively prepared a vaccine for *Bacillus anthracis* using the spore tetrasaccharide to develop antibodies against anthrax for imaging and detection purposes [131].

Mulard and associates used the three repeating units of *Shigella flexneri* 2a LPS' O-specific polysaccharide domain to prepare a synthetic version of pentadecasaccharide (Figure 6b). This carbohydrate conjugated tetanus toxoid (TT) showed the improved response of anti-LPS 2a antibody in serum in a mice model compared to a small length of synthetic O-specific polysaccharide sequences[132]. After immunization, the mice synthesized anti-LPS antibodies (glycoconjugate-induced) which provided protection during SF2a infection. Based on pre-clinical data, Phalipon et al. showed that these results are also valid for treatment in humans [133]. Unlike previous developments, which were typically conjugation methods based on monovalent epitopes, the modern approach is to utilize the constructs of a multivalent epitope. β-mannan trisaccharide epitope attached polymer which was isolated from the cell wall of *Candida albicans* chemically attached with chicken serum albumin showed significantly higher antibody production in a mice model compared to the trisaccharide-TT vaccine[134]. Later, Bundle and associates used this IgG to classify a vaccine with minimal disaccharide epitope to protect the *C. albicans* infected rabbit (Figure 6f)[135]. Reports from Nifantiev and Pier labs showed that a number of synthetic glycan-based vaccines can detect the β-(1→6)-poly-*N*-acetyl-d-glucosamine (PNAG) which is a basic component of various pathogenic bacteria's capsular polysaccharide. Antibodies isolated from immunized rabbits

were used to protect *S. aureus* and *E. coli* infected mice [136]. Over the past decade, a major focus of glycobiology research was to target the high mannose (Man_9 carbohydrate) domain of gp120, the envelope protein of HIV via roughly neutralizing antibodies [137]. One of the most popular candidates is the 2G12 clone which deactivates via deceleration of the admission process of the virus into the host cells. After the passive transfer of the virus into patients, it can also hinder the replication of the virus [75b]. Another successful example is the PG9 clone which can bind to the carbohydrates present in both gp 140 and gp 120 envelope protein[138]. During the early stages of HIV vaccine development, researchers tried to induce an immune response by targeting the gp120's high mannose epitope in bovine serum albumin conjugate in a rabbit model. However, they observed that the antibodies produced showed neither an affinity towards native gp120 N-glycans nor a deactivation of the virus[139].

Based on these background findings, the Davis research group developed a vaccine using a synthetic equivalent of mannose to produce a non-self-epitope with more immunogenic properties (Figure 6e). Doores et al. showed that this synthetic mannose analog conjugated virus-like particle Qβ has a high binding affinity towards 2G12 and can cause a better antibody-mediated immune response that is only comparable to the native mannose conjugated vaccines [140]. Rabbits were immunized to produce an antibody that can bind to the cowpea mosaic virus conjugated with Man_8 and Man_9. However, these antibodies showed no affinity towards gp120 or a decrease in virus infection indicating that the carrier protein also plays a crucial role in neutralizing antibody production. Aussedat et al. recently showed the synthesized glycopeptide having two closely spaced N-glycans at Asn^{156} and Asn^{160} of gp120 that can bind more strongly with PG9 antibody than to glycan alone as a ligand [141].

Several research groups have observed the changes in glycosylation of transformed cancer cells[142]. It was noted that the changes in glycosylation tend to increase in the case of highly sialylated and branched constructs which is usually followed by a rise in mucinous proteins viz., MUC16 and MUC1[143]. For cancer vaccine, tumor-associated carbohydrate antigens (TACAs) and unique repeated glycan epitopes have been a major target[144] (Figure 6a). Being an early pioneer in the field, Danishefsky et al. developed the first synthetic-based Globo H vaccine for clinical application [145]. Later, his research group synthesized the KLH carrier protein conjugated five breasts and prostate cancer-associated glycan-based antigen Tn, TF, Globo-H and GM2[8a] (Figure 6a). These candidates showed successful IgG/IgM-based

immune response in a mice model and currently, in clinical trials. Huang et al. tested different adjuvants as well as carrier proteins expanding the safety profile and immunogenicity to prepare an optimized version of a vaccine against hexasaccharide Globo-H [146]. A mixture of α-galactosylceramide analog adjuvant and Globo-H attached diphtheria toxoid elicited a strong IgG response against Globo-H as well as against other interrelated compounds specifically observed in cancer stem cell and breast cancer. Ingale et al. reported a multi-component vaccine made of a tumor-associated glycopeptide and a TLR2 agonist that are able to produce a strong IgG response in a mice model which was further identified by the tumor-linked glycans on cancer cells [147]. Due to the presence of MUC1 in a wide variety of cancers, it was accepted as a cancer vaccine target[148]. But the initial approaches are mostly dependent on the traditional method of vaccine development using unglycosylated epitopes [145a]. The three-component method consists of the Pam_3CysSK_4 TLR agonist, the T-helper epitope and a GalNAc-glycosylated MUC1 peptide, developed by the Boons research group showed that it could produce a higher IgG response in a mice model, i.e. it could elicit both cellular and humoral immunity [149]. Wilkinson et al. also developed a self-assembled nanoparticle-based three-component vaccine for adjuvant purposes [150]. Inspired by the synthetic multivalent based approach, Dumy and associates prepared a regioselectively addressable functionalized templates (RAFT) attached self-adjuvanting glyco-lipopeptides [151]. RAFT is composed of a universal $CD4^+$ T-helper epitope and a $CD8^+$ T cell epitope and four α-GalNAc molecules (Tn antigen) linked to the Pam adjuvant[152]. It was observed that this biomaterial elicits a strong IgG/IgM response in a mice model and is able to identify tumor cell lines. It was also found that this construct was able to reduce the size of the tumor in mice that is inoculated using a syngeneic murine cancer cell (MO5). Huang et al. developed a self-adjuvating based system using glycosylated MUC1 peptides attached to self-assembling fibrils from the peptide domain of Q11 which can elicit an adjuvant-free response[153].

Gene delivery

Gene therapy shows great promise and potential in the treatment of currently incurable genetic and acquired diseases[154]. However, safe and effectual delivery of therapeutic genes to the desired target remains a fundamental challenge in gene therapy. One of the promising candidates to overcome these issues is polyethylenimine (PEI) which has shown effective endocytotic pathway uptake and endosomal escape capacity along with DNA

condensation[155]. However, researchers have observed that the high transfection efficiency of PEI is allied to a high molecular weight which in turn shows a high cytotoxic effect[156]. Also, the targeted delivery of DNA with PEI is still a challenge due to the lack of specificity of PEI towards targeted cells. To overcome these issues, PEI was conjugated with mannose which showed higher transfection efficiency, higher cell-specific targeting and reduced toxicity than that of unconjugated PEI[157].

Macrophages are vital to carry out immune functions such as antigen presentation[158]. Targeted delivery of genes to macrophages is a challenge for the treatment of genetic metabolic diseases such as Gaucher's disease or for the inhibition of HIV replication[159]. The use of non-viral vectors for *in vivo* gene delivery is being considered because of their simplicity and safety over viral vector systems[160]. Cationic liposomes are among the promising non-viral gene delivery systems; however, they do not exhibit any cell specificity in vivo. To overcome this drawback, galactosylated cholesterol-derived gene delivery systems have been synthesized[161].

Protein and peptide delivery

Proteins and peptides are promising biopharmaceutical drugs in the prevention and cure of numerous diseases. However, the manufacturing and storage of these therapeutics are challenging due to the issues relating to their stability, such as inactivation and aggregation. To resolve the issues pertaining to solid-phase stability of proteins after lyophilization, a chemical glycosylation strategy was applied for the development of a stable formulation of α-chymotrypsin (α-CT)[162]. A study showed that glycans (lactose and dextran) were covalently attached to the protein (α-CT) surface and prevented the electrostatic reaction between water molecules and interior protein by the formation of steric hindrance. This led to a reduction in the moisture-induced aggregation and activity loss of α-CT regardless of the size of the glycans.

One of the hallmarks of cancer is the avoidance of apoptosis[163]. Cytochrome c (Cyt c), is a small mitochondrial heme protein that activates the intrinsic apoptotic pathway upon the discharge into the cytosol [164]. In cancer, sustained angiogenesis and insufficient lymphatic damage observed in tumor leads to the enhanced permeability and retention (EPR) effect which is exploited for the accumulation of nanoparticles in tumors[165]. Mesoporous silica

nanoparticles were used for the stimulus-responsive intracellular delivery of Cyt c but the treatment was unsuccessful in the induction of apoptosis in human urine cancer cells [166]. However, the further study demonstrated that the attachment of lactose to the surface of Cyt c not only improved the apoptotic activity and thermodynamic stability of the drug but also protected the drug from protease degradation.

Multifunctional gold glyconanoparticles incorporating sialyl-Tn and Lewis^y antigens, T-cell helper peptides (TT) and glucose were developed as a novel platform for anticancer vaccines[167]. These multifunctional hybrid nanoparticles with defined average chemical composition were prepared in a one-step procedure. This methodology opens the door for the creation of potential polyvalent vaccine candidates and polyvalent drug carriers with chemically defined average composition[168].

Diagnostic reagent delivery

Glycosylated nanocarriers have shown great potential as diagnostic tools. Sentinel lymph nodes (SLNs) play an important part in the metastasis of primary tumors as they are the first lymph nodes to be reached by cancer cells during metastasis[169]. Therefore, the biopsy evaluation of SLNs is important for the determination of tumor metastasis for which the accurate localization of SLNs is required[170]. To overcome the drawbacks in current imaging methods for SLNs such as non-selective biodistribution, a need for invasive surgery, expensive equipment, and complex preoperative preparation procedures, a new glycoconjugate-based method was developed[171]. The dextran-based nanogels conjugated with a wide spectra emitting fluorescent agents (FDNGs) were developed with an optimum size for specific SLN imaging[172]. The results showed that FDNG could be used as highly efficient molecular imaging probes for stable, specific, selective, safe and non-invasive SLN mapping and thus have the potential for use in bioimaging of SLN for detection of cancer metastasis.

The recognition, labeling, and imaging of target cells have been widely applied in many areas of medicine and cell biology for the early diagnosis of disease, cancer metastasis, cell development, cell separation and targeted drug delivery[173]. Magnetic resonance imaging (MRI), an imaging technique, is widely used for the study of neurologic diseases in humans. In brain diseases, antibody-mediated detection of broad-spectrum inflammation biomarkers is being replaced by carbohydrate-mediated detection of brain-specific inflammation markers

(such as CD62 proteins) because of the numerous drawbacks associated with the former technique[174]. The affinity of CD62 proteins for their cognate ligand molecule type, carbohydrate- sialyl Lewis^X (sLe^x), was exploited to design T_2-type nano-sized glycosylated contrast agents GNP-sLe^x [100]. This GNP-MRI method selectively detects CD62E as a biomarker which is displayed on the "blood-side" of the blood-brain barrier (BBB) but is indicative of pathology on the "brain-side". Also, the in vivo model did not show any BBB breakdown and the GNPs efficiently cleared post-detection without the induction of toxicity. Moreover, Galactose conjugated fluorescent nanoparticles have shown promising effectiveness in identifying liver cancer cells in the blood and this was demonstrated by laser confocal scanning microscopy and flow cytometric analysis[175]. A summary of glycoconjugate nanocarriers used for application in imaging techniques is given in Table 3.

Table 3: Overview of bioimaging nanoscale devices developed using glycans as grafting molecules

Application	Nature of nanoparticle	Binding sugar	Target cells/tissue	Model	Imaging Technique	Properties	Ref
Quantum dots	Cadmium sulfide	Lactobionic acid	Hepatocytes	*In vitro*	Fluorescence bioimaging	Enhanced biocompatibility	[176]
Contrast enhancer	Dendrimer	Lactobionic acid	HCC	*In vivo*	Computer tomography	Enhanced biocompatibility and stable colloidal formulation	[177]
	Dendrimer	Lactobionic acid	HCC	*In vivo*	Computer tomography	High in-vivo biodistribution	[178]
	Fe_3O_4	Lactobionic acid	Hepatocytes	*In vivo*	Magnetic resonance	High liver specificity, low non-specific distribution	[179]
	Fe_3O_4	Dextran	Brain, tumor cells	*In vivo*	Magnetic resonance	Specificity to tumor cells and macrophages	[180]
	Fe_3O_4	Lactobionic acid	HCC	*In vivo*	Magnetic resonance	High colloidal stability, biocompatibility, hemocompatibility	[181]
	Fe_3O_4	Sialyl Lewis^X (sLe^x)	Brain inflammation	*In vivo*	Magnetic resonance	biocompatibility, no blood brain barrier breakage, high specificity	[100]
	Polymeric	Lactobionic acid	HepG2	*In vitro*	Magnetic resonance	Targeted drug delivery, bioimaging	[182]
	Polymeric	Lactobionic acid	Hepatocytes	*In vivo*	Computer tomography	Specific targeting, Low non-specific distribution	[183]
	Polymeric	Lactobionic acid	Hepatocytes	*In vivo*	Computer tomography	Enhanced specificity to liver	[184]
Fluorescent agent	Carbon nanospheres	Lactobionic acid	Hepatocytes	*In vitro*	Fluorescence bioimaging	Enhanced stability, low cytotoxicity	[185]
	Chitin nanocrystals	Mannose	-	-	Fluorescence bioimaging	Enhanced targetability	[186]
	Nanogels	Dextran	Sentinel lymph nodes	*In vivo*	Fluorescence bioimaging	specific, stable, selective, non-invasive and safe SLN mapping	[172]
	Liposomes	Mannose	Macrophages	*In vivo*	PET Imaging	Specific targeting, low non-specific distribution	[187]
	Gold	Lactobionic acid	HepG2	*In vitro*	Fluorescence bioimaging	Low non-specific targeting	[188]
	Silica	Lactobionic acid	BEL-7404	*In vitro*	Fluorescence bioimaging	Increased signal amplification, enhanced photostability	[175]
	Polymeric	Lactobionic acid	CHO	*In vitro*	Fluorescence bioimaging	Increased binding capacity	[189]

Lectin-mediated drug targeting

Targeted delivery of drugs is crucial in enhancing the efficacy of medical therapy. Sugar-binding proteins – lectins - are expressed on the surface of a large variety of mammalian cells where they may act as signaling molecules, recognition molecules and adhesion molecules for the targeted delivery of bioactive components by exploiting their binding affinity to specific monosaccharides or oligosaccharides. The therapeutic applications of carbohydrates as a drug carrier system are outlined in Table 4 and are additionally illustrated in Figure 7.

