Chapter-1

Carbohydrates in Therapeutics
1.1 Importance of Carbohydrates

Carbohydrates are ubiquitous in nature as one of the major class of biopolymers. They play significant roles in each and every part of life, be it in kitchen to clothes that we wear and the metabolic processes occurring in our living system to the paper on which these words are written. A plethora of roles of carbohydrates can be found in our day to day life. In the past carbohydrates were considered only for energy storage and as skeletal components. During last 30 years the exciting developments in the field of glycoscience have started to reveal that they are involved in an enormous range of very precise and sophisticated processes. The study of carbohydrates within the biological systems has illustrated that they play a key role in a number of fundamental biological processes such as cell-cell recognition and cell-external agent interaction. These interactions can initiate beneficial biological events such as fertilization, cell growth and differentiation (for example during embryogenesis) and immune responses as well as disease processes such as inflammation, viral and bacterial infections and cancer metastasis (Figure 1). Carbohydrates of even short sequences are used for carrying biological information, for example the human blood groups are differentiated by relatively simple changes in the oligosaccharide structures.

1.2 What are Carbohydrates?

Chemically carbohydrates are polyhydroxy aldehydes or ketones or their derivatives containing carboxyl, amino or other groups which can be hydrolyzed to them. Most often carbohydrates are referred to as saccharides. The term saccharide comes from the Greek word saccharon meaning sugar. The carbohydrates that can not be hydrolyzed into simpler compounds are called “monosaccharides” such as glucose, fructose, etc. A carbohydrate that can be hydrolyzed to two monosaccharides is called a disaccharide. e.g. sucrose, maltose, lactose, melibiose, cellobiose, etc., while those composed of many monosaccharide units are called “polysaccharides”. The carbohydrates with an aldehydic group are known as “aldoses” and those with a ketonic functionality as “ketoses”. Depending upon the number of carbon atoms it contains, the monosaccharide is termed as tetrose, pentose, hexose and so on.

The polysaccharides can be classified into homopolysaccharide and heteropolysaccharide depending on the monosaccharide units present. Homopolysaccharides are those containing only one type of monosaccharide units, e.g. glucans, mannans, galactans, arabinans, etc. Heteropolysaccharides contain two or more different monosaccharide units, e.g.
Figure 1. Examples of the roles of carbohydrate in cell-surface interactions.

glucomannans, galactomannans, arabinogalactans, etc. Depending on the functional properties, polysaccharides can be divided into two parts. One is fibrous polysaccharide, *e.g.* cellulose in higher plants and some algae, chitin in yeast and fungi. The other one is the polysaccharide having matrix forming property and is characterized by their gel forming characteristics which confers flexibility on the structural assembly. Arabinoxylans, galactomannans, *etc.* of plant origin represent this group. Carbohydrates can conjugate with its counterpart proteins to form glycoproteins and with lipids to form glycolipids. These glycoproteins and glycolipids are collectively known as glycoconjugates.²⁰

### 1.3 Carbohydrate based Therapeutic Agents

Given the prevalent role of carbohydrates in a wide range of biological processes it is quite surprising that only a few carbohydrate-based therapeutics and diagnostics are used in the
clinics. This may be explained by considering two factors: (a) an under-estimation of the biological importance of carbohydrate molecules, and (b) difficulties associated with the synthesis of carbohydrate derivatives and to their poor pharmacological profiles.

1.3.1 Carbohydrate based Antibiotics

The largest group of carbohydrate containing drugs is antibiotics. In general, antibiotics are the products that inhibit the growth of other organisms.\(^2\) The cell wall of bacteria plays an important role for its survival which is largely composed of oligosaccharides. It is therefore not surprising that various existing antibiotics are based on carbohydrates, that is, either they contain a glycan portion in their structure or they are non-carbohydrate molecules that target an enzyme receptor associated with carbohydrate synthesis, metabolism or recognition. Antibiotics can be divided into two categories according to their mode of action. First one consists of those which act by targeting the cell wall of bacteria either through interfering with biosynthesis of lipopolysaccharide or peptidoglycan and the second one comprise of prokaryotic protein biosynthesis inhibitors.

\[ \text{Figure 2. Examples of nucleoside antibiotics.} \]
Several class of nucleoside antibiotics inhibit the biosynthesis of peptidoglycan layer by inhibiting the phospho-MurNAc-pentapeptide translocase (MraY). These include tunicamycin, liposidomycin, and mureidomycins, etc. (Figure 2). Ramoplanin, a glycodepsipeptide, is the only known naturally occurring inhibitor of the GlcNAc transferase (MurG) (Figure 2).

