# Carbohydrate-Based Bioactive Compounds for Medicinal Chemistry Applications

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**Abstract:** In this article we review our work over the years on carbohydrates and carbohydrate mimetics and their applications in medicinal chemistry. In the first part of the review innovative synthetic methods, such as the chemoselective glycosylation method originally developed by our group and its applications to the synthesis of neoglycoconjugates (neoglycopeptides, oligosaccharide mimetics, neoglycolipids, etc…) will be presented. The high density of functional groups (hydroxyls) on the monosaccharides and the structural role of sugars forming the core of complex glycans in scaffolding and orienting the external sugar units for the interaction with receptors, inspired us and others to use sugars as scaffolds for the construction of pharmacologically active compounds. In the second part of this review, we will present some examples of bioactive and pharmacologically active compounds obtained by decorating monosaccharide scaffolds with pharmacophore groups. Sugar-derived protein ligands were also used as chemical probes to study the interaction of their target with other proteins in the cell. In this context, sugar mimetics and sugar-derived compounds have been employed as tools for exploring biology according to the "chemical genetic" approach.

**Keywords:** Carbohydrates, glycobiology, glycomimetics, peptidomimetics, glycidic scaffolds, glycoconjugates, medicinal chemistry, glycochemistry.

## 1. INTRODUCTION

### The Sugar Code in Medicinal Glycoscience

Biological information storage and transfer have been classically associated to two types of biomolecules: nucleic acids and proteins. Sugar molecules have been nearly exclusively assigned as biochemical fuels in energy metabolism or building blocks of polymers with structural functions in the cell wall (for example cellulose and chitin). Only recently, with the advent of modern glycobiology, carbohydrates have become important molecules to contemplate in relation to the life of a cell, and new light has been shed on the role of sugars in the transmission of biological information [1]. The glycans constituting the sugar code of a cell protrude from its surface and are covalently anchored to membrane proteins or lipids. Protein/lipid glycosylation is highly sensitive to alteration in cellular function, and abnormal glycosylation is diagnostic for a number of diseases states, including cancer, autoimmune and genetic diseases [2-4]. For this reason, several glycoprocessing enzymes have been recognized as important targets for therapeutic intervention [5,6]. This concept inspired the development of important classes of therapeutic, such as anti flu drugs that inhibit influenza virus neuraminidase [7], glycosidase inhibitors against HIV [8], Gaucher's disease, hepatitis, and cancer [9]. However, because of the structural complexity of oligosaccharides, the molecular details of biological recognition processes are still not fully understood, and consequently the pace of development of carbohydrate-based therapeutics has been relatively slow. This is mainly due to the difficulty of the synthesis of oligosaccharide structures and to the problems associated with undesirable physical chemical properties of sugars as drug candidates. Sugars are sensitive to chemical and enzymatic hydrolysis and present an elevated number of hydrogen bond donor and acceptor hydroxyl groups, so that their pharmacokinetic properties are generally poor in terms of membrane and tissue penetration. However, these problems can be circumvented with the use of carbohydrate mimetics, that is, the development of small molecules that mimic the structure and the function of native complex carbohydrates, with the hope that more active, more stable, and perhaps orally active small molecules can be developed. Once unravelled the complex relationship between oligosaccharide 3D shape and their biological function, it is in principle possible to reproduce the same function with mimetics that have been rationally designed in the perspective of a structural simplification. Besides the pharmacological reasons previously mentioned, one of the major advantage in doing mimetics is the simplification of the synthesis. The formation of the natural glycosidic bond is a challenging reaction that requires stereochemical control. The introduction into the molecule of non-natural glycosidic bonds (C-glycosides, S-glycosides, hydroxylamino-glycosides) might help to simplify the synthesis. Non-natural glycosidic bond are also resistant to glycosidase and, especially in the case of C-glycosides, to acid hydrolysis, thus improving pharmacokinetic properties and oral activity.