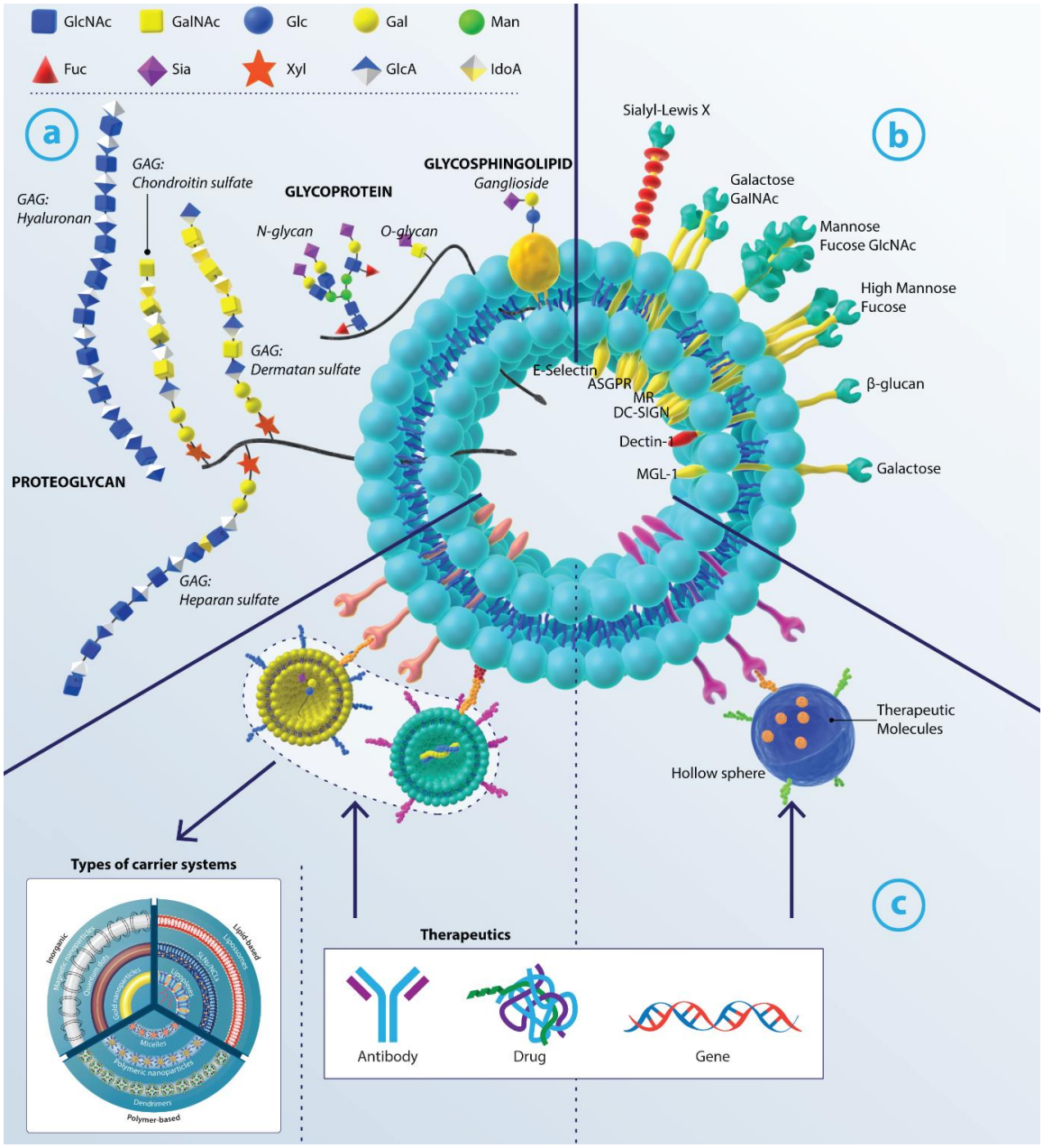


Figure 7: Graphical illustration of cell surface glycans, lectin receptors and glycan-based therapeutic systems. A) Glycans can be found attached to proteins and lipids on the cell

surface. Some of the glyco-conjugates depicted here are glycoproteins and proteoglycans in which glycans are attached to proteins and glycosphingolipids involving lipid-based glyco-conjugates. B) Schematic representation of C-type lectin receptors that bind to carbohydrates in a calcium-dependent manner. Conserved carbohydrate recognition domains mediate the carbohydrate recognition activity of these lectin receptors. C) Glyco-therapeutics can be broadly divided into two subclasses – glycoconjugates as therapeutics and glycoconjugates as systems for therapeutic application.

Disease-specific applications of glycoconjugates

Human immunodeficiency virus (HIV)

Human immunodeficiency virus (HIV) is a type of RNA virus more specifically known as a retrovirus which causes irreparable damage to the immune system of the host. The antiretroviral drugs synthesized for the treatment of HIV infections have been found successful in inhibiting reverse transcriptase and thus preventing the replication of HIV by halting the synthesis of viral DNA. However, one of the major obstacles in making these drugs available clinically is their dose-limiting toxicity. Recent research shows that this limitation can be overcomed by entrapping these drugs in colloidal carrier systems like liposomes which are preferentially uptaken by cells of the mononuclear phagocyte system (MPS) - Macrophages[190]. Cells of macrophage lineage contribute to the pathogenesis of HIV infection substantially owing to their ability to spread virus particles to CD4-lymphocytes and their explicit dynamics of HIV replication which makes them an ideal target for the delivery of HIV drugs[191]. It has been observed that the macrophages and the other cells of MPS express various glycan-specific lectin-like receptors such as asialoglycoprotein receptors and Fc receptors on their surface. Thus, when the delivery system is conjugated with a glycan moiety such as mannose, galactose, lactose or fructose, it would effectively deliver the drug to the infected macrophages via the lectin-carbohydrate interaction[192].

When liposomes coated with chemically modified galactose were synthesized, they showed prolonged carrier potential that was greater than the unmodified liposomes with significantly low AZT drug release in 24 h[193]. Along with sustained drug release, the cellular uptake of the encapsulated drug was significantly increased in the case of glycan-modified liposomes.

Similarly, the galactosylated liposomal vesicular system showed prolonged release carrier potential along with the alteration in the biodistribution of the stavudine drug by targeted delivery to liver, spleen, and lungs that was more than that of the uncoated liposomal formulation[194]. On labeling the drug, plain liposomes and galactosylated liposomes with ^{99m}Tc to study their biodistribution profiles *in vivo,* it was observed that the drug had a very short plasma half-life and this can be increased by using liposomes as they were able to target and be retained in the liver for longer. It was also observed that the galactosylation of liposomes increased their accumulation in liver and decreased the bone-marrow cytotoxicity of the drug[195]. Liposome systems were also utilized for the delivery of genes to macrophages for the inhibition of DNA replication[161b]. Although the liposomes showed promising results as non-viral gene delivery vehicles on their own, conjugation with galactose further enhanced their targetability towards macrophages.

Dendrimers are actively being used as a controlled and targeted delivery system and one such example is poly (propylene imine) (PPI). PPI dendrimers were synthesized and used for the targeted delivery of the anticancer drug efavirenz to macrophages[196]. Similarly, Mannosylated fifth-generation Poly(propylene imine) dendrimers (MPPI) were studied as a potential drug carrier for targeted and sustained delivery of the antiretroviral drug lamivudine (3TC) in contrast to plain PPI dendrimers[32a]. Both PPI and MPPI, when loaded with a drug, were observed to display significant anti-HIV activity compared to the free drug. MPPI showed a substantial increase in the entrapment of the drug over that of PPI. Also, the steric crowding at the periphery in MPPI allowed sustained drug release up to 144 h as compared to 24 h in the case of PPI.

Cancer

Our body undergoes many molecular changes in any disease condition and glycosylation of proteins and lipids is one of these [197]. In cancer, atypical glycosylation of affected tissue is often accompanied by the expression of several lectin receptors present on the cell surface displaying an affinity for ligands containing glycan moiety[33b]. With this in mind, researchers have designed glycoconjugated carriers for targeted delivery and a higher uptake of anticancer drugs into the cancerous tissues and cells[198].

Conjugation of polypropylene imine (PPI) dendrimers with high molecular weight dextran was utilized to prevent the renal elimination of PPI and thus to enhance its retention time in the body[199]. The formulation not only depicted enhanced permeation and retention (EPR) but also caused an efficient release of the drug in an acidic environment in tumor vessels as compared to normal tissues and this was clearly observed from *in vitro* drug release studies. Similarly, conjugation of galactose to paclitaxel-loaded nanoparticles was seen to help internalization of the particles in the cells via the asialoglycoprotein (ASGP) receptors present on the surface of hepatoma cells[200]. This interaction can thus be useful in the efficient targeting of hydrophobic anticancer drugs to liver cancer cells.

In most advanced cases of gastric cancer, peritoneal dissemination of tumor cells is a common therapeutic problem as it is associated with trans-lymphatic metastasis. Effective inhibition of peritoneal dissemination by Man/CpG DNA lipoplex observed both *in vitro* and *in vivo* was suggested to be obtained by the activation of immunocompetent cells through mannose receptor-mediated CpG DNA transfer[201]. Additionally, oligomannose-coated liposomes (OMLs) were explored as a delivery system for the anticancer drug and observed to be successfully up taken by mouse peritoneal macrophages and the drug was carried and delivered specifically to metastatic sites in the peritoneal cavity in mice[202]. The clinical application of the OMLs showed similar results in which the peripheral blood-derived healthy human monocytes/macrophages and the peritoneal washes of patients with gastric cancer took up OMLs[203]. The results of this study suggest that the OML-based drug delivery system is effective for the targeted delivery of the drug to peritoneal micrometastasis in gastric cancer patients and thus can be evaluated as a platform for novel intraperitoneal chemotherapy with a minimum dose of drug administration.

Active targeting of cancer cells for the delivery of cytotoxic agents carried by nanoparticles is enabled by selective overexpression of specific membrane receptors present on the cell surface of cancer cells[204]. This approach not only increases the specificity of the drug delivery systems towards cancer cells but also maximizes the efficacy of the drug therapeutic by reducing serious side effects associated with the drug by non-specific delivery of the drug[205]. Active targeting of cancer cell-specific receptors results in the drug delivery by endocytosis of the receptor-bound carriers. Following endocytosis, the carrier system is transported from

early endosomes to lysosomes where the cytotoxic cargo of the carrier system is finally released.

One of the interesting strategies of antitumor therapies that have been explored is the use of lysosomotropic ligand-targeted therapeutics (LTTs) for the delivery of molecules which destabilize lysosomes by induction of lysosomal membrane permeabilization and leakage of hydrolytic enzymes such as cathepsins into the cytoplasm. Cathepsins are lysosomal peptidases belonging to the papain family, which upon re-localization in the cytoplasm, may cause caspase-dependent or independent cell death with or without the involvement of the mitochondria[206].

Mannose-6-phosphate/insulin-like growth factor receptor (M6P/IGF-IIR) participates in the transportation of cellular proteins from the cell surface/trans-Golgi network to lysosomes. The receptor specifically recognizes the mannose-6-phosphate (M6P) carbohydrate moiety and exclusively internalizes systems presenting the M6P group. By taking into consideration the overexpression of IGF-IIR in several human carcinoma cells and its high internalization rate of M6P systems, IGF-IIR has been successfully exploited for the design and development of cancer-targeted drug delivery systems such as M6P functionalized liposomes [207]. The IGF-IIR expression during fibrosis has also been studied and it was observed that the receptor expression was preferentially higher on fibrogenic cells[208]. Thus, researchers have used IGF-IIR as a target for the delivery of drugs for liver fibrosis[208-209].

The conjugation of mannose derivative to the solid lipid nanoparticles for the delivery of large doses of anti-cancer drug doxorubicin (DOX) not only minimized the side effects associated with a large dose of the drug and depicted the sustained release nature *in vitro* but also showed an increase in hemocompatibility and biocompatibility of the system *in vivo* which facilitated targeted delivery of the drug to the tumor site[210].

Drug delivery systems play a vital role in enhancing the therapeutic efficacy of cancer treatments by increasing the safety, stability and efficiency of the drug[211]. However, the drug delivery efficiency remains limited by non-specific targeting of the drug. Among distinct targeting strategies[211a], most promising is the glycosylation-mediated one [212]. In comparison to healthy cellular glycosylation, in cancer, tumor cells often express high levels of sialylation, N-linked glycans, truncated glycans and glycosphingolipids[213]. By taking the

aberrant glycosylation in tumor into consideration, various glycan moieties have been attached to the nanocarriers to acquire tumor-targeting abilities[214] and thus provide new therapeutic opportunities for cancer treatments[215].