Natural product that inhibit transglycosylation step of peptidoglycan can be categorized into two classes: (i) those that directly inhibit the enzymes (e.g. moenomycin, Figure 3), (ii) those that bind to lipid II, the substrate of transglycosylation e.g. type II antibiotics, glycopeptide antibiotics, etc.

![Moenomycin A](image)

**Figure 3.**

Glycopeptide antibiotics, vancomycin and teicoplanin, are in clinical use and are often referred to as ultimate drugs, because of their utility in treatment of infections by bacteria that are resistant to many other classes of antibiotics (Figure 4).

![Vancomycin and Teicoplanin](image)

**Figure 4.** Representative examples of the antibiotic belonging to the class of glycopeptides antibiotics.
The second class of antibiotics targets the prokaryotic protein biosynthesis. Macrolide antibiotics consist of a branched chain fatty acids formed into a macrocyclic ring. Typically one or more deoxy sugars are attached to the macrolide core structure, and the sugar moiety is essential for the activities. Erythromycin A, a 14-membered macrolide antibiotic and its analogs have been known for long time and has provided effective antibiotic therapy against gram positive pathogens much of the time, especially as an alternative for patients that are allergic to β-lactam antibiotics (Figure 5). The lincosamides are another class of RNA- binding antibiotics that include a carbohydrate in their structure (Figure 5). The semisynthetic clindamycin has replaced the naturally occurring lincomycin clinically because of its higher activity and reduced side effects.

Aminoglycosides consist of a six membered carbocyclic nucleus (aminocyclitol) with a varying number of sugar substituents (Figure 6 and 7). This core is most commonly 2-deoxystreptamine, but other aminocyclitols such as streptidine (streptomycins), actinamine (spectinomycins), and fortamine (fortimicins) are also abundant. The aminoglycosides are generally classified according to their attached amino sugar groups. The aminoglycosides function as inhibitors of protein translation by binding to the highly conserved A-site sequence of the small 30S ribosomal subunit.
The everninomycins (Figure 8), for example, everninomycin 13,384-1, are a class of complex oligosaccharide antibiotics. They are part of the orthosomycin family, which feature one or more orthoester linkages. Everninomycin antibiotics are thought to inhibit RNA to protein translation by preventing the assembly of the initiation complex. Everninomycins are active both in vitro and in vivo against methicillin resistant S. aureus and vancomycin resistant enterococci.
1.3.2 Carbohydrates as Antiviral Agents

Carbohydrates are also useful antiviral agents, for example, the compound 4-guanidino Neu-5Ac2en (Zanamivir, Figure 9) is an analogue of neuraminic acid and is currently being developed as anti-influenza A and B drug. Tamiflu\textsuperscript{30,31} (oseltamivir phosphate; Roche) is another monosaccharide-inspired drug in the market for the treatment of influenza. Both compounds have been found to be highly potent inhibitors (IC\textsubscript{50} = 1 ng ml\textsuperscript{-1}) of the influenza neuraminidase, to inhibit influenza A and B virus replication \textit{in vitro} and \textit{in vivo} (mice, ferrets), to be well tolerated, and to be both prophylactically (significant reduction in number of ill subjects) and therapeutically (significant reduction in duration of illness) effective against influenza A and B virus infection in humans.

1.3.3 Carbohydrates based Anticoagulants

Heparin, the oldest carbohydrate based drug isolated from animal organs has been used clinically as an anti-thrombin agent since 1940's (Figure 10a). Heparin activates the serine protease inhibitor antithrombin III, which blocks thrombin and factor Xa in the coagulation cascade.\textsuperscript{32} This drug is a highly heterogeneous mixture of polysaccharides and is associated with severe side effects, including heparin-induced thrombocytopenia, bleeding and allergic reactions. Chemically or enzymatically fragmented heparins (low-molecular-weight heparins, LMWHs) are also heterogeneous, but are more bio-available, with a longer half-life, a more predictable
anticoagulant activity and fewer side effects *in vivo*. After the specific pentasaccharide responsible for the anticoagulant property was identified in the early 1980s\(^3\) (Figure 10b), a Herculean effort lasting more than 10 years was begun to establish a structure function relationship using synthetic oligosaccharides.\(^4\) As a result of this drug-development effort, a synthetic pentasaccharide known as Arixtra (fondaparinux sodium; GlaxoSmithKline) has been available since 2002.\(^5\)

![Figure 10](image)

Figure 10. (a) Heparin, an oldest carbohydrate based anticoagulant agent. (b) A pentasaccharide sequence of heparin. This sequence is responsible for binding to antithrombin III.