### Scope of the Review

The scope of this article is to review our work on sugar mimetics and scaffolds since 2000. In the first part of this review our original contribution to the development of sugar mimetics will be presented. Innovative methodologies such

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as the chemoselective glycosylation method will also be discussed and exemplified in the case of the synthesis of neoglycoconjugates (neoglycopeptides, oligosaccharide mimetics, neoglycolipids, etc…). The high density of functional groups (hydroxyls) on the monosaccharides inspired us and others to use sugars as scaffolds for the construction of pharmacologically active compounds. In the second part of this review, we will present some examples of bioactive and pharmacologically active compounds obtained by decorating monosaccharide scaffolds with pharmacophore groups.

## 2. SUGAR MIMETICS

The deeper understanding over the years of carbohydrate-protein interactions have allowed the development of a new class of small-molecule drugs known as glycomimetics [10-12]. These compounds should mimic native carbohydrate function, but at the same time they might overcome the drawbacks of carbohydrate leads, such as their low activity and scarce drug-like properties, and several modification of the sugar backbone can be made towards glycomimetics. Thus, to date several glycomimetic therapeutics are commercially available, such as the anti flu drugs Zanamivir and Oseltamivir, both inhibitors of the viral neuraminidase.

### 2.1. Inhibitors of Carbohydrate Processing Enzymes: Iminosugars

Oligosaccharide processing enzymes such as glycosidases and glycosyltransferases are important classes of biocatalysts involved in synthesising specific oligosaccharide structures on proteins and lipids. These enzymes are known to be entailed in a wide range of important biological processes, such as intestinal digestion, post-translational processing of glycoproteins, lysosomal catabolism of glycoconjugates, and inter-cellular recognition events [13]. Inhibition of these enzymes can disrupt biosynthesis of oligosaccharides, thus interfering in all these processes. Hence, "glycoenzyme" inhibitors might have enormous therapeutic potential in many diseases such as viral infection, cancer and diabetes. These molecules could, in fact, act as inhibitors of carbohydrate processing enzymes, provided that the functional groups involved in the enzyme-catalysed process are modified. Interference in oligosaccharide processing is the basis for the design of inhibitors as polyhydroxylated nitrogen heterocycles, usually referred to as iminosugars [14]. In this review we will focus on our recent efforts towards iminosugar derivatives.

Iminosugars are carbohydrate mimetics in which the endocyclic oxygen is replaced by an aminic nitrogen, able to inhibit glycosidases since the protonated nitrogen can mime the oxonium ion formed in the transition state intermediate in the enzymatic reaction [15]. The natural iminosugar nojirimycin (**1**, Fig. (**1**)), discovered in 1966 as the first glucose mimic [16] has shown inhibitory activity towards α- and β-glucosidases, but because of the lability of the hemiaminal function, chemists' interest shifted to the stable, and even more powerful, inhibitor, 1-deoxynojirimycin (DNJ, **2**) and to a variety of its derivatives, such as its *N*-alkylated derivatives. The iminosugar C-glycoside α-homonojirimycin (**3**), first synthesized by Liu [17] and thereafter isolated from natural sources [18] has proven to be a potent and, more significantly, selective inhibitor of α-glycosidases from the mouse gut and human intestine.

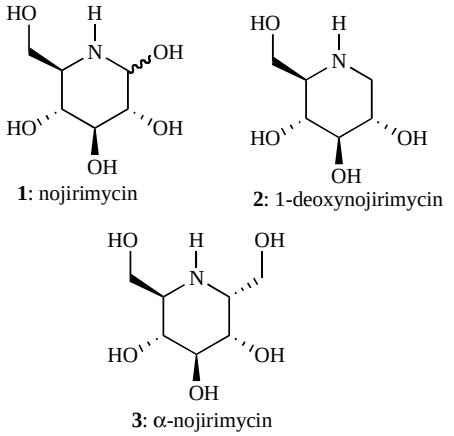
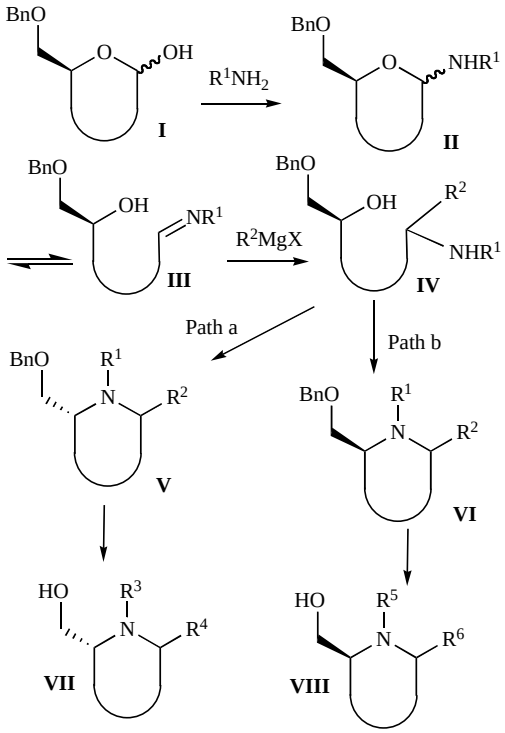