Hepatocellular Carcinoma and Hepatitis

Hepatocytes are liver parenchymal cells that constitute 60-80% of the total mass of the liver tissue. Hepatocytes have unique cell surface receptors called asialoglycoprotein receptors (ASGPr) which can bind specifically to β-D-galactose or N-acetylgalactosamine residues[216]. Carriers modified with these glycan moieties thus have an ability to target hepatocytes via the ASGPr-mediated pathway[217]. One of the examples of these types of carriers shows that liposomes containing lactobionic acid possess enhanced liver targetability and cause accumulation of the drug in the liver by specifically targeting parenchymal cells expressing ASGPr receptors[218]. However, to overcome the rapid elimination of the drug-containing liposomes from blood circulation, the liposomes were modified with PEG to create a steric stabilization effect[219].

Glycodendrimers have been introduced as drug carriers in recent years and can be classified as dendrimers with carbohydrate moieties coated, incorporated or attached to a carbohydrate-based, carbohydrate-centered or carbohydrate-coated structure[220]. The coating of carbohydrates on the dendrimeric system showed a reduction in the hemolytic toxicity associated with the terminal NH_2 groups by neutralizing the charges via conjugation with terminal amine groups[221].

Methotrexate conjugated to the mannosylated human serum albumin (Man-HSA) has been used for specific delivery to liver Kupffer cells thus reaching the liver macrophages in large quantities with an improved pharmacokinetic profile to act on *Leishmania* parasites[222]. This glycoprotein-based delivery system carries and releases the bioactive compound to the suitable diseased site through a receptor-mediated endocytotic process. Similarly, a liver cell-specific drug delivery system was developed using biotinylated poly(ethylene glycol) conjugated with galactose containing lactobionic acid[223]. These galactose conjugated nanoparticles when studied as a delivery system for the anti-cancer hydrophobic drugs showed pseudo-zero-order release kinetics over a period of one month.

Block copolymers containing carbohydrates have been synthesized via a metal-free organocatalytic ring-opening polymerization (ROP) reaction of functional trimethylene carbonate (TMC) derivatives[224]. In aqueous solutions, these sugar-functionalized polycarbonate block copolymers can self-assemble into micelles. A study showed that cytotoxicity of doxorubicin (DOX) against HepG2 liver cancer cells significantly increased in galactose-containing micelles over that of glucose-containing micelles and free drug.

Digestive disorders and gastrointestinal diseases

Nanoparticles are often used to increase the bioavailability of orally administrated drugs as nanoparticles interact with the gastrointestinal surface by developing adhesive bonds with different components of the mucosa[225]. The adhesion of nanoparticles to the inner lining of the gut results in localization of delivery of the drug in a specific region of the gut along with an increase in the retention time of the drug in the mucosa[226]. The adhesion interaction with the mucosa for conventional nanoparticles is non-specific and appears mainly because of the Physico-chemical properties of the drug delivery system[227]. To overcome this limitation, the delivery systems enriched in mannose are being extensively studied to exploit their high binding affinity to the mannose-binding lectins (MBL) expressed on the lymphoid and non-lymphoid cells of the gut[228]. Although the conjugation of Acyclovir (ACV)- entrapped liposomes (ACV-lip) with mannose did not result in an increase in entrapment efficiency and loading efficiency of the system, it nevertheless enhanced the bioavailability of the poorly absorbable drug significantly when studied *in vitro*[229]. This result accords with the hypothesis of the interaction of the mannose containing system with MBLs expressed by gut cells which could promote substantial drug transport through the gi-tract. In a similar study, mannosylated nanoparticles showed a strong capacity to adhere to the outer layer of the ileum (mucus layer) region of the small intestine and penetrate the Peyer's patches[226]. The strong bioadhesive capacity and tissue affinity to gut-associated lymphoid tissue of mannosylated nanoparticles after their oral administration suggests that these particles can be used as a promising vehicle for oral drug delivery. In addition, non-peptidic ligands and glycan moieties attached to nanoparticles for targeted delivery to M cells have shown promising results as delivery systems for oral immunization[230].

Table 4: Summary of therapeutic applications of glycoconjugates

Targeted disease	Type of carrier	Conjugate sugar	Carrier compound	Active compound/ drug	Targeted moiety/ cells	In vitro model	In vivo model	Rote of administration	Effect of glycosylation/ Application	Ref.
HIV	Liposomes	Galactose	Egg PC: CH: PE in different molar ratios	Azidothymidine	Monocytes/ macrophages Liver, spleen, lymph nodes and lungs	-	Male albino rats (SD strain) (100 ± 20 g)	Intravenous injection	Enhanced cellular uptake, sustained drug release, high distribution of drug and reduction in side-effects associated with the drug	[193]
	Liposomes	Galactose Fucose Mannose	Lipid mixture of egg PC, CH, DMPE (Gal-DMPE) in molar ratio 7:2:1	Stavudine	Macrophages and Hepatocytes	-	Male albino rats (SD stain) (100 ± 20 g)	Intravenous injection	Enhanced entrapment efficiency, smaller particle size, sustained release of drug, decrease in surface charge of liposomes, localized delivery of the drug.	[194]
	Liposomes	Galactose	Lipid mixture of egg PC, CH, DMPE (Gal-DMPE) in molar ratio 7:2:1	Radio-labeled stavudine	Macrophages	HIV-1 Infected MT2 cell line	Strain A mice (25-30 g) (8-12 weeks old)	Intravenous injection	Increase in accumulation of liposomes in the liver, decrease in bone-marrow cytotoxicity of the drug	[195]
	Liposomes	Mannose	Man-4-Chol/DOPE (6:4), DC-Chol/DOPE (6:4), DOTMA/DOPE (1:1), Man-4-Chol/DC-Chol/DOPE (3:3:4)	Plasmid DNA encoding luciferase gene (pCMV-Luc)	Macrophages	Mice macrophages	ICR mice (5 weeks old)	Intravenous injection	Selective delivery of plasmid DNA to the liver, higher transfection activity, applicability of the system for targeted delivery to splenic macrophages, highly efficient non-viral gene transfer both in vivo and in vitro via recognition by mannose receptors	[161b]
	Dendrimers	Mannose	Fifth-generation poly(propylene imine) dendrimers	Lamivudine (3TC)	Macrophages	MT2 cell line	-	-	Improved safety and efficacy of the drug, a significant increase in entrapment efficiency, sustained drug release, significant increase in cellular uptake	[32a]
	Dendrimers	Mannose	Poly(propylene imine) dendrimer	Efavirenz	Monocytes/ macrophages	Hepatoma (Hep G2) cell line	-	-	Negligible cytotoxicity, high drug entrapment efficiency, scanty hemotoxicity and a significant increase in cellular uptake	[196]

Cancer	Liposomes	Mannose-6-phosphate	DPPC and CH (50:50), DPPC: CH: Chol-M6P (50:30:20)	C6-Ceramide	Tumor cells	HDF and MCF7 cells	-	-	Selective induction of apoptosis in cancer cells	[207d]
	Liposomes	Mannose	-	Plasmid DNA	Dendritic cells	Primary mbmDCs from C57Bl/6J mice	C57Bl/6J mice	Subcutaneous injection	A promising application for direct *in vivo* targeting to DCs. Observed long-lasting antitumor response and significant memory response	[231]
	Liposome	Mannose	L-α-Phosphatidylcholine, cholesterol	^{64}Cu	Tumor-associated macrophages	Murine bone marrow-derived macrophages	Female FVB mice (6-8 weeks old)	Intravenous injection	Confirmation of the role of M2 subtype of macrophages in cancer progression, targeted delivery of radioactive agents	[187]
	Liposomes	D-Mannose	2X3- DOPE	Plasmid DNA and RNA	Dendritic cells	DC progenitor cells, immature DC cells obtained from the bone marrow of C57Bl/6J mice	Male C57Bl/6J mice (10-14 weeks old)	Intravenous injection	Efficient and targeted delivery of pDNA and RNA observed in vitro, the ability to deliver RNA in vivo with the induction of antitumor response	[232]
	Liposomes	Mannose	Man/CpG DNA lipoplex	CpG DNA	Peritoneal macrophages	Mouse peritoneal macrophages	Male Balb/c mice (4 weeks old)	Intraperitoneal administration	The increased survival rate of mice treated with mannosylated lipoplex, efficient immunotherapy Inhibition of tumor cells proliferation in the greater omentum and the mesentery.	[201]
	Liposomes	Fucose	DPPC, Chol, ganglioside, DCP, DPPE mixed in different molar ratios	Cisplatin	Pancreatic cancer cells	BxPC-3 cell line	Nude mice (4-6 weeks old)	Intravenous injection	Targeted delivery of the drug, effective inhibition of CA19-9 producing cancer cells in vitro and tumor growth in vivo	[233]
	Nanoparticles	Mannan	Poly (ε-caprolactone)-PEG-poly(ε-	Human basic fibroblast growth	Dendritic cells	-	Female C57BL/6 mice (5 weeks old)	Subcutaneous injection)	Improved humoral immunity due to targeted delivery to dendritic cells.	[234]

			caprolactone) (PCEC) polymer	factor (bFGF)						
	Nanoparticles	Mannose	Chitosan	Plasmid DNA encoding murine IL-12	Dendritic cells	NCTC 3749 cell line	BALB/c mice (6-8 weeks old)	Injected in the tumor	Small size of mannosylated particles, low cytotoxicity, sustained gene expression.	[235]
	Nanoparticles	Analogue of Mannose-6-Phosphate (M6C)	Mesoporous silica nanoparticles (MSN)	One- and two-photon photosensitizers	Prostate cancer cells	LNCaP cell line	-	-	Efficient targeting, imaging and photodynamic therapy	[207b]
	Polymer conjugate	Mannan	Methotrexate (MTX)	Methotrexate	Lung	A549 cell line, B16 cell line	Female and Male (BALB/c X DBA/2)F1 (CD_2F_1) mice (12- 24 weeks old)	Intraperitoneal administration	Improved antitumor activity compare to free methotrexate with intraperitoneal administration of the drug, potential application for the treatment of advanced ovarian cancer.	[236]
	Polymer conjugate	Fucose	pLK-Fucose	Phosphorothioate oligonucleotide	Lung	A549 cell line	-	-	Reduction in toxicity of carrier system, selective inhibition of gene expression, increase in uptake	[237]
	Human serum albumin	Mannose-6-Phosphate	HSA-M6P	Doxorubicin	Subcutaneous tissue	B16-F10 cell line, C26 cell line	Male C57BL/6 and Balb/c mice (20-25 g)	Intravenous injection	Targeted therapy of tumors through IGF-IIR receptors was achieved, high cellular uptake, increase in internalization and enhanced efficacy of the drug	[207a]
Hepatocellular carcinoma (HCC)	Liposomes	Lactobionic acid	Lipid mixture of HSPC: CH: CHS-ED-LA (60:40:0/10)	Doxorubicin	Liver	-	Female KM mice (18-22 g)	Intravenous injection	Enhanced liver targetability and accumulation of liposomes in liver	[218]
	Liposomes	Lactobionic acid	Lipid mixture of HSPC:CH: PEG_{2000}-CHEMS (60:40:2), HSPC: CH: CHS-ED-LA: PEG_{2000}-CHEMS (60:30:10:2)	Doxorubicin	Liver	-	Female KM mice (18 – 22 g)	Intravenous injection	Controlled drug delivery to hepatocytes, Efficient liver targetability, enhanced accumulation of the drug in liver, enhanced therapeutic benefits such as the decrease in liver damage and enhanced therapeutic efficiency	[219]

	Nanoparticles	Galactose	Bovine serum albumin (BSA)	Oridonin	Hepatocytes	-	-	-	Sustained-release profile in vitro, crosslinking degree influences the rate of release	[238]
	Nanoparticles	Galactose	PBLG/PEG	Paclitaxel	Hepatocytes	P388 cell line, SK-Hep01 cell line, HepG2 cell line	-	-	Selective delivery of the drug to HepG2 cells via ASGP receptors.	[239]
Liver fibrosis	Polymeric micelle	Mannose-6-Phosphate	GDC-0449	PEG-PCD	Liver	-	C57BL/6 male mice (10-12 weeks old)	Intravenous injection	Increased retention and accumulation of drug in the target site (Liver)	[209]
	Human serum albumin	Mannose-6-Phosphate	M6PHSA	Doxorubicin	Hepatic stellate cells (HSC)	HSC isolate from male Wistar rats (>400 g)	Male Wistar rats (220-240 g)	Intravenous injection	Significant inhibition of HSC proliferation in vitro, successful delivery to HSCs in vivo	[240]
	Human serum albumin	Mannose-6-Phosphate	HSA-M6P	Mycophenolic acid (MPA)	Liver	3T3 fibroblasts cell line	Male wistar rats (220-240 g)	Intravenous injection	IGF-IIR receptor upregulated in fibroblast-like cells in fibrotic liver, selective drug delivery at early stage of fibrotic diseases	[208]
Hepatitis B	Microcapsules	Galactose	Poly(vinyl galactose ester-co-methacryloxyethyl trimethylammonium chloride) (PGEDMC) / poly(styrenesulfonate) (PSS)	Acyclovir	Hepatocytes	-	-	-	Sustained drug release, promising system to encapsulate and deliver various therapeutic agents to hepatic cells.	[241]
	Vesicles – nonionic surfactant based vesicles (niosomes)	Mannan	Span 60: Ch: stearylamine in molar ratios 6: 3: 1	Plasmid DNA	Gut associated lymphoid tissue (GALT) Dendritic cells	-	BALB/c mice (4-8 week old)	Oral administration	Stabilization of vesicles in GI environment, safe, economic and stable system with potential for application in oral delivery of vaccines	[242]