### 1.3.4 Carbohydrate based Anti-inflammatory Agents

Selectins are cell surface carbohydrate binding proteins present in platelets, endothelial cells and leucocytes (white blood cells).\(^6\)\(^7\)\(^8\) They are unique in their functions that they specifically bind to restricted type of carbohydrates. They are of three types L-selectin (expressed on leucocytes), E-selectin (expressed on endothelial cells) and P-selectin (expressed on platelets). Leucocytes are important species for the repair of tissue damage and self defense against microbial infection. Leukocytes are brought to the site of injury by a complex series of steps, referred to as the inflammatory cascade.\(^9\) The cascade begins when damaged tissues release cytokines that stimulate the endothelium to express E-\(^10\) and P-selectin\(^11\) transiently on the endothelial lining.\(^12\)\(^13\)\(^14\) Selectins E and P recognize sialyl Lewis X (sLe\(^X\)) and related oligosaccharides on the surface of the leukocytes\(^15\) and promote leukocyte adhesion to the affected endothelial cells.\(^16\)\(^17\)\(^18\) L-selectin\(^19\)\(^20\) is constitutively expressed on leukocytes\(^21\) and it recognizes similar carbohydrate ligands displayed on the endothelium.\(^22\)\(^23\) Although leukocyte-
endothelium cell attachment is a normal component of immune response against microbial infection, errant leukocyte recruitment is a hallmark of acute diseases, such as stroke and reperfusion injury during surgery and organ transplantation, and chronic inflammatory diseases such as psoriasis and rheumatoid arthritis. The widespread participation in both acute and chronic inflammatory leukocyte recruitment makes the selectins attractive targets for broad spectrum anti-inflammatory agents. The tetrasaccharide antigen sialyl Lewis X (sLe\(^x\)) glycosides\(^49\) (Figure 11), synthesized chemically\(^{58-61}\) and enzymatically,\(^62\) have shown selectin antagonist activity\(^63,64\) in several animal models for both acute and chronic inflammation and are currently being evaluated in clinical trials. The search for glycomimetics based on sLe\(^x\) has been motivated by this realization and several molecules\(^65,66\) with equal or greater selectin inhibitory potency have been reported in the past few years.

![Figure 11. Sialylated and fucosylated tetrasaccharide Sialyl Lewis X (sLe\(^x\)) glycoside, a potent selectin binder molecule.](image)

In doing so, a hexasaccharide\(^67\) (Figure 12) has been synthesized having 5 to 6 fold better activity than sialyl Lewis X tetrasaccharide in binding to E- and P-selectin.

![Figure 12. A hexasaccharide having 5 to 6 fold better activity than sialyl Lewis X tetrasaccharide.](image)
However, these complex oligosaccharides have some disadvantages like unfavorable pharmacokinetics, poor metabolic stability and poor oral absorption. Therefore the research efforts are directed towards designing templates having required functional groups in their preferred orientation. A series of biphenyl derivative have also been synthesized to act as selectin inhibitors (Figure 13). The α-D-mannopyranosyloxybiphenyl substituted carboxylic acid, TBC265, is such a compound that displays these functionalities in the required orientation. This compound has a greater in vitro potency than the parent sLe\textsuperscript{x} tetrasaccharide and an in vivo efficiency in small animal models of inflammation.\textsuperscript{68} Bimosiamose or TBC 1269, a biphenyl analogue of sialyl Lewis\textsuperscript{x} has been found to exhibit a better binding profile than its natural counterpart. Synthesis and in vitro studies measuring inhibition of binding of selectin immunoglobulin fusion protein to HL-60 human myelogenous leukemia cells showed that TBC-1269 has an IC\textsubscript{50} value of 70 μM for P-selectin compared with 2.6 mM for sLe\textsuperscript{x} tetrasaccharide.\textsuperscript{69,70}

![Figure 13. Biphenyl derivatives as selectin inhibitor.](image)