**Fig. (1).** Structure of a few natural imino sugars.

Over the years, our research group developed a new synthetic strategy for the synthesis of iminosugar *C*-glycosides *via* glycosyl amines, that lastly was applied to the synthesis of a small library of nojirimycin-derived bicyclic structures [19,20]. The general synthetic scheme can be outlined as follows, as illustrated in Scheme (**1**):




**Scheme 1.** Synthetic strategy for the preparation of iminosugar derivatives.

1. Introduction of the desired amino functionality by formation of the suitably protected glycosyl amine **II** in equilibrium with the open-chain imine **III**;

2. Introduction of the desired *C*-glycosidic appendage in a stereoselective way by Grignard reaction on the imine to the open-chain amino alcohol **IV**;

3. Stereoselective cyclisation (either by nucleophilic substitution to **V**, or by reductive amination to **VI**) to the target iminosugar;

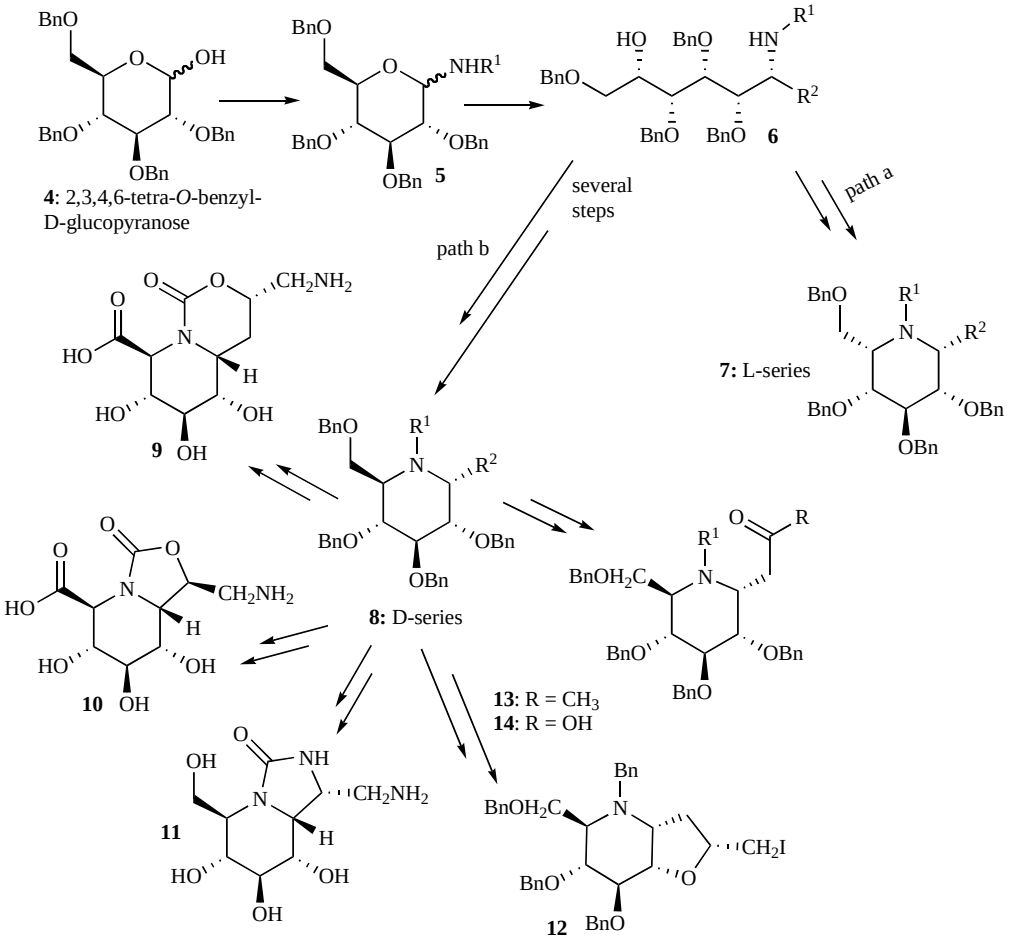
4. Modification of the *C*-glycosidic appendage and/or exocyclic nitrogen substituents in order to introduce pharmacologically relevant functional groups or additional cycles (structures **VII/VIII)**.