Malaria	Dendrimers	Galactose	Fourth generation poly-L-lysine dendrimer	Chloroquine phosphate	macrophages	-	Male albino rats (SD strain) (130 ± 10 g)	Intravenous injection	Reduction in hemolytic toxicity of poly-L-dendrimers.	[221]
Tuberculosis	Dendrimers	Mannose	5G EDA PPI dendrimer	Rifampicin	Alveolar macrophages	Vero cells (ATCC-CCL -81)	-	-	Biocompatible system with site-specific delivery and enhanced cellular uptake.	[243]
Pulmonary diseases	Liposomes	Mannose	DSPC, CH, F-DHPE and Man-C4-Chol in different molar ratios	-	Alveolar macrophages	Primary culture rat alveolar macrophages	Male wistar rats (250 – 300 g) (8 weeks old)	Intratracheal administration	Efficient and selective targeting to alveolar macrophages, high cellular uptake with increase in mannose content	[244]
Rheumatoid arthritis	Liposomes	Mannose	ACHx/DC-Chol/DOPE	Plasmid DNA expressing human IL-10	Tumor-associated macrophages	-	-	-	Successfully synthesized neoglycolipids, high mannose type neoglycolipids actively targeted macrophages	[245]
AM-associated diseases	Liposomes	Mannose	HSPC, CH, DCP and Mannose in different molar ratios	-	Alveolar macrophages	SD rat alveolar macrophages	Male SD rats (190-220 g)	Sprayed into the lungs with liquid MicroSprayer™	Higher cellular uptake of mannosylated liposomes by NR8383, efficient and targeted aerosolized delivery to alveolar macrophage	[246]
-	Liposomes	Mannose	Egg PC: M_3-DPPE/M_1-DPPE in two different molar ratios	Protein	Dendritic cells	Human MoDCs, murine bone-marrow derived DCs	-	-	Increase in uptake for tri-mannose derivatives, increase in proliferation of primed T cells in vitro, no increase in dendritic cell activation	[247]
-	Liposomes	Galactose	Lipid mixture of soybean phosphatidylcholine (SPC) : Cholesterol : NGPE in molar ratios of 55 : 44: 11.2	-	Liver – Parenchymal cells, Hepatocytes	-	Male swiss albino mice (28 – 30 g)	Intravenous injection	Significant increase in uptake in liver, decrease in uptake of drug in spleen, specific binding to parenchymal cells. The system effective for delivery of drugs, enzymes, genetic materials, anti-sense oligonucleotides selectively to liver parenchymal cells.	[248]

-	Liposomes	Mannose	Lipid mixture of soybean phosphatidylcholine (SPC): Cholesterol: NGPE in molar ratios of 55:44: 11.2	-	Liver – Parenchymal cells, Hepatocytes	-	Male swiss albino mice (28 – 30 g)	Intravenous injection	Significant increase in uptake in liver, decrease in uptake of drug in spleen, specific binding to parenchymal cells. The system effective for selective delivery of therapeutic agents to liver parenchymal cells.	[248]
-	Superparamagnetic nanoparticles	Galactose	Fe_3O_4	-	Hepatocytes	HepG2 cell line			Low toxicity, high cellular uptake.	[249]
-	Nanoparticles	Galactose	Polyphosphoramidate (PPA)	DNA	Hepatocyte	HeLa cell line, HepG2 cell line, Primary rat hepatocytes	-	-	Lower DNA compaction capacity with increase in galactose substitution, significantly higher gene transfection efficiency observed for ternary nanoparticles and reduction in cytotoxicity was observed with increase in galactose substitution of nanoparticles.	[250]

Concluding remarks and future perspectives

Glycosylation is emerging as a versatile technique for achieving effective therapy and diagnosis as a result of the distinctive recognition between the sugar molecules and the respective receptors. The knowledge of various lectin receptors present in our body, the cells/tissues they are expressed on and the glycans that can be recognized by them along with different glycosylation strategies that can be employed to achieve the desired glycoconjugation of the materials in focus is essential for the design of efficient glycotherapeutics. Recent advances in glycotheraputics show that the researchers have exploited this knowledge for the design of various glycan-based drug molecules and drug delivery systems. However, lectins have mostly been used as target moieties in this process. There is a huge area of research yet to be explored by reversing the role of glycans and lectins and using glycan moieties in our body as potential targets.

It is important to understand that the recognition of multiple glycan moieties by targeted receptors is an important issue associated with glycan – lectin interaction at the moment and one that could significantly affect the targeting efficiency of the system. For example, a serum-type mannan-binding protein, a specific lectin for the innate immune system, could also recognize D-mannose, L-fucose, and N-acetyl-D-glucosamine[251]. This limitation could significantly reduce the therapeutic efficiency of the delivery system and may also cause undesirable effects of the carrier drug *in vivo*. Thus, glycan-lectin interaction needs to be explored in further detail. It will be useful if research is done to understand the changes incurred in the interactions based on the location of the receptors in the body to engineer the materials to obtain site-tailored interaction. This will greatly improve the specificity of the glycoconjugated delivery systems and thus the therapeutic efficiency of the drug along with a decrease in the side effects associated with non-specific targeting.

The reason carbohydrates are being acknowledged as a promising tool for forming targeted delivery systems is because of their multiple hydroxyl groups and the ability to be modified in a facile manner. However, several issues need to be addressed for the design of glycosylated delivery systems for practical applications. One such issue is the bioconjugation of delivery systems by glycosylation. This may result in a variation in physicochemical properties of delivery vehicles such as size distribution, surface charge, and biocompatibility as well[252]. Although studies have shown that glycoconjugation has improved the targetability of the

delivery systems, the effect on encapsulation efficiency, size and thus the application has not been correlated. Also, the interaction of glycosylated nanocarriers with other cells, tissues and organs beyond those targeted impose a limitation on the system. Recent studies have shown that the glycosylation might affect other organs by off-target effect, however, further investigation is still required[253].

The clinical application of glycosylated strategies is challenging because of the safety and reproducibility concerns[213a]. Recent studies have shown that the combination of glycosylation strategies and immunological therapy demonstrates a potential for cancer therapy[254]. The strategy can be exploited to enhance the properties and the clinical applicability of the glycosylated systems for various applications including cancer.

In summary, we have reviewed different strategies that can be used for functionalising glycans to materials, a variety of lectin receptors and their role in glycotherapy, and the therapeutic applications of different glycoconjugates that have been synthesized over the last few decades. Although the research in glycotherapeutics has exponentially increased in last few years and novel and efficient systems for exploiting the understanding of glycan-lectin interactions have been developed to achieve the ultimate goal of clinical translation, further innovations are crucial in the field of discovery of more-specific receptors as well as in their carbohydrate ligands and the development of practical fabrication methods for glycosylated drugs and delivery systems.

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References:

[1] a) T. Ju, V. I. Otto, R. D. Cummings, *Angewandte Chemie International Edition* **2011**, 50, 1770; b) F.-G. Hanisch, *Biological chemistry* **2001**, 382, 143; c) T. Wennekes, R. J. van den Berg, R. G. Boot, G. A. van der Marel, H. S. Overkleeft, J. M. Aerts, *Angewandte Chemie International Edition* **2009**, 48, 8848; d) R. Apweiler, H. Hermjakob, N. Sharon, *Biochimica et Biophysica Acta (BBA)-General Subjects* **1999**, 1473, 4; e) C. Somerville, *Annu. Rev. Cell Dev. Biol.* **2006**, 22, 53.

[2] a) A. Mohanty, M. a. Misra, G. Hinrichsen, *Macromolecular materials and Engineering* **2000**, 276, 1; b) S. Weinbaum, J. M. Tarbell, E. R. Damiano, *Annu. Rev. Biomed. Eng.* **2007**, 9, 121.

[3] a) C. R. Bertozzi, L. L. Kiessling, *Science* **2001**, 291, 2357; b) T. Rademacher, R. Parekh, R. Dwek, *Annual review of biochemistry* **1988**, 57, 785; c) A. Varki, *Glycobiology* **1993**, 3, 97.

[4] a) J. W. Dennis, I. R. Nabi, M. Demetriou, *Cell* **2009**, 139, 1229; b) R. B. Parker, J. J. Kohler, *ACS chemical biology* **2009**, 5, 35; c) K. J. Yarema, C. R. Bertozzi, *Genome Biology* **2001**, 2, reviews0004. 1.

[5] a) R. D. Cummings, *Molecular BioSystems* **2009**, 5, 1087; b) H. J. Gabius, H. C. Siebert, S. André, J. Jiménez-Barbero, H. Rüdiger, *ChemBioChem* **2004**, 5, 740; c) S. M. Muthana, C. T. Campbell, J. C. Gildersleeve, *ACS chemical biology* **2012**, 7, 31.

[6] a) R. S. Haltiwanger, J. B. Lowe, *Annual review of biochemistry* **2004**, 73, 491; b) Y. Van Kooyk, G. A. Rabinovich, *Nature immunology* **2008**, 9, 593; c) K. Ohtsubo, J. D. Marth, *Cell* **2006**, 126, 855; d) R. G. Spiro, *Glycobiology* **2002**, 12, 43R.

[7] B. Ernst, J. L. Magnani, *Nature Reviews Drug Discovery* **2009**, 8, 661.

[8] a) X. Zhu, R. R. Schmidt, *Angewandte Chemie International Edition* **2009**, 48, 1900; b) T. J. Boltje, T. Buskas, G.-J. Boons, *Nature chemistry* **2009**, 1, 611; c) B. Lepenies, J. Yin, P. H. Seeberger, *Current opinion in chemical biology* **2010**, 14, 404.

[9] a) J. Zaia, *Chemistry & biology* **2008**, 15, 881; b) W. R. Alley Jr, B. F. Mann, M. V. Novotny, *Chemical reviews* **2013**, 113, 2668.

[10] S. T. Laughlin, C. R. Bertozzi, *Proceedings of the National Academy of Sciences* **2009**, 106, 12.

[11] T. Okajima, K. D. Irvine, *Cell* **2002**, 111, 893.

[12] A. Helenius, M. Aebi, *Science* **2001**, 291, 2364.

[13] R. Kannagi, *Current opinion in structural biology* **2002**, 12, 599.

[14] K. Nishiwaki, Y. Kubota, Y. Chigira, S. K. Roy, M. Suzuki, M. Schvarzstein, Y. Jigami, N. Hisamoto, K. Matsumoto, *Nature cell biology* **2004**, 6, 31.

[15] J. D. Marth, *Nature cell biology* **2008**, 10, 1015.

[16] S. Smyth, A. Heron, *Nat Med* **2006**, 12, 75.

[17] W. Watkins, *Transfusion medicine* **2001**, 11, 243.

[18] a) E. A. Kabat, S. Leskowitz, *Journal of the American Chemical Society* **1955**, 77, 5159; b) W. Morgan, W. M. Watkins, *British journal of experimental pathology* **1953**, 34, 94.

[19] a) J. McLean, *American Journal of Physiology-Legacy Content* **1916**, 41, 250; b) R. Lever, B. Mulloy, C. P. Page, *Heparin-a century of progress*, Springer Science & Business Media, **2012**.

[20] a) K. Brinkhous, H. Smith, E. Warner, W. Seegers, *American Journal of Physiology-Legacy Content* **1939**, 125, 683; b) U. Lindahl, G. Bäckström, M. Höök, L. Thunberg, L.-A. Fransson, A. Linker, *Proceedings of the National Academy of Sciences* **1979**, 76, 3198.

[21] D. E. Humphries, G. W. Wong, D. S. Friend, M. F. Gurish, W.-T. Qiu, C. Huang, A. H. Sharpe, R. L. Stevens, *Nature* **1999**, 400, 769.

[22] A. R. Dochez, O. T. Avery, *Journal of Experimental Medicine* **1917**, 26, 477.

[23] M. Heidelberger, O. T. Avery, *The Journal of experimental medicine* **1923**, 38, 73.

[24] a) M. Heidelberger, M. M. Dilapi, M. Siegel, A. W. Walter, *The Journal of Immunology* **1950**, 65, 535; b) C. M. MAcLEOD, R. G. Hodges, M. Heidelberger, W. G. Bernhard, *Journal of Experimental Medicine* **1945**, 82, 445; c) T. Francis, W. S. Tillett, *Journal of Experimental Medicine* **1930**, 52, 573.

[25] M. A. Barocchi, S. Censini, R. Rappuoli, *Vaccine* **2007**, 25, 2963.