**1.3.5 Carbohydrate based Vaccine**

As antibiotic resistance began to evolve ever-more rapidly in the 1970s, the development of antibacterial carbohydrate vaccines was pursued, building on fundamental discoveries in immunology. It has been found that the carbohydrates present on the cell surface of parasites are different from host and are more evolutionarily stable than the proteins.\textsuperscript{71} These specific carbohydrate antigens on the cell surface of particular types of cells provide the option of creating vaccines. Vaccination is one of the most important scientific inventions in the control and eradication of microbial infections. Earlier, vaccines were based on the whole organism or on protein toxin isolated from different microbial sources. In the past few years, intense efforts focused on the development of completely defined, synthetic carbohydrate vaccines. Several
vaccines based on the purified cell-wall polysaccharides (CPS) or neoglycoconjugates are now either commercially available or under development, which include, vaccines against *Neisseria meningitidis*, *Streptococcus pneumoniae*, *Haemophilus influenzae* type b (Hib), 72-74 *Shigella flexneri*, 75,76 *Salmonella typhi*, 71,77,78 *Vibrio cholerae* 79,80 to name just a few.

*Neisseria meningitidis*, a Gram-negative organism causing meningitis worldwide in children and adults have been serologically classified into several types, A, B, C, 29e, W-135, X, Y and Z, based on their capsular polysaccharide. 81-85 Groups A, B and C are responsible for most of the meningococcal infections and a simplified tetravalent polysaccharide vaccine comprised of group A, C, W-135 and Y polysaccharides has been successful in the worldwide prevention of meningococcal meningitis. 86

*Streptococcus pneumoniae* is the main cause of middle ear infections in children and lower respiratory tract infections in adults. The organism has capsular polysaccharides of different serotypes and a common group of antigen called C-substance. 87-89 Emergence of antibiotic resistance has prompted researchers for the development of effective conjugate vaccines. It has been found that a combination vaccine, containing capsular polysaccharides of seven of the most prevalent *S. pneumoniae* serotypes conjugated to CRM197 would be efficacious in the prevention of the invasive disease, lower respiratory tract infection and otitis media. 90,91

*Haemophilus influenzae* are Gram-negative organisms that are classified into six serotypes (types a, b, c, d, e, f) based on the structure of their capsular polysaccharides. 92-95 Type b *H. influenzae* is the cause of meningitis, the most serious infection among infants, leading to severe and permanent neurological defects in survivors. 96 The synthetic carbohydrate vaccine against *Haemophilus influenzae* type b is now commercially available as Quimi-Hib. It is a conjugate of a polyribosylribitol phosphate oligosaccharide and a carrier protein 97 (Figure 14).

![Figure 14. Structure of Quimi-Hib, the vaccine against meningitis.](image-url)
Malaria is caused by single-celled parasites *Plasmodium* genus, of which *Plasmodium falciparum* is the most pathogenic. The *P. falciparum* expresses a large amount of glycosylphosphatidylinositol (GPI) on its cell surface. After entering into the host, this parasitic GPI triggers an inflammatory response, which contributes to morbidity and mortality of the host. For the unequivocal preparation of the immunogen against malaria toxin and evaluate its potential use as a new vaccine, a hexasaccharide has been conjugated to the maleimide-activated carrier protein KEYHOLE LIMPET HAEMOCYANIN (KLH) through a spacer linker. The preclinical model revealed that this non-toxic GPI oligosaccharide coupled to a carrier protein (Figure 15a) is immunogenic and provides significant protection against malarial pathogenesis. The antitoxic antipathogenesis oligosaccharide vaccine against malaria is currently in advanced preclinical evaluation.

Leishmaniasis, a tropical disease that afflict more than 12 million people worldwide, is spread by the bite of infected sandflies. Lipophosphoglycans (LPGs) are ubiquitous on the cell surface of the parasites and are composed of GPI anchor, a repeating phosphorylated disaccharide with different oligosaccharides on the cap. Although the phosphoglycan part is antigenic in nature, its high molecular mass and heterogeneity preclude its use as a vaccine. Therefore, preparation of a structurally well-defined carbohydrate vaccine based on the tetrasaccharide cap (Figure 15b) for Leishmaniasis was focused. The branched tetrasaccharide was conjugated either to the carrier protein KLH or virosomal particles. The initial results of the immunological evaluation of vaccine against Leishmaniasis in mice were found promising.