Key stereoselective steps of the synthetic scheme are the Grignard reaction and the cyclisation reaction to the iminosugar moiety. In the first case, the Grignard addition proceeds with high stereoselection, due to the formation of a Cram-chelate intermediate, in which the magnesium coordinates to the nitrogen of the imine **III**, in equilibrium with the glycosyl amine **II**, and the oxygen at C-2, allowing the attack of the nucleophilic carbon preferentially from the less hindered face. Regarding the cyclisation, two different path can be followed: (a) inversion of configuration at the carbon undergoing nucleophilic substitution by the nitrogen atom or (b) retention of configuration by reductive amination, after oxidation of the sole unprotected hydroxyl group of **IV** to the corresponding ketone. It's worth of note that the nitrogen atom should be protected with a suitable electron-withdrawing protecting group prior to hydroxyl oxidation.

The synthetic approach was recently used for the synthesis of *C*-glycosides of nojirimycin and novel nojirimycin-derived bicyclic structures, Scheme (**2**), containing cyclic carbamate, urea and guanidine moieties obtained from suitably protected α-*C*-vinylnojirimycin and α-*C*-allylnojirimycin, respectively. Bicyclic derivatives were assayed against different glycosidases, as antibacterial agents, and against insect trehalase.

### 2.2. Lipid A Mimetics as Immunotherapeutics

We were involved in a project aimed at synthesizing new mimetics of the lipodisaccharide named lipid A (Fig. (**2**)), that is the toxic part of bacterial lipopolysaccharide (LPS) or endotoxin. LPS from Gram-negative bacteria are responsible for acute sepsis and septic shock, serious clinical syndromes linked with high mortality rates and still lacking efficient pharmacological treatment [21]. Endotoxins are unique and very abundant surface glycolipids (i.e., lipopolysaccharides, LPS and lipooligosaccharides, LOS) of Gram-negative bacteria. They are amphipathic molecules, comprising a generally conserved lipid A region that contains a β-1→6 linked disaccharide of N-acetylglucosamine linked by ester or amide bonds to 3-OH-fatty acids that may be further substituted with non-hydroxylated fatty acids in an acyloxyacyl linkage (Fig. (**2**)). The toxic action of LPS is due to a complex cascade of extracellular protein-LPS and protein-protein interactions, leading finally to the activation of membrane-bound Toll Like Receptor 4 (TLR4) [22].