[26] A. Schatz, E. Bugle, S. A. Waksman, *Proceedings of the Society for Experimental Biology and Medicine* **1944**, 55, 66.
[27] L.-X. Wang, J. V. Lomino, *ACS chemical biology* **2011**, 7, 110.
[28] a) G. J. Kelloff, J. M. Hoffman, B. Johnson, H. I. Scher, B. A. Siegel, E. Y. Cheng, B. D. Cheson, J. O'Shaughnessy, K. Z. Guyton, D. A. Mankoff, *Clinical Cancer Research* **2005**, 11, 2785; b) M. Reivich, D. Kuhl, A. Wolf, J. Greenberg, M. a. Phelps, T. Ido, V. Casella, J. Fowler, E. Hoffman, A. Alavi, *Circulation research* **1979**, 44, 127.
[29] S. M. Ametamey, M. Honer, P. A. Schubiger, *Chemical reviews* **2008**, 108, 1501.
[30] a) G.-J. Boons, *Carbohydrate chemistry* **2010**, 36, 152; b) L. Cipolla, F. Peri, C. Airoldi, *Anti-Cancer Agents in Medicinal Chemistry (Formerly Current Medicinal Chemistry-Anti-Cancer Agents)* **2008**, 8, 92; c) P. V. Chang, C. R. Bertozzi, *Chemical Communications* **2012**, 48, 8864.
[31] a) M. Monsigny, A.-C. Roche, P. Midoux, R. Mayer, *Advanced Drug Delivery Reviews* **1994**, 14, 1; b) C. Unverzagt, S. André, J. Seifert, S. Kojima, C. Fink, G. Srikrishna, H. Freeze, K. Kayser, H.-J. Gabius, *Journal of medicinal chemistry* **2002**, 45, 478; c) C. Bies, C.-M. Lehr, J. F. Woodley, *Advanced Drug Delivery Reviews* **2004**, 56, 425; d) M. S. Wadhwa, K. G. Rice, *Journal of drug targeting* **1995**, 3, 111.
[32] a) T. Dutta, N. K. Jain, *Biochimica et Biophysica Acta (BBA)-General Subjects* **2007**, 1770, 681; b) J. Wang, T. Duan, L. Sun, D. Liu, Z. Wang, *Analytical Biochemistry* **2009**, 392, 77.
[33] a) B. G. Davis, M. A. Robinson, *Current Opinion in Drug Discovery and Development* **2002**, 5, 279; b) A. Agarwal, S. Saraf, A. Asthana, U. Gupta, V. Gajbhiye, N. K. Jain, *International journal of pharmaceutics* **2008**, 350, 3.
[34] E. Kolomiets, M. A. Swiderska, R. U. Kadam, E. M. Johansson, K. E. Jaeger, T. Darbre, J. L. Reymond, *ChemMedChem: Chemistry Enabling Drug Discovery* **2009**, 4, 562.
[35] a) T. Darbre, J.-L. Reymond, *Current topics in medicinal chemistry* **2008**, 8, 1286; b) E. Kolomiets, E. M. Johansson, O. Renaudet, T. Darbre, J.-L. Reymond, *Organic letters* **2007**, 9, 1465.
[36] S. Magnusson, T. Berg, *Biochemical Journal* **1989**, 257, 651.
[37] a) B. Friedman, K. Vaddi, C. Preston, E. Mahon, J. R. Cataldo, J. M. McPherson, *Blood* **1999**, 93, 2807; b) U. Gazi, L. Martinez-Pomares, *Immunobiology* **2009**, 214, 554; c) E. J. McKenzie, P. R. Taylor, R. J. Stillion, A. D. Lucas, J. Harris, S. Gordon, L. Martinez-Pomares, *The Journal of Immunology* **2007**, 178, 4975.
[38] a) C. K. Tang, J. Lodding, G. Minigo, D. S. Pouniotis, M. Plebanski, A. Scholzen, I. F. McKenzie, G. A. Pietersz, V. Apostolopoulos, *Immunology* **2007**, 120, 325; b) S. S. Diebold, M. Kursa, E. Wagner, M. Cotten, M. Zenke, *Journal of Biological Chemistry* **1999**, 274, 19087.
[39] a) A. Lin, Y. Liu, Y. Huang, J. Sun, Z. Wu, X. Zhang, Q. Ping, *International journal of pharmaceutics* **2008**, 359, 247; b) M. Nishikawa, M. Yamauchi, K. Morimoto, E. Ishida, Y. Takakura, M. Hashida, *Gene therapy* **2000**, 7, 548; c) L. Fiume, G. Di Stefano, *European Journal of Pharmaceutical Sciences* **2010**, 40, 253.
[40] a) D. Fiete, V. Srivastava, O. Hindsgaul, J. U. Baenziger, *Cell* **1991**, 67, 1103; b) G. Kempka, P. Roos, V. Kolb-Bachofen, *The Journal of Immunology* **1990**, 144, 1004.
[41] a) P. Opanasopit, K. Shirashi, M. Nishikawa, F. Yamashita, Y. Takakura, M. Hashida, *American Journal of Physiology-Gastrointestinal and Liver Physiology* **2001**, 280, G879; b) V. L. Shepherd, Y. Lee, P. H. Schlesinger, P. D. Stahl, *Proceedings of the National Academy of Sciences* **1981**, 78, 1019; c) P. Opanasopit, M. Nishikawa, F. Yamashita, Y. Takakura, M. Hashida, *Journal of drug targeting* **2001**, 9, 341.
[42] T. J. Nicholls, K. L. Green, D. J. Rogers, J. D. Cook, S. Wolowacz, J. D. Smart, *International journal of pharmaceutics* **1996**, 138, 175.
[43] P. Nantwi, D. Cook, D. Rogers, J. Smart, *Journal of drug targeting* **1997**, 5, 45.
[44] E. L. Vodovozova, E. V. Moiseeva, G. K. Grechko, G. P. Gayenko, N. Nifant'Ev, N. Bovin, J. Molotkovsky, *European Journal of Cancer* **2000**, 36, 942.

[45] C. M. Fehres, H. Kalay, S. C. Bruijns, S. A. Musaafir, M. Ambrosini, L. Van Bloois, S. J. Van Vliet, G. Storm, J. J. Garcia-Vallejo, Y. Van Kooyk, *Journal of Controlled Release* **2015**, 203, 67.
[46] S. Park, G.-H. Kim, S.-H. Park, J. Pai, D. Rathwell, J.-Y. Park, Y.-S. Kang, I. Shin, *Journal of the American Chemical Society* **2015**, 137, 5961.
[47] M. A. Boks, M. Ambrosini, S. C. Bruijns, H. Kalay, L. Van Bloois, G. Storm, J. J. Garcia-Vallejo, Y. Van Kooyk, *Journal of Controlled Release* **2015**, 216, 37.
[48] a) W.-J. Lin, M. H. Chen, *Carbohydrate polymers* **2007**, 67, 474; b) K. Sagara, S. W. Kim, *Journal of Controlled Release* **2002**, 79, 271.
[49] a) S. GORDON, V. H. Perry, S. RABINOWITZ, L.-P. CHUNG, H. ROSEN, *J Cell Sci* **1988**, 1988, 1; b) C. Kelly, C. Jefferies, S.-A. Cryan, *Journal of drug delivery* **2011**, 2011; c) A. Regnier-Vigouroux, *International review of cytology* **2003**, 321; d) H. Takagi, M. Numazaki, T. Kajiwara, Y. Abe, M. Ishii, C. Kato, N. Kojima, *Glycobiology* **2008**, 19, 258.
[50] T. E. Wileman, M. R. Lennartz, P. D. Stahl, *Proceedings of the National Academy of Sciences* **1986**, 83, 2501.
[51] a) B. S. Egan, R. Abdolrasulnia, V. L. Shepherd, *Archives of biochemistry and biophysics* **2004**, 428, 119; b) J. M. Irache, H. H. Salman, C. Gamazo, S. Espuelas, *Expert opinion on drug delivery* **2008**, 5, 703.
[52] X. Zhou, B. Liu, X. Yu, X. Zha, X. Zhang, Y. Chen, X. Wang, Y. Jin, Y. Wu, Y. Chen, *Journal of Controlled Release* **2007**, 121, 200.
[53] F. Berti, R. Adamo, *Chemical Society Reviews* **2018**, 47, 9015.
[54] B. K. Nunnally, V. E. Turula, R. D. Sitrin, **2015**.
[55] R. Dagan, J. Poolman, C.-A. Siegrist, *Vaccine* **2010**, 28, 5513.
[56] E. J. Grayson, G. J. Bernardes, J. M. Chalker, O. Boutureira, J. R. Koeppe, B. G. Davis, *Angewandte Chemie International Edition* **2011**, 50, 4127.
[57] A. Habeeb, in *Methods in enzymology*, Vol. 25, Elsevier **1972**, p. 457.
[58] K. Pachamuthu, R. R. Schmidt, *Chemical reviews* **2006**, 106, 160.
[59] O. Boutureira, N. Martínez-Sáez, K. M. Brindle, A. A. Neves, F. Corzana, G. J. Bernardes, *Chemistry–A European Journal* **2017**, 23, 6483.
[60] B. Lee, S. Sun, E. Jiménez-Moreno, A. A. Neves, G. J. Bernardes, *Bioorganic & medicinal chemistry* **2018**, 26, 3060.
[61] D. P. Gamblin, P. Garnier, S. van Kasteren, N. J. Oldham, A. J. Fairbanks, B. G. Davis, *Angewandte Chemie International Edition* **2004**, 43, 828.
[62] J. M. Chalker, S. B. Gunnoo, O. Boutureira, S. C. Gerstberger, M. Fernández-González, G. J. Bernardes, L. Griffin, H. Hailu, C. J. Schofield, B. G. Davis, *Chemical science* **2011**, 2, 1666.
[63] A. M. Freedy, M. J. Matos, O. Boutureira, F. Corzana, A. Guerreiro, P. Akkapeddi, V. J. Somovilla, T. Rodrigues, K. Nicholls, B. Xie, *Journal of the American Chemical Society* **2017**, 139, 18365.
[64] G. Stefanetti, Q. Y. Hu, A. Usera, Z. Robinson, M. Allan, A. Singh, H. Imase, J. Cobb, H. Zhai, D. Quinn, *Angewandte Chemie International Edition* **2015**, 54, 13198.
[65] H. Ban, J. Gavrilyuk, C. F. Barbas III, *Journal of the American Chemical Society* **2010**, 132, 1523.
[66] D. Rabuka, *Current opinion in chemical biology* **2010**, 14, 790.
[67] A. B. Lowe, *Polymer Chemistry* **2014**, 5, 4820.
[68] O. Ito, R. Omori, M. Matsuda, *Journal of the American Chemical Society* **1982**, 104, 3934.
[69] a) A. Ogawa, H. Tanaka, H. Yokoyama, R. Obayashi, K. Yokoyama, N. Sonoda, *The Journal of Organic Chemistry* **1992**, 57, 111; b) T. Taniguchi, T. Fujii, A. Idota, H. Ishibashi, *Organic letters* **2009**, 11, 3298.
[70] a) N. Asano, *Glycobiology* **2003**, 13, 93R; b) J. R. Brown, B. E. Crawford, J. D. Esko, *Critical reviews in biochemistry and molecular biology* **2007**, 42, 481.
[71] M. von Itzstein, W.-Y. Wu, G. B. Kok, M. S. Pegg, J. C. Dyason, B. Jin, T. Van Phan, M. L. Smythe, H. F. White, S. W. Oliver, *Nature* **1993**, 363, 418.
[72] Z. Shriver, R. Raman, K. Viswanathan, R. Sasisekharan, *Chemistry & biology* **2009**, 16, 803.

[73] M. N. Matrosovich, T. Y. Matrosovich, T. Gray, N. A. Roberts, H.-D. Klenk, *Journal of virology* **2004**, 78, 12665.
[74] J.-H. Kim, R. Resende, T. Wennekes, H.-M. Chen, N. Bance, S. Buchini, A. G. Watts, P. Pilling, V. A. Streltsov, M. Petric, *Science* **2013**, 340, 71.
[75] a) O. Abian, P. Alfonso, A. Velazquez-Campoy, P. Giraldo, M. Pocovi, J. Sancho, *Molecular pharmaceutics* **2011**, 8, 2390; b) F. M. Platt, G. R. Neises, G. Reinkensmeier, M. J. Townsend, V. H. Perry, R. L. Proia, B. Winchester, R. A. Dwek, T. D. Butters, *Science* **1997**, 276, 428.
[76] A. Imberty, Y. M. Chabre, R. Roy, *Chemistry–A European Journal* **2008**, 14, 7490.
[77] L. Thunberg, G. Bäckström, U. Lindahl, *Carbohydrate research* **1982**, 100, 393.
[78] a) R. J. Linhardt, *Journal of medicinal chemistry* **2003**, 46, 2551; b) R. J. Linhardt, S. A. Ampofo, J. Fareed, D. Hoppensteadt, J. Folkman, J. B. Mulliken, *Biochemistry* **1992**, 31, 12441.
[79] a) M. Petitou, C. A. van Boeckel, *Angewandte Chemie International Edition* **2004**, 43, 3118; b) C. A. van Boeckel, M. Petitou, *Angewandte Chemie International Edition in English* **1993**, 32, 1671; c) J. M. Walenga, J. Fareed, W. P. Jeske, F. X. Frapaise, R. L. Bick, M. M. Samama, *Turk J Haematol* **2002**, 19, 137.
[80] M. Maccarana, U. Lindahl, *Glycobiology* **1993**, 3, 271.
[81] Y. Xu, S. Masuko, M. Takieddin, H. Xu, R. Liu, J. Jing, S. A. Mousa, R. J. Linhardt, J. Liu, *Science* **2011**, 334, 498.
[82] H. Liu, Z. Zhang, R. J. Linhardt, *Natural product reports* **2009**, 26, 313.
[83] R. Copeland, A. Balasubramaniam, V. Tiwari, F. Zhang, A. Bridges, R. J. Linhardt, D. Shukla, J. Liu, *Biochemistry* **2008**, 47, 5774.
[84] a) M. Bevilacqua, E. Butcher, B. Furie, B. Furie, M. Gallatin, M. Gimbrone, J. Harlan, K. Kishimoto, L. Lasky, R. McEver, *Cell* **1991**, 67, 233; b) L. A. Lasky, *Annual review of biochemistry* **1995**, 64, 113.
[85] B. A. Imhof, M. Aurrand-Lions, *Nature Reviews Immunology* **2004**, 4, 432.
[86] M. J. Schultz, A. F. Swindall, S. L. Bellis, *Cancer and Metastasis Reviews* **2012**, 31, 501.
[87] P.-C. Pang, P. C. Chiu, C.-L. Lee, L.-Y. Chang, M. Panico, H. R. Morris, S. M. Haslam, K.-H. Khoo, G. F. Clark, W. S. Yeung, *Science* **2011**, 333, 1761.
[88] K. M. KERR, W. R. AUGER, J. J. MARSH, R. M. COMITO, R. L. FEDULLO, G. J. SMITS, D. P. KAPELANSKI, P. F. FEDULLO, R. N. CHANNICK, S. W. JAMIESON, *American journal of respiratory and critical care medicine* **2000**, 162, 14.
[89] a) T. P. Kogan, B. Dupré, H. Bui, K. L. McAbee, J. M. Kassir, I. L. Scott, X. Hu, P. Vanderslice, P. J. Beck, R. A. Dixon, *Journal of medicinal chemistry* **1998**, 41, 1099; b) J. Chang, J. T. Patton, A. Sarkar, B. Ernst, J. L. Magnani, P. S. Frenette, *Blood* **2010**, 116, 1779.
[90] J. Egger, C. l. Weckerle, B. Cutting, O. Schwardt, S. Rabbani, K. Lemme, B. Ernst, *Journal of the American Chemical Society* **2013**, 135, 9820.
[91] a) M. R. Bond, J. A. Hanover, *Annual review of nutrition* **2013**, 33, 205; b) C. Slawson, R. Copeland, G. W. Hart, *Trends in biochemical sciences* **2010**, 35, 547.
[92] S. A. Yuzwa, M. S. Macauley, J. E. Heinonen, X. Shan, R. J. Dennis, Y. He, G. E. Whitworth, K. A. Stubbs, E. J. McEachern, G. J. Davies, *Nature chemical biology* **2008**, 4, 483.
[93] a) J. D. Diot, I. G. Moreno, G. Twigg, C. O. Mellet, K. Haupt, T. D. Butters, J. Kovensky, S. G. Gouin, *The Journal of organic chemistry* **2011**, 76, 7757; b) S. B. Ferreira, A. C. Sodero, M. F. Cardoso, E. S. Lima, C. R. Kaiser, F. P. Silva Jr, V. F. Ferreira, *Journal of medicinal chemistry* **2010**, 53, 2364; c) M. Weïwer, C. C. Chen, M. M. Kemp, R. J. Linhardt, *European journal of organic chemistry* **2009**, 2009, 2611.
[94] a) B. La Ferla, C. Airoldi, C. Zona, A. Orsato, F. Cardona, S. Merlo, E. Sironi, G. D'Orazio, F. Nicotra, *Natural product reports* **2011**, 28, 630; b) B. Ostash, X. Yan, V. Fedorenko, A. Bechthold, in *Natural Products via Enzymatic Reactions*, Springer **2010**, p. 105.
[95] M. Nahar, T. Dutta, S. Murugesan, A. Asthana, D. Mishra, V. Rajkumar, M. Tare, S. Saraf, N. K. Jain, *Critical Reviews™ in Therapeutic Drug Carrier Systems* **2006**, 23.
[96] J. E. Hudak, C. R. Bertozzi, *Chemistry & biology* **2014**, 21, 16.