**Figure 15.** Parasitic vaccine candidates: (a) Malaria vaccine candidate and (b) Leishmaniasis vaccine candidate.
Anthrax, a disease related to the serious concern of bio-terrorism, is caused by the infection of *Bacillus anthracis*. Recently it was discovered that *Bacillus anthracis* spores display a unique carbohydrate structure on their surface for infecting the host.\(^{106}\) The tetrasaccharide found on the surface of glycoprotein BC1A of *Bacillus anthracis*\(^{107,108}\) has been used to generate anti-carbohydrate antibodies towards the preparation of carbohydrate vaccines against anthrax (Figure 16). The immunological studies on the carbohydrate vaccines are under investigation.

\[
\begin{align*}
\text{H} & \text{H} - \text{N} - \text{O} \\
\text{O} & \text{O} - \text{O} \\
\text{O} & \text{O} - \text{O} \\
\text{O} & \text{O} - \text{O} \\
\text{Anthrose} & \\
\text{R} & = \text{H or 4-pentynyl}
\end{align*}
\]

*Figure 16.* Tetrasaccharide structure used to prepare carbohydrate vaccine against anthrax.

*Candida albicans*, is the causative agent of the fungal infection called candiasis.\(^{109}\) Several phosphomannan oligosaccharides are found in the cell-wall of *Candida albicans*. Due to the immunogenicity of the phosphomannan cell wall antigen, they have been considered as the potential target for vaccine preparation against candiasis.\(^{109}\) This has led to the synthesis of a glycoconjugate vaccine for *Candida albicans*\(^{110}\) (Figure 17).

\[
\begin{align*}
\text{HO} & \text{O} - \text{O} \\
\text{HO} & \text{O} - \text{O} \\
\text{HO} & \text{O} - \text{O} \\
\text{HO} & \text{O} - \text{O} \\
\text{O} & \text{O} - \text{O} \\
\text{HO} & \text{O} - \text{O} \\
\text{HO} & \text{O} - \text{Tetanus Toxoid} \\
\text{N} & \text{A}
\end{align*}
\]

*Figure 17.* Carbohydrate vaccine candidate against candiasis.
In addition to carbohydrate-based vaccines for bacterial, viral and parasitic infections discussed above, the carbohydrate-based cancer vaccines are also under development. The expression of specific types of glycolipids and glycoproteins present on normal cells increases in certain tumor cells. This high level of expression on tumor cells causes an antibody response, and renders these cell-surface glycoconjugates tumor-associated antigens which form the basis for the development of antitumor vaccines. Carbohydrate antigens such as sialyl Lewis X and Globo-H are suitable targets for both active and passive anticancer immunotherapies because they have been carefully characterized as being over expressed on the surface of malignant cells (Figure 18). Globo-H is found on the cell surface of breast, prostate and ovarian cancer cells, whereas sialyl Lewis X is observed in various types of cancer. A successful Phase I clinical trial of a Globo H oligosaccharide-protein conjugate along with an adjuvant (immune activator) was recently carried out. Immunization of ovarian cancer patients with Le\(^{\text{y}}\) antigen is also under phase I clinical trial. Another carbohydrate-based anti-cancer vaccine called GMK induces anti-GM\(_{2}\) antibodies that target melanoma cells in a very specific manner. This vaccine is currently in Phase III trials for malignant melanoma.

Figure 18.

Theratope is a carbohydrate-based vaccine against breast cancer completed its Phase III clinical trial (Figure 19). The vaccine is a conjugate of a carbohydrate tumor antigen called STn and a carrier protein.
Another carbohydrate-based vaccine in the preclinical development is BGLP 40, a small synthetic segment of a mucin type glycoprotein expressed on cancer cells and includes multiple carbohydrate and peptide epitopes. Now the focus is on the synthesis of a unimolecular multivalent vaccine by connecting all antigens involved in the carcinoma. It would be a single glycopeptide (Figure 20) that contains five antigens as Globo-H, Le\(^\gamma\), STn, Tn and TF and thereby inducing antibodies against all of them. Construction of a multivalent antigen could be more effective against tumor cells containing a range of different monovalent antigens.\(^{120}\)

**Figure 19.** Theratope: A vaccine against breast cancer.

**Figure 20.** A proposed multivalent anti cancer vaccine.
In order to develop an anti HIV vaccine, glycosylation of the HIV envelope proteins have been considered as one of the major impediment. Human monoclonal antibody 2g12 binds the viral coat protein gp120, and plays a vital role in inhibiting HIV type-1 infection. Burton and coworkers co-crystallized 2g12 with high-mannose type oligosaccharide Man9GlcNAc2 and demonstrated that the high mannose type glycan may provide a recognition motif for the binding of antibody. A synthetic oligosaccharide cluster (Figure 21) was found to be 46-fold more effective than the subunit Man9GlcNAc2Asn in inhibiting 2g12-binding to immobilized gp120.