**Scheme 2.** Synthesis of nojirimycin *C*-glycosides and some bicyclic structures derived thereof.

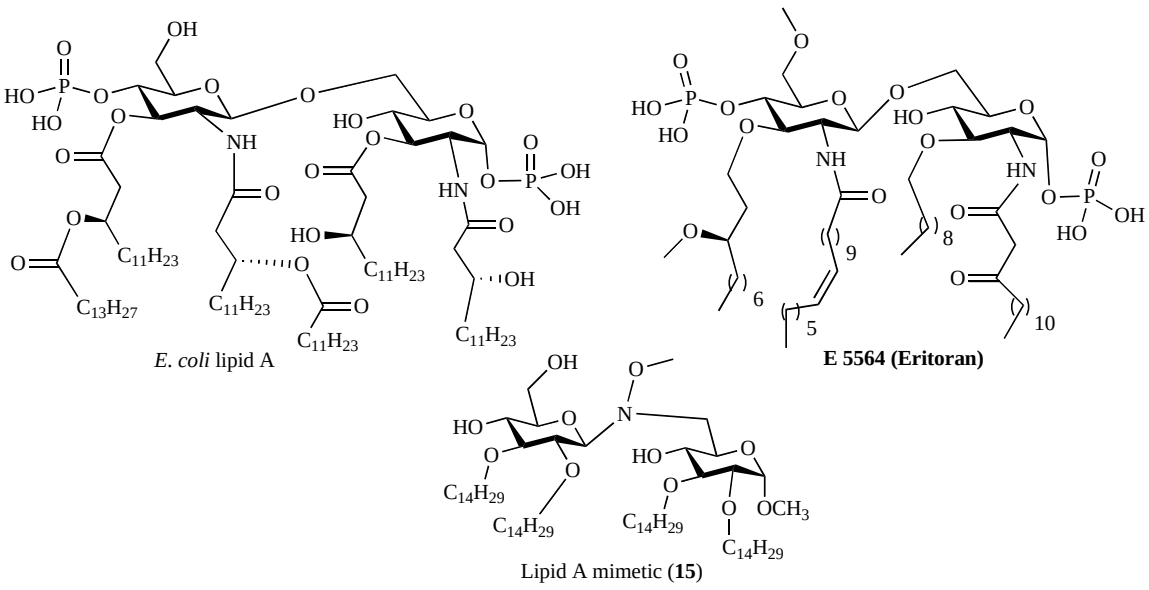



**Fig. (2).** Upper, on the left: structure of *E. coli* lipid A, on the right: structure of synthetic antagonist Eritoran (E 5564); compound 15: synthetic lipid A mimetic with non natural glycoside bond.

Other receptors are sequentially involved in LPS sensing and recognition: lipid-binding protein (LBP) first binds to LPS aggregates and transfers LPS to CD14, that in turns presents LPS to the MD-2 receptor both free of TRL4-bound. The final event of the cascade, the TLR4 activation, leads to intracellular signalling, and synthesis of pro-inflammatory cytokines.

Several synthetic lipid A and lipid A mimetics have been synthesized with both agonist or antagonist activity against TLR4. Antagonists have potential as anti-sepsis and anti-inflammatory agents while agonists are rather leads for the development of vaccine adjuvant or stand-alone immuno-therapeutics. The majority of small molecules so far proposed as leads for drug development are synthetic mimic of the lipid A or naturally occurring under-acylated forms of lipid A that act as antagonists on the TLR4 [23]. Among these compounds, worth of note is compounds E5564 also called Eritoran [24] a synthetic lipid A analogue that is currently in phase III clinical trials (Fig. (**2**)).

The hexa-acylated lipid A has a conical shape with higher cross-section of the hydrophobic moiety than the hydrophilic one and in physiological conditions forms non-lamellar inverted aggregates that are associated to highly endotoxic activity, while under-acylated forms micellar or lamellar aggregates that have been associated to an antagonistic action on TLR4 [25]. According to this rationale, (that also inspired the synthesis of Eritoran) we designed and synthesized lipid A antagonist 15, Fig. (**2**), composed by two glucose units bearing four C14 linear chain ethers linked by a non-natural β-(1→6)-*N*(*O*Me) bond [26]. This compound was active in inhibiting in a dose-dependent way lipid A-induced cytokine production in murine macrophages, that are representative cells of the innate immunity. In the attempt to obtain this disaccharide by simple condensation of unprotected monosaccharide building blocks, we serendipitously obtained the amino-monosaccharide (**17**) (Fig. (**3**)). Compound (**17**), and a series