[97] P. I. Kitov, J. M. Sadowska, G. Mulvey, G. D. Armstrong, H. Ling, N. S. Pannu, R. J. Read, D. R. Bundle, *Nature* **2000**, 403, 669.

[98] a) A. V. Pukin, H. M. Branderhorst, C. Sisu, C. A. Weijers, M. Gilbert, R. M. Liskamp, G. M. Visser, H. Zuilhof, R. J. Pieters, *ChemBioChem* **2007**, 8, 1500; b) C. Sisu, A. J. Baron, H. M. Branderhorst, S. D. Connell, C. A. Weijers, R. de Vries, E. D. Hayes, A. V. Pukin, M. Gilbert, R. J. Pieters, *ChemBioChem* **2009**, 10, 329.

[99] S. M. Rele, W. Cui, L. Wang, S. Hou, G. Barr-Zarse, D. Tatton, Y. Gnanou, J. D. Esko, E. L. Chaikof, *Journal of the American Chemical Society* **2005**, 127, 10132.

[100] S. I. van Kasteren, S. J. Campbell, S. Serres, D. C. Anthony, N. R. Sibson, B. G. Davis, *Proceedings of the National Academy of Sciences* **2009**, 106, 18.

[101] a) S.-K. Wang, P.-H. Liang, R. D. Astronomo, T.-L. Hsu, S.-L. Hsieh, D. R. Burton, C.-H. Wong, *Proceedings of the National Academy of Sciences* **2008**, 105, 3690; b) O. Martínez-Ávila, L. M. Bedoya, M. Marradi, C. Clavel, J. Alcamí, S. Penadés, *ChemBioChem* **2009**, 10, 1806.

[102] Y. van Kooyk, T. B. Geijtenbeek, *Nature Reviews Immunology* **2003**, 3, 697.

[103] a) C. Jandus, H.-U. Simon, S. von Gunten, *Biochemical pharmacology* **2011**, 82, 323; b) M. K. O'Reilly, J. C. Paulson, *Trends in pharmacological sciences* **2009**, 30, 240; c) O. Blixt, S. Han, L. Liao, Y. Zeng, J. Hoffmann, S. Futakawa, J. C. Paulson, *Journal of the American Chemical Society* **2008**, 130, 6680; d) C. D. Rillahan, E. Schwartz, R. McBride, V. V. Fokin, J. C. Paulson, *Angewandte Chemie International Edition* **2012**, 51, 11014.

[104] P. I. Kitov, G. L. Mulvey, T. P. Griener, T. Lipinski, D. Solomon, E. Paszkiewicz, J. M. Jacobson, J. M. Sadowska, M. Suzuki, K.-i. Yamamura, *Proceedings of the National Academy of Sciences* **2008**, 105, 16837.

[105] a) B. G. Davis, R. C. Lloyd, J. B. Jones, *The Journal of organic chemistry* **1998**, 63, 9614; b) S. I. van Kasteren, H. B. Kramer, H. H. Jensen, S. J. Campbell, J. Kirkpatrick, N. J. Oldham, D. C. Anthony, B. G. Davis, *Nature* **2007**, 446, 1105.

[106] M. Rawat, C. I. Gama, J. B. Matson, L. C. Hsieh-Wilson, *Journal of the American Chemical Society* **2008**, 130, 2959.

[107] J. M. Brown, J. Xia, B. Zhuang, K.-S. Cho, C. J. Rogers, C. I. Gama, M. Rawat, S. E. Tully, N. Uetani, D. E. Mason, *Proceedings of the National Academy of Sciences* **2012**, 109, 4768.

[108] a) C. R. Becer, *Macromolecular rapid communications* **2012**, 33, 742; b) D. Deniaud, K. Julienne, S. G. Gouin, *Organic & biomolecular chemistry* **2011**, 9, 966; c) J. J. Lundquist, E. J. Toone, *Chemical reviews* **2002**, 102, 555.

[109] a) Y. M. Chabre, R. Roy, *Current topics in medicinal chemistry* **2008**, 8, 1237; b) M. Marradi, F. Chiodo, I. García, S. Penadés, *Chemical Society Reviews* **2013**, 42, 4728.

[110] M. A. Mintzer, E. L. Dane, G. A. O'Toole, M. W. Grinstaff, *Molecular pharmaceutics* **2011**, 9, 342.

[111] a) G. R. Newkome, C. D. Shreiner, *Polymer* **2008**, 49, 1; b) G. R. Newkome, C. Shreiner, *Chemical reviews* **2010**, 110, 6338.

[112] C. L. Yuan, *Carbohydrate research* **1978**, 67, 509.

[113] a) A. Berzi, J. J. Reina, R. Ottria, I. Sutkeviciute, P. Antonazzo, M. Sanchez-Navarro, E. Chabrol, M. Biasin, D. Trabattoni, I. Cetin, *Aids* **2012**, 26, 127; b) S. Sattin, A. Daghetti, M. Thépaut, A. Berzi, M. Sánchez-Navarro, G. Tabarani, J. Rojo, F. Fieschi, M. Clerici, A. Bernardi, *ACS chemical biology* **2010**, 5, 301.

[114] P. M. Rendle, A. Seger, J. Rodrigues, N. J. Oldham, R. R. Bott, J. B. Jones, M. M. Cowan, B. G. Davis, *Journal of the American Chemical Society* **2004**, 126, 4750.

[115] R. Ribeiro-Viana, M. Sánchez-Navarro, J. Luczkowiak, J. R. Koeppe, R. Delgado, J. Rojo, B. G. Davis, *Nature communications* **2012**, 3, 1303.

[116] J. J. García-Vallejo, M. Ambrosini, A. Overbeek, W. E. van Riel, K. Bloem, W. W. Unger, F. Chiodo, J. G. Bolscher, K. Nazmi, H. Kalay, *Molecular immunology* **2013**, 53, 387.

[117] S. K. Singh, J. Stephani, M. Schaefer, H. Kalay, J. J. García-Vallejo, J. den Haan, E. Saeland, T. Sparwasser, Y. van Kooyk, *Molecular immunology* **2009**, 47, 164.

[118] a) J.-K. Rhee, M. Baksh, C. Nycholat, J. C. Paulson, H. Kitagishi, M. Finn, *Biomacromolecules* **2012**, 13, 2333; b) W. C. Chen, G. C. Completo, D. S. Sigal, P. R. Crocker, A. Saven, J. C. Paulson, *Blood* **2010**, 115, 4778; c) W. C. Chen, N. Kawasaki, C. M. Nycholat, S. Han, J. Pilotte, P. R. Crocker, J. C. Paulson, *PLoS One* **2012**, 7, e39039; d) C. M. Nycholat, C. Rademacher, N. Kawasaki, J. C. Paulson, *Journal of the American Chemical Society* **2012**, 134, 15696.

[119] a) J. E. Gestwicki, C. W. Cairo, L. E. Strong, K. A. Oetjen, L. L. Kiessling, *Journal of the American Chemical Society* **2002**, 124, 14922; b) L. L. Kiessling, J. C. Grim, *Chemical Society Reviews* **2013**, 42, 4476.

[120] a) S. A. Busch, J. Silver, *Current opinion in neurobiology* **2007**, 17, 120; b) R. Lin, T. W. Rosahl, P. J. Whiting, J. W. Fawcett, J. C. Kwok, *PLoS One* **2011**, 6, e21499.

[121] V. Vázquez-Dorbatt, J. Lee, E. W. Lin, H. D. Maynard, *ChemBioChem* **2012**, 13, 2478.

[122] Q. Zhang, J. Collins, A. Anastasaki, R. Wallis, D. A. Mitchell, C. R. Becer, D. M. Haddleton, *Angewandte Chemie International Edition* **2013**, 52, 4435.

[123] a) P. H. Seeberger, D. B. Werz, *Nature* **2007**, 446, 1046; b) M. A. Tarp, H. Clausen, *Biochimica et Biophysica Acta (BBA)-General Subjects* **2008**, 1780, 546.

[124] L. Morelli, L. Poletti, L. Lay, *European journal of organic chemistry* **2011**, 2011, 5723.

[125] D. P. Gamblin, E. M. Scanlan, B. G. Davis, *Chemical reviews* **2008**, 109, 131.

[126] V. Pozsgay, *Current topics in medicinal chemistry* **2008**, 8, 126.

[127] O. Ouerfelli, J. D. Warren, R. M. Wilson, S. J. Danishefsky, *Expert review of vaccines* **2005**, 4, 677.

[128] L. Wang, *Current opinion in drug discovery & development* **2006**, 9, 194.

[129] V. Verez-Bencomo, V. Fernandez-Santana, E. Hardy, M. E. Toledo, M. C. Rodríguez, L. Heynngnezz, A. Rodriguez, A. Baly, L. Herrera, M. Izquierdo, *Science* **2004**, 305, 522.

[130] a) M. C. Hewitt, D. A. Snyder, P. H. Seeberger, *Journal of the American Chemical Society* **2002**, 124, 13434; b) L. Schofield, M. C. Hewitt, K. Evans, M.-A. Siomos, P. H. Seeberger, *Nature* **2002**, 418, 785.

[131] M. Tamborrini, D. B. Werz, J. Frey, G. Pluschke, P. H. Seeberger, *Angewandte Chemie International Edition* **2006**, 45, 6581.

[132] a) F. Bélot, K. Wright, C. Costachel, A. Phalipon, L. A. Mulard, *The Journal of organic chemistry* **2004**, 69, 1060; b) A. Phalipon, C. Costachel, C. Grandjean, A. Thuizat, C. Guerreiro, M. Tanguy, F. Nato, B. Vulliez-Le Normand, F. Bélot, K. Wright, *The Journal of Immunology* **2006**, 176, 1686.

[133] A. Phalipon, M. Tanguy, C. Grandjean, C. Guerreiro, F. Bélot, D. Cohen, P. J. Sansonetti, L. A. Mulard, *The Journal of Immunology* **2009**, 182, 2241.

[134] T. Lipinski, P. I. Kitov, A. Szpacenko, E. Paszkiewicz, D. R. Bundle, *Bioconjugate chemistry* **2010**, 22, 274.

[135] D. R. Bundle, C. Nycholat, C. Costello, R. Rennie, T. Lipinski, *ACS chemical biology* **2012**, 7, 1754.

[136] M. L. Gening, T. Maira-Litrán, A. Kropec, D. Skurnik, M. Grout, Y. E. Tsvetkov, N. E. Nifantiev, G. B. Pier, *Infection and immunity* **2010**, 78, 764.

[137] a) L. E. McCoy, R. A. Weiss, *Journal of Experimental Medicine* **2013**, 210, 209; b) L. M. Walker, S. K. Phogat, P.-Y. Chan-Hui, D. Wagner, P. Phung, J. L. Goss, T. Wrin, M. D. Simek, S. Fling, J. L. Mitcham, *Science* **2009**, 326, 285; c) R. Pejchal, K. J. Doores, L. M. Walker, R. Khayat, P.-S. Huang, S.-K. Wang, R. L. Stanfield, J.-P. Julien, A. Ramos, M. Crispin, *Science* **2011**, 334, 1097.