Few Manα1-2Man-containing oligomannoses (Figure 22) have also been synthesized which can effectively inhibit the binding of 2g12 to gp120 and the inhibitory efficiency is better than Man9GlcNAc2. Therefore, these compounds are expected to be potential candidates for the development of HIV vaccine.

![Figure 21. An oligosaccharide cluster towards the development of anti HIV vaccine.](image)

![Figure 22. Oligomannose derivatives for the preparation of anti HIV vaccine.](image)
Apart from this, synthesis of fragments corresponding to 2G12 carbohydrate epitope has been undertaken for its use in the immunological characterization. Among the synthetic glycopeptides, two compounds showed promising results in the binding studies using Surface Plasmon Resonance technique. A dimeric oligomannose derivative (a, Figure 23) exhibited the binding capability of 78 in the said assay while a monomeric high oligomannose derivative (b, Figure 23) showed a binding capacity of 75. These two compounds have been proposed to be the ideal candidates in the preparation of anti HIV vaccines for inhibiting the binding of gp120 to 2G12.

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Figure 23. Oligomannose clustered oligosaccharides candidates for anti HIV vaccine.
1.3.6 Carbohydrate based Therapeutics for Metabolic Diseases

The enzymes involved in the assembling of carbohydrates e.g. glycosyltransferases and degradation of oligosaccharides e.g. glycosidases are usually targeted in this strategy. Practically, this is an indirect approach in the pharmacological manipulation of oligosaccharides. In the past, a series of carbohydrates or carbohydrate derived compounds have been exploited in biosynthesis modulation for treatment of metabolic diseases (Figure 24).

Acarbose (Pecose, Glucobay; Bayer) (a, Figure 24), a pseudo oligosaccharide isolated from microbial origin, is a blockbuster anti-diabetic drug which inhibit α-glucosidase and α-amylase and controls the rate of absorption of monosaccharides in the intestine and hence influences the carbohydrate metabolism.\(^{127}\) Trezhazolins (b, Figure 24) exhibit powerful inhibitory activity of various trehalases, Mannostatins (c, Figure 24) inhibit α-mannosidases and Allosamidines (d, Figure 24) inhibit various chitinases very strongly. Thus, each class of compounds inhibits mainly one group of enzymes.\(^{128}\) The N-butyldeoxynojirimycin (NBJ) (e, Figure 24) has been used as a glucosyltransferase inhibitor in the treatment of Tay–Sachs disease by preventing the accumulation of ganglioside GM\(_2\) in the brain. It was also reported that NBJ resulted in a reduction of a number of glycoforms of the HIV surface protein gp120.\(^{129}\) The proposed mode of action of NBJ suggested that it inhibits the early glucosidase trimming in the HIV glycoprotein biosynthesis and provides a poorly processed N-glycans corresponding to gp120.\(^{130}\) Apart from these, miglitol (f, Figure 24), an oral alpha-glucosidase inhibitor has been used in the management of non-insulin-dependent diabetes mellitus (NIDDM).\(^{131}\) Kotanol (g, Figure 24), Salacinol (h, Figure 24) and Nagstatine (i, Figure 24) are some other glucosidase inhibitors reported in the literature.\(^{132,133}\) Isofagomine (j, Figure 24) has been recognized as a potential drug in the treatment of type-II diabetes for its powerful inhibitory activity against glycogen phosphorylase.\(^{134}\) The indolizidine alkaloid swainsonine, (k, Figure 24)\(^{135}\) reduces tumor cell metastasis, enhances cellular immune responses, and slows down tumor cell growth in mice acting as an inhibitor of Golgi α-mannosidase II.\(^{136}\)
1.3.7 Carbohydrates in Drug Delivery System