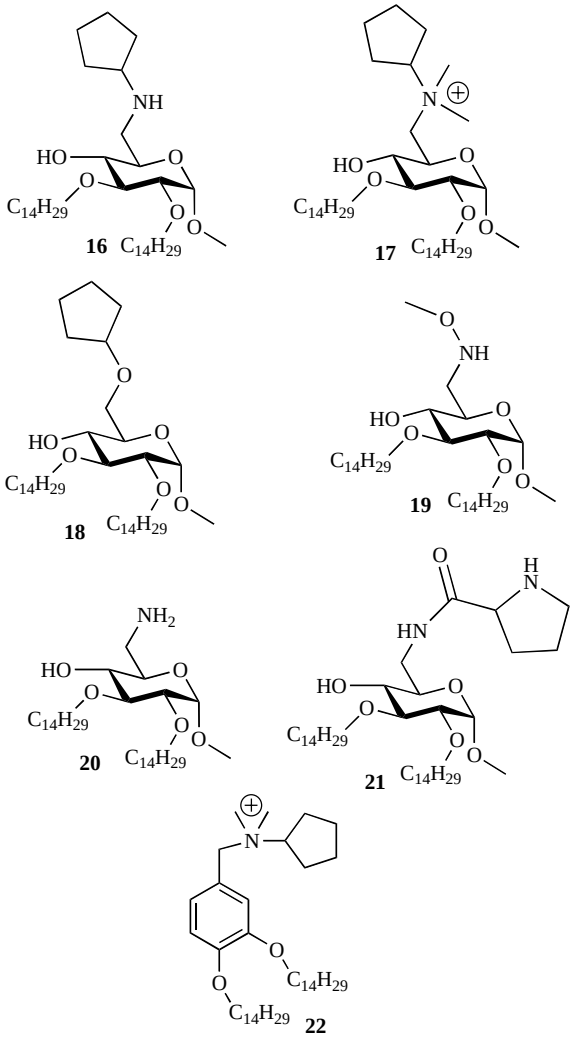



**Fig. (3).** Glycolipids (16)-(21) and benzylamonium lipid (22) targeting LPS signalling.

of structurally related compounds, such as compounds **16**, **20**-**22**, Fig. (**3**), were able to inhibit the lipid A-induced cytokine production in important cells of the innate immune system, such as dendritic cells and macrophages. [27]. Compounds (**16**), (**17**), (**20**), (**21**), (**22**) (Fig. (**3**)) were also active in inhibiting the lipid A-induced NF-kB activation of HEK cells selectively transfected with the human TLR4 and protected mice from LPS-induced lethality [28]. Compounds (**18**) and (**19**) lacking the positive charge or a protonatable nitrogen were totally inactive in both the tests on HEK cells and macrophages and were unable to contrast *in vivo* the LPS-induced septic shock in animal models. Recent evidence indicates a pivotal role of TLR4 in the development and maintenance of some other pathologies such inflammation or painful neuropathies. We observed an interesting activity of compound (**17**) in inhibiting both neuropathic pain [29] and carrageenan-induced local inflammation *in vivo* [28].

The molecular mechanism of action of compound (**17**) was recently investigated. It was found that the capacity of this and related compounds to inhibit endotoxin-triggered TLR4 activation is due to a selective antagonistic effect on CD14 receptor [30]. Biochemical experiments together with NMR measurements confirmed that glycolipid (**17**) binds to CD14 thus competing with endotoxin for the receptor. Due to the absence of antisepsis and anti-inflammatory agents targeting selectively CD14, we are investigating the possible application of compound (**17**) and related molecules as novel immunotherapeutics.

### 2.3. Glycopeptide Mimetics (Neoglycopeptides) by Chemoselective Ligation

Glycoproteins, that is proteins with covalently linked glycans, have important pharmaceutical properties, but the production of drugs based on these molecules has been limited by the variability of the sugar part (referred as microetherogeneity) when glycoproteins are produced by heterologous expression in cells. For this reason, the chemical or chemo-enzymatic synthesis of glycoproteins is the only possibility to produce these molecules in a chemical pure (homogeneous) form. A variety of methods have been developed for the synthesis of natural *O*- and *N*-linked glycopeptides and glycoproteins: sugars are generally introduced into peptides during solid phase synthesis (SPPS) by means of glycosyl amino acid building blocks [31]. This "linear" synthetic approach is generally laborious, requires extensive use of protecting groups and allows the preparation of one glycoform per synthesis. The alternative "convergent" approach is based either on the enzymatic elongation of the oligosaccharide chain of a pre-synthesized glycopeptide or glycosyl amino acid [32, 33], or on the conjugation of a fully elaborated, complex saccharide to short synthetic peptides [34].

Chemoselective ligation, first described by protein chemists as the coupling of two mutually and uniquely reactive groups in an aqueous environment, provides access to complex neoglycoconjugates in an elegant and convergent