[138] J.-P. Julien, J. H. Lee, A. Cupo, C. D. Murin, R. Derking, S. Hoffenberg, M. J. Caulfield, C. R. King, A. J. Marozsan, P. J. Klasse, *Proceedings of the National Academy of Sciences* **2013**, 110, 4351.

[139] a) R. D. Astronomo, H.-K. Lee, C. N. Scanlan, R. Pantophlet, C.-Y. Huang, I. A. Wilson, O. Blixt, R. A. Dwek, C.-H. Wong, D. R. Burton, *Journal of virology* **2008**, 82, 6359; b) J. G. Joyce, I. J. Krauss, H. C. Song, D. W. Opalka, K. M. Grimm, D. D. Nahas, M. T. Esser, R. Hrin, M. Feng, V. Y. Dudkin, *Proceedings of the National Academy of Sciences* **2008**, 105, 15684.

[140] K. J. Doores, Z. Fulton, V. Hong, M. K. Patel, C. N. Scanlan, M. R. Wormald, M. Finn, D. R. Burton, I. A. Wilson, B. G. Davis, *Proceedings of the National Academy of Sciences* **2010**, 107, 17107.

[141] B. Aussedat, Y. Vohra, P. K. Park, A. Fernández-Tejada, S. M. Alam, S. M. Dennison, F. H. Jaeger, K. Anasti, S. Stewart, J. H. Blinn, *Journal of the American Chemical Society* **2013**, 135, 13113.
[142] a) M. Fukuda, *Cancer research* **1996**, 56, 2237; b) T. M. Kloppel, T. W. Keenan, M. J. Freeman, D. J. Morré, *Proceedings of the National Academy of Sciences* **1977**, 74, 3011; c) S. Sell, *Human pathology* **1990**, 21, 1003.
[143] a) M. A. Hollingsworth, B. J. Swanson, *Nature Reviews Cancer* **2004**, 4, 45; b) D. W. Kufe, *Nature Reviews Cancer* **2009**, 9, 874.
[144] A. D. Pashov, B. Monzavi-Karbassi, T. Kieber-Emmons, *Human vaccines* **2011**, 7, 156.
[145] a) T. Gilewski, S. Adluri, G. Ragupathi, S. Zhang, T.-J. Yao, K. Panageas, M. Moynahan, A. Houghton, L. Norton, P. O. Livingston, *Clinical Cancer Research* **2000**, 6, 1693; b) S. Slovin, G. Ragupathi, S. Adluri, G. Ungers, K. Terry, S. Kim, M. Spassova, W. Bornmann, M. Fazzari, L. Dantis, *Proceedings of the National Academy of Sciences* **1999**, 96, 5710.
[146] Y.-L. Huang, J.-T. Hung, S. K. Cheung, H.-Y. Lee, K.-C. Chu, S.-T. Li, Y.-C. Lin, C.-T. Ren, T.-J. R. Cheng, T.-L. Hsu, *Proceedings of the National Academy of Sciences* **2013**, 110, 2517.
[147] S. Ingale, M. A. Wolfert, J. Gaekwad, T. Buskas, G.-J. Boons, *Nature chemical biology* **2007**, 3, 663.
[148] M. A. Cheever, J. P. Allison, A. S. Ferris, O. J. Finn, B. M. Hastings, T. T. Hecht, I. Mellman, S. A. Prindiville, J. L. Viner, L. M. Weiner, *Clinical Cancer Research* **2009**, 15, 5323.
[149] V. Lakshminarayanan, P. Thompson, M. A. Wolfert, T. Buskas, J. M. Bradley, L. B. Pathangey, C. S. Madsen, P. A. Cohen, S. J. Gendler, G.-J. Boons, *Proceedings of the National Academy of Sciences* **2012**, 109, 261.
[150] B. L. Wilkinson, S. Day, R. Chapman, S. Perrier, V. Apostolopoulos, R. J. Payne, *Chemistry–A European Journal* **2012**, 18, 16540.
[151] O. Renaudet, P. Dumy, *Organic letters* **2003**, 5, 243.
[152] I. Bettahi, G. Dasgupta, O. Renaudet, A. A. Chentoufi, X. Zhang, D. Carpenter, S. Yoon, P. Dumy, L. BenMohamed, *Cancer immunology, immunotherapy* **2009**, 58, 187.
[153] Z.-H. Huang, L. Shi, J.-W. Ma, Z.-Y. Sun, H. Cai, Y.-X. Chen, Y.-F. Zhao, Y.-M. Li, *Journal of the American Chemical Society* **2012**, 134, 8730.
[154] a) D. H. Kim, J. J. Rossi, *Nature reviews genetics* **2007**, 8, 173; b) T. P. O'Connor, R. G. Crystal, *Nature reviews genetics* **2006**, 7, 261.
[155] K. Itaka, A. Harada, Y. Yamasaki, K. Nakamura, H. Kawaguchi, K. Kataoka, *The Journal of Gene Medicine: A cross-disciplinary journal for research on the science of gene transfer and its clinical applications* **2004**, 6, 76.
[156] R. Deng, Y. Yue, F. Jin, Y. Chen, H.-F. Kung, M. C. Lin, C. Wu, *Journal of Controlled Release* **2009**, 140, 40.
[157] Y. Hu, B. Xu, Q. Ji, D. Shou, X. Sun, J. Xu, J. Gao, W. Liang, *Biomaterials* **2014**, 35, 4236.
[158] G. Dietrich, A. Bubert, I. Gentschev, Z. Sokolovic, A. Simm, A. Catic, S. H. Kaufmann, J. Hess, A. A. Szalay, W. Goebel, *Nature biotechnology* **1998**, 16, 181.
[159] T. Ohashi, S. Boggs, P. Robbins, A. Bahnson, K. Patrene, F.-S. Wei, J.-F. Wei, J. Li, L. Lucht, Y. Fei, *Proceedings of the National Academy of Sciences* **1992**, 89, 11332.
[160] R. I. Mahato, Y. Takakura, M. Hashida, *Critical Reviews™ in Therapeutic Drug Carrier Systems* **1997**, 14.
[161] a) S. Kawakami, F. Yamashita, M. Nishikawa, Y. Takakura, M. Hashida, *Biochemical and biophysical research communications* **1998**, 252, 78; b) S. Kawakami, A. Sato, M. Nishikawa, F. Yamashita, M. Hashida, *Gene therapy* **2000**, 7, 292.
[162] G. M. Flores-Fernández, M. Pagán, M. Almenas, R. J. Solá, K. Griebenow, *BMC biotechnology* **2010**, 10, 57.
[163] D. Chandra, D. G. Tang, in *Apoptosis*, Springer **2009**, p. 65.
[164] a) S. B. Bratton, G. S. Salvesen, *J Cell Sci* **2010**, 123, 3209; b) A. M. Chinnaiyan, *Neoplasia* **1999**, 1, 5.

[165] a) H. Maeda, J. Fang, T. Inutsuka, Y. Kitamoto, *International immunopharmacology* **2003**, 3, 319; b) F. Danhier, O. Feron, V. Préat, *Journal of Controlled Release* **2010**, 148, 135; c) R. Ranganathan, S. Madanmohan, A. Kesavan, G. Baskar, Y. R. Krishnamoorthy, R. Santosham, D. Ponraju, S. K. Rayala, G. Venkatraman, *International journal of nanomedicine* **2012**, 7, 1043; d) D. Hanahan, R. A. Weinberg, *Cell* **2011**, 144, 646.

[166] J. Méndez, M. Morales Cruz, Y. Delgado, C. M. Figueroa, E. A. Orellano, M. Morales, A. Monteagudo, K. Griebenow, *Molecular pharmaceutics* **2013**, 11, 102.

[167] R. Ojeda, J. L. de Paz, A. G. Barrientos, M. Martín-Lomas, S. Penadés, *Carbohydrate research* **2007**, 342, 448.

[168] U. Lungwitz, M. Breunig, T. Blunk, A. Göpferich, *European Journal of Pharmaceutics and Biopharmaceutics* **2005**, 60, 247.

[169] a) K.-R. Kim, Y.-D. Lee, T. Lee, B.-S. Kim, S. Kim, D.-R. Ahn, *Biomaterials* **2013**, 34, 5226; b) M. Skobe, T. Hawighorst, D. G. Jackson, R. Prevo, L. Janes, P. Velasco, L. Riccardi, K. Alitalo, K. Claffey, M. Detmar, *Nature medicine* **2001**, 7, 192; c) A. E. Giuliano, D. M. Kirgan, J. M. Guenther, D. L. Morton, *Annals of surgery* **1994**, 220, 391.

[170] X. Huang, F. Zhang, S. Lee, M. Swierczewska, D. O. Kiesewetter, L. Lang, G. Zhang, L. Zhu, H. Gao, H. S. Choi, *Biomaterials* **2012**, 33, 4370.

[171] E. Tanaka, H. S. Choi, H. Fujii, M. G. Bawendi, J. V. Frangioni, *Annals of surgical oncology* **2006**, 13, 1671.

[172] T. Dai, S. Zhou, C. Yin, S. Li, W. Cao, W. Liu, K. Sun, H. Dou, Y. Cao, G. Zhou, *Biomaterials* **2014**, 35, 8227.

[173] a) S. A. Stacker, C. Caesar, M. E. Baldwin, G. E. Thornton, R. A. Williams, R. Prevo, D. G. Jackson, S.-i. Nishikawa, H. Kubo, M. G. Achen, *Nature medicine* **2001**, 7, 186; b) D. Wang, J. He, N. Rosenzweig, Z. Rosenzweig, *Nano Letters* **2004**, 4, 409; c) D. S. Steinwaerder, C. A. Carlson, D. L. Otto, Z.-Y. Li, S. Ni, A. Lieber, *Nature medicine* **2001**, 7, 240.

[174] a) N. R. Sibson, A. M. Blamire, M. Bernades-Silva, S. Laurent, S. Boutry, R. N. Muller, P. Styles, D. C. Anthony, *Magnetic Resonance in Medicine: An Official Journal of the International Society for Magnetic Resonance in Medicine* **2004**, 51, 248; b) P. A. Barber, T. Foniok, D. Kirk, A. M. Buchan, S. Laurent, S. Boutry, R. N. Muller, L. Hoyte, B. Tomanek, U. I. Tuor, *Annals of neurology* **2004**, 56, 116; c) S. Boutry, C. Burtea, S. Laurent, G. Toubeau, L. Vander Elst, R. N. Muller, *Magnetic Resonance in Medicine: An Official Journal of the International Society for Magnetic Resonance in Medicine* **2005**, 53, 800; d) R. Weissleder, K. Kelly, E. Y. Sun, T. Shtatland, L. Josephson, *Nature biotechnology* **2005**, 23, 1418.

[175] J. Peng, K. Wang, W. Tan, X. He, C. He, P. Wu, F. Liu, *Talanta* **2007**, 71, 833.

[176] K. K. Selim, Z.-C. Xing, H. Guo, I.-K. Kang, *Journal of Materials Science: Materials in Medicine* **2009**, 20, 1945.

[177] Y. Cao, Y. He, H. Liu, Y. Luo, M. Shen, J. Xia, X. Shi, *Journal of Materials Chemistry B* **2015**, 3, 286.

[178] H. Liu, H. Wang, Y. Xu, R. Guo, S. Wen, Y. Huang, W. Liu, M. Shen, J. Zhao, G. Zhang, *ACS applied materials & interfaces* **2014**, 6, 6944.

[179] K. K. Selim, Y.-S. Ha, S.-J. Kim, Y. Chang, T.-J. Kim, G. H. Lee, I.-K. Kang, *Biomaterials* **2007**, 28, 710.

[180] A. Moore, E. Marecos, A. Bogdanov Jr, R. Weissleder, *Radiology* **2000**, 214, 568.

[181] Y. Hu, R. Wang, J. Li, L. Ding, X. Wang, X. Shi, M. Shen, *Particle & Particle Systems Characterization* **2017**, 34, 1600113.

[182] J. An, X. Zhang, Q. Guo, Y. Zhao, Z. Wu, C. Li, *Colloids and Surfaces A: Physicochemical and Engineering Aspects* **2015**, 482, 98.

[183] Y. Jung, H. S. Hwang, K. Na, *Biomaterials* **2018**, 160, 15.

[184] E.-M. Kim, H.-J. Jeong, S.-L. Kim, M.-H. Sohn, J.-W. Nah, H.-S. Bom, I.-K. Park, C.-S. Cho, *Nuclear medicine and biology* **2006**, 33, 529.

[185] X. Liu, Z. Wei, M. Wu, X. Zhang, D. Zhang, B. Li, J. Liu, *RSC Advances* **2016**, 6, 37472.