Carbohydrates may also be used in other pharmaceutical applications. Saccharides, which are recognized by cell-specific proteins, may be used as drug delivery systems. It has been demonstrated that Naproxen, an anti-inflammatory drug and Arabinoside-AMP, an antiviral agent can be delivered to the target efficiently by attaching it to the lactosaminylated and galactosylated human serum albumin through various degradable linkers,\textsuperscript{137-139} which are absorbed into hepatic cells through receptor-mediated endocytosis.\textsuperscript{140} Moreover, use of cyclodextrin (Figure 25a) in drug delivery has also been well documented. These glycosides along with their various derivatives have been used to enhance oral bioavailability and to extend the half-life of a number of water-insoluble drugs in serum.\textsuperscript{141} Several amphiphilic glycosylated
bile acid derivatives (Figure 25b)\textsuperscript{142} have been synthesized as a carrier of non polar drugs for the improvement of their permeability and bioavailability.

\[ \text{Glc}(\alpha 1-4)-\text{Glc}(\alpha 1-4) \]
\[ \text{Glc}(\alpha 1-4) \]
\[ \text{Glc}(\alpha 1-4)-\text{Glc}(\alpha 1-4) \]

(a) Cyclodextrin

\[ \text{CH}_3 \]
\[ \text{CH}_3 \]
\[ \text{OH} \]
\[ \text{OH} \]
\[ \text{OH} \]
\[ \text{OH} \]
\[ \text{OH} \]
\[ \text{OH} \]
\[ \text{OH} \]
\[ \text{COO}^-\text{K}^+ \]

(b) Bile acid derivative

**Figure 25.** Carbohydrate derivatives used in drug delivery.

It has been demonstrated that the presence of sialic acid in higher levels on the termini of glycoprotein glycans can increase circulation half life of drug molecules.\textsuperscript{143,144} Nishimura et al. has made a successful attempt in glycosylating insulin,\textsuperscript{145,146} a protein hormone used to treat hyperglycaemia. They successfully prepared sialyllactose bound artificial insulins (Figure 26) which showed more prolonged activity in comparison to the wild type insulin.

**Figure 26.** Sialyllactose conjugated Insulin.

Carbohydrates may also be used in other pharmaceutical applications. Carbohydrates can be used as a plasma substitute. The original idea of using partially hydrolyzed dextran as a plasma substitute dates back to 1942.\textsuperscript{147} Continued studies and development of dextran-based products have kept their position as an important plasma substitute with several interesting additional therapeutic benefits (e.g. antithrombotic activity). Starting from the technology base of dextran some other interesting pharmaceuticals were developed. The most important is probably Debrisan, a wound-cleansing agent, prepared by cross-linking of dextran. The product acts by absorbing wound exudates in secreting wounds and shortens the healing time.\textsuperscript{148}
1.4 Conclusion

For many years the lack of tools for studying glycobiology prevented biologists and medical researchers from addressing research problems that involve carbohydrates. During the past decade, sequencing and synthesis technologies that are commonly used to study nucleic acids and proteins have become available for glycomics as well. Automated solid-phase synthesis, \textsuperscript{71,149,150} improved methods for solution-phase oligosaccharide assembly,\textsuperscript{151} enzymatic methods\textsuperscript{152} and the use of engineered cells have complemented each other, allowing oligosaccharide synthesis to take a big step forward by granting access to different classes of glycoconjugates.

Involvement of oligosaccharides at key positions of signaling pathways is beginning to emerge and a molecular understanding of carbohydrate binding to proteins is evolving. Detailed structural studies including studies of protein–carbohydrate interactions using X-ray crystallography will become commonplace in the near future. Further improvements in the methods by which oligosaccharides can be synthesized will be required for their routine synthesis. Diagnosis of bacterial and viral infections can be achieved using carbohydrate microarrays.\textsuperscript{153,154} Several glycoconjugate vaccines against infectious diseases and cancers are undergoing preclinical and clinical trials. The trend to produce defined carbohydrate based vaccines using chemical and enzymatic methods, as well as engineered cells, is likely to increase.

As our understanding of carbohydrate involvement in signaling cascades expands, in particular of those that involve glycosaminoglycans, carbohydrate-mediated processes will become the target of drug-development efforts using small organic molecules. Glycomics has just gone beyond the initial proof-of-principle studies for diagnostics and therapeutic candidates. The excitement of glycomics is just beginning, with many discoveries to be made and applications to be developed.
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Chapter 1

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Chapter 1


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