[186] J. Zhou, N. r. Butchosa, H. S. N. Jayawardena, Q. Zhou, M. Yan, O. Ramström, *Bioconjugate chemistry* **2014**, 25, 640.
[187] L. W. Locke, M. W. Mayo, A. D. Yoo, M. B. Williams, S. S. Berr, *Biomaterials* **2012**, 33, 7785.
[188] P. Rathinaraj, K. Lee, Y. Choi, S.-Y. Park, O. H. Kwon, I.-K. Kang, *Journal of Nanoparticle Research* **2015**, 17, 311.
[189] J. Jacobs, A. Byrne, N. Gathergood, T. Keyes, J. Heuts, A. Heise, *Macromolecules* **2014**, 47, 7303.
[190] N. Düzgüneş, S. Simões, V. Slepushkin, E. Pretzer, D. Flasher, I. I. Salem, G. Steffan, K. Konopka, M. C. P. de Lima, in *Methods in enzymology*, Vol. 391, Elsevier **2005**, p. 351.
[191] S. Aquaro, R. Caliò, J. Balzarini, M. C. Bellocchi, E. Garaci, C. F. Perno, *Antiviral research* **2002**, 55, 209.
[192] K. Shigeta, S. Kawakami, Y. Higuchi, T. Okuda, H. Yagi, F. Yamashita, M. Hashida, *Journal of Controlled Release* **2007**, 118, 262.
[193] M. Garg, N. K. Jain, *Journal of drug targeting* **2006**, 14, 1.
[194] M. Garg, T. Dutta, N. K. Jain, *European Journal of Pharmaceutics and Biopharmaceutics* **2007**, 67, 76.
[195] M. Garg, B. R. Garg, S. Jain, P. Mishra, R. K. Sharma, A. K. Mishra, T. Dutta, N. K. Jain, *European Journal of Pharmaceutical Sciences* **2008**, 33, 271.
[196] T. Dutta, H. B. Agashe, M. Garg, P. Balasubramanium, M. Kabra, N. K. Jain, *Journal of drug targeting* **2007**, 15, 89.
[197] J. W. Dennis, M. Granovsky, C. E. Warren, *Biochimica et Biophysica Acta (BBA)-General Subjects* **1999**, 1473, 21.
[198] P. Kesharwani, R. K. Tekade, V. Gajbhiye, K. Jain, N. K. Jain, *Nanomedicine: Nanotechnology, Biology and Medicine* **2011**, 7, 295.
[199] A. Agarwal, U. Gupta, A. Asthana, N. K. Jain, *Biomaterials* **2009**, 30, 3588.
[200] H.-F. Liang, C.-T. Chen, S.-C. Chen, A. R. Kulkarni, Y.-L. Chiu, M.-C. Chen, H.-W. Sung, *Biomaterials* **2006**, 27, 2051.
[201] Y. Kuramoto, S. Kawakami, S. Zhou, K. Fukuda, F. Yamashita, M. Hashida, *The Journal of Gene Medicine: A cross-disciplinary journal for research on the science of gene transfer and its clinical applications* **2008**, 10, 392.
[202] Y. Ikehara, T. Niwa, L. Biao, S. K. Ikehara, N. Ohashi, T. Kobayashi, Y. Shimizu, N. Kojima, H. Nakanishi, *Cancer Research* **2006**, 66, 8740.
[203] M. Matsui, Y. Shimizu, Y. Kodera, E. Kondo, Y. Ikehara, H. Nakanishi, *Cancer science* **2010**, 101, 1670.
[204] a) S. P. Vyas, A. Singh, V. Sihorkar, *Critical reviews in therapeutic drug carrier systems* **2001**, 18, 1; b) N. K. Mehra, V. Mishra, N. K. Jain, *Therapeutic delivery* **2013**, 4, 369.
[205] a) P. P. Deshpande, S. Biswas, V. P. Torchilin, *Nanomedicine* **2013**, 8, 1509; b) P. Siafaka, N. Üstündağ Okur, E. Karavas, D. Bikiaris, *International journal of molecular sciences* **2016**, 17, 1440; c) T. M. Allen, *Nature Reviews Cancer* **2002**, 2, 750; d) S. A. Slavoff, A. Saghatelian, *Nature biotechnology* **2012**, 30, 959.
[206] a) P. Boya, G. Kroemer, *Oncogene* **2008**, 27, 6434; b) T. Kirkegaard, M. Jäättelä, *Biochimica et Biophysica Acta (BBA)-Molecular Cell Research* **2009**, 1793, 746.
[207] a) J. Prakash, L. Beljaars, A. K. Harapanahalli, M. Zeinstra-Smith, A. de Jager-Krikken, M. Hessing, H. Steen, K. Poelstra, *International journal of cancer* **2010**, 126, 1966; b) O. Vaillant, K. E. Cheikh, D. Warther, D. Brevet, M. Maynadier, E. Bouffard, F. Salgues, A. Jeanjean, P. Puche, C. Mazerolles, *Angewandte Chemie International Edition* **2015**, 54, 5952; c) E. Crucianelli, P. Bruni, A. Frontini, L. Massaccesi, M. Pisani, A. Smorlesi, G. Mobbili, *RSC Advances* **2014**, 4, 58204; d) C. Minnelli, L. Cianfruglia, E. Laudadio, R. Galeazzi, M. Pisani, E. Crucianelli, D. Bizzaro, T. Armeni, G. Mobbili, *Journal of drug targeting* **2018**, 26, 242.
[208] R. Greupink, H. I. Bakker, H. van Goor, M. H. de Borst, L. Beljaars, K. Poelstra, *Pharmaceutical research* **2006**, 23, 1827.

[209] R. Dutta, V. Kumar, Y. Peng, R. E. Evande, J. L. Grem, R. I. Mahato, *Pharmaceutical research* **2017**, 34, 564.
[210] A. Jain, A. Agarwal, S. Majumder, N. Lariya, A. Khaya, H. Agrawal, S. Majumdar, G. P. Agrawal, *Journal of Controlled Release* **2010**, 148, 359.
[211] a) N. Bertrand, J. Wu, X. Xu, N. Kamaly, O. C. Farokhzad, *Advanced drug delivery reviews* **2014**, 66, 2; b) W. Sun, Q. Hu, W. Ji, G. Wright, Z. Gu, *Physiological reviews* **2016**, 97, 189.
[212] a) H. Wang, J. Wu, L. Xu, K. Xie, C. Chen, Y. Dong, *Chemical Communications* **2017**, 53, 2618; b) S. V. Moradi, W. M. Hussein, P. Varamini, P. Simerska, I. Toth, *Chemical science* **2016**, 7, 2492; c) N. C. Reichardt, M. Martín-Lomas, S. Penadés, *Chemical Society Reviews* **2013**, 42, 4358.
[213] a) S. S. Pinho, C. A. Reis, *Nature Reviews Cancer* **2015**, 15, 540; b) Y. Van Kooyk, H. Kalay, J. J. Garcia-Vallejo, *Frontiers in immunology* **2013**, 4, 451.
[214] Z. Wu, K. Jiang, H. Zhu, C. Ma, Z. Yu, L. Li, W. Guan, Y. Liu, H. Zhu, Y. Chen, *Bioconjugate chemistry* **2016**, 27, 1972.
[215] a) B. Kang, T. Opatz, K. Landfester, F. R. Wurm, *Chemical Society Reviews* **2015**, 44, 8301; b) A. Martinez, C. O. Mellet, J. M. G. Fernández, *Chemical Society Reviews* **2013**, 42, 4746.
[216] J. Wu, M. H. Nantz, M. A. Zern, *Front Biosci* **2002**, 7, d717.
[217] A. Murao, M. Nishikawa, C. Managit, J. Wong, S. Kawakami, F. Yamashita, M. Hashida, *Pharmaceutical research* **2002**, 19, 1808.
[218] S.-n. Wang, Y.-h. Deng, H. Xu, H.-b. Wu, Y.-k. Qiu, D.-w. Chen, *European Journal of Pharmaceutics and Biopharmaceutics* **2006**, 62, 32.
[219] S. Wang, H. Xu, J. Xu, Y. Zhang, Y. Liu, Y.-h. Deng, D. Chen, *Aaps Pharmscitech* **2010**, 11, 870.
[220] a) D. Bhadra, A. Yadav, S. Bhadra, N. Jain, *International journal of pharmaceutics* **2005**, 295, 221; b) W. B. Turnbull, J. F. Stoddart, *Reviews in Molecular Biotechnology* **2002**, 90, 231; c) N. Röckendorf, T. K. Lindhorst, in *Dendrimers IV*, Springer **2001**, p. 201; d) E. K. Woller, M. J. Cloninger, *Biomacromolecules* **2001**, 2, 1052.
[221] P. Agrawal, U. Gupta, N. Jain, *Biomaterials* **2007**, 28, 3349.
[222] R. Sett, H. S. Sarkar, P. K. Das, *Journal of Antimicrobial Chemotherapy* **1993**, 31, 151.
[223] I.-S. Kim, S.-H. Kim, *International journal of pharmaceutics* **2003**, 257, 195.
[224] F. Suriano, R. Pratt, J. P. Tan, N. Wiradharma, A. Nelson, Y.-Y. Yang, P. Dubois, J. L. Hedrick, *Biomaterials* **2010**, 31, 2637.
[225] G. Ponchel, J.-M. Irache, *Advanced drug delivery reviews* **1998**, 34, 191.
[226] H. H. Salman, C. Gamazo, M. A. Campanero, J. M. Irache, *Journal of nanoscience and nanotechnology* **2006**, 6, 3203.
[227] a) G. P. Carino, J. S. Jacob, E. Mathiowitz, *Journal of Controlled Release* **2000**, 65, 261; b) P. Arbós, M. A. Campanero, M. A. Arangoa, J. M. Irache, *Journal of Controlled Release* **2004**, 96, 55.
[228] S. Wagner, N. J. Lynch, W. Walter, W. J. Schwaeble, M. Loos, *The Journal of Immunology* **2003**, 170, 1462.
[229] P. Pukanud, P. Peungvicha, N. Sarisuta, *Drug delivery* **2009**, 16, 289.
[230] V. Fievez, L. Plapied, A. des Rieux, V. Pourcelle, H. Freichels, V. Wascotte, M.-L. Vanderhaeghen, C. Jerôme, A. Vanderplasschen, J. Marchand-Brynaert, *European Journal of Pharmaceutics and Biopharmaceutics* **2009**, 73, 16.
[231] A. Garu, G. Moku, S. K. Gulla, A. Chaudhuri, *Molecular Therapy* **2016**, 24, 385.
[232] O. V. Markov, N. L. Mironova, E. V. Shmendel, R. N. Serikov, N. G. Morozova, M. A. Maslov, V. V. Vlassov, M. A. Zenkova, *Journal of Controlled Release* **2015**, 213, 45.
[233] M. Yoshida, R. Takimoto, K. Murase, Y. Sato, M. Hirakawa, F. Tamura, T. Sato, S. Iyama, T. Osuga, K. Miyanishi, *PloS one* **2012**, 7, e39545.
[234] M. Gou, M. Dai, X. Li, L. Yang, M. Huang, Y. Wang, B. Kan, Y. Lu, Y. Wei, Z. Qian, *Colloids and Surfaces B: Biointerfaces* **2008**, 64, 135.
[235] T. H. Kim, H. Jin, H. W. Kim, M.-H. Cho, C. S. Cho, *Molecular cancer therapeutics* **2006**, 5, 1723.

[236] R. Budzynska, D. Nevozhay, U. Kanska, M. Jagiello, A. Opolski, J. Wietrzyk, J. Boratynski, *Oncology Research Featuring Preclinical and Clinical Cancer Therapeutics* **2007**, 16, 415.
[237] A. Stewart, C. Pichon, L. Meunier, P. Midoux, M. Monsigny, A. Roche, *Molecular pharmacology* **1996**, 50, 1487.
[238] C. Li, D. Zhang, H. Guo, L. Hao, D. Zheng, G. Liu, J. Shen, X. Tian, Q. Zhang, *International journal of pharmaceutics* **2013**, 448, 79.
[239] Y.-I. Jeong, S.-J. Seo, I.-K. Park, H.-C. Lee, I.-C. Kang, T. Akaike, C.-S. Cho, *International journal of pharmaceutics* **2005**, 296, 151.
[240] R. Greupink, H. I. Bakker, W. Bouma, C. Reker-Smit, D. K. Meijer, L. Beljaars, K. Poelstra, *Journal of Pharmacology and Experimental Therapeutics* **2006**, 317, 514.
[241] F. Zhang, Q. Wu, Z.-C. Chen, M. Zhang, X.-F. Lin, *Journal of colloid and interface science* **2008**, 317, 477.
[242] S. Jain, P. Singh, V. Mishra, S. Vyas, *Immunology letters* **2005**, 101, 41.
[243] P. V. Kumar, A. Asthana, T. Dutta, N. K. Jain, *Journal of drug targeting* **2006**, 14, 546.
[244] W. Wijagkanalan, S. Kawakami, M. Takenaga, R. Igarashi, F. Yamashita, M. Hashida, *Journal of Controlled Release* **2008**, 125, 121.
[245] A. Düffels, L. G. Green, S. V. Ley, A. D. Miller, *Chemistry–A European Journal* **2000**, 6, 1416.
[246] S. Chono, K. Kaneko, E. Yamamoto, K. Togami, K. Morimoto, *Drug development and industrial pharmacy* **2010**, 36, 102.
[247] K. L. White, T. Rades, R. H. Furneaux, P. C. Tyler, S. Hook, *Journal of pharmacy and pharmacology* **2006**, 58, 729.
[248] A. Nag, P. C. Ghosh, *Journal of drug targeting* **1999**, 6, 427.
[249] G. Huang, J. Diakur, Z. Xu, L. I. Wiebe, *International journal of pharmaceutics* **2008**, 360, 197.
[250] X.-Q. Zhang, X.-L. Wang, P.-C. Zhang, Z.-L. Liu, R.-X. Zhuo, H.-Q. Mao, K. W. Leong, *Journal of Controlled Release* **2005**, 102, 749.
[251] C. Auriti, G. Prencipe, M. Moriondo, I. Bersani, C. Bertaina, V. Mondì, R. Inglese, *Journal of immunology research* **2017**, 2017.
[252] S. Kawakami, M. Hashida, *Journal of Controlled Release* **2014**, 190, 542.
[253] W. Yu, N. Zhang, C. Li, *Current pharmaceutical design* **2009**, 15, 3826.
[254] a) R. Jefferis, *Nature reviews Drug discovery* **2009**, 8, 226; b) T. Kieber-Emmons, L. Hutchins, P. Emanuel, A. Pennisi, E. Siegel, F. Jousheghany, B. Karbassi, I. Makhoul, AACR, 2017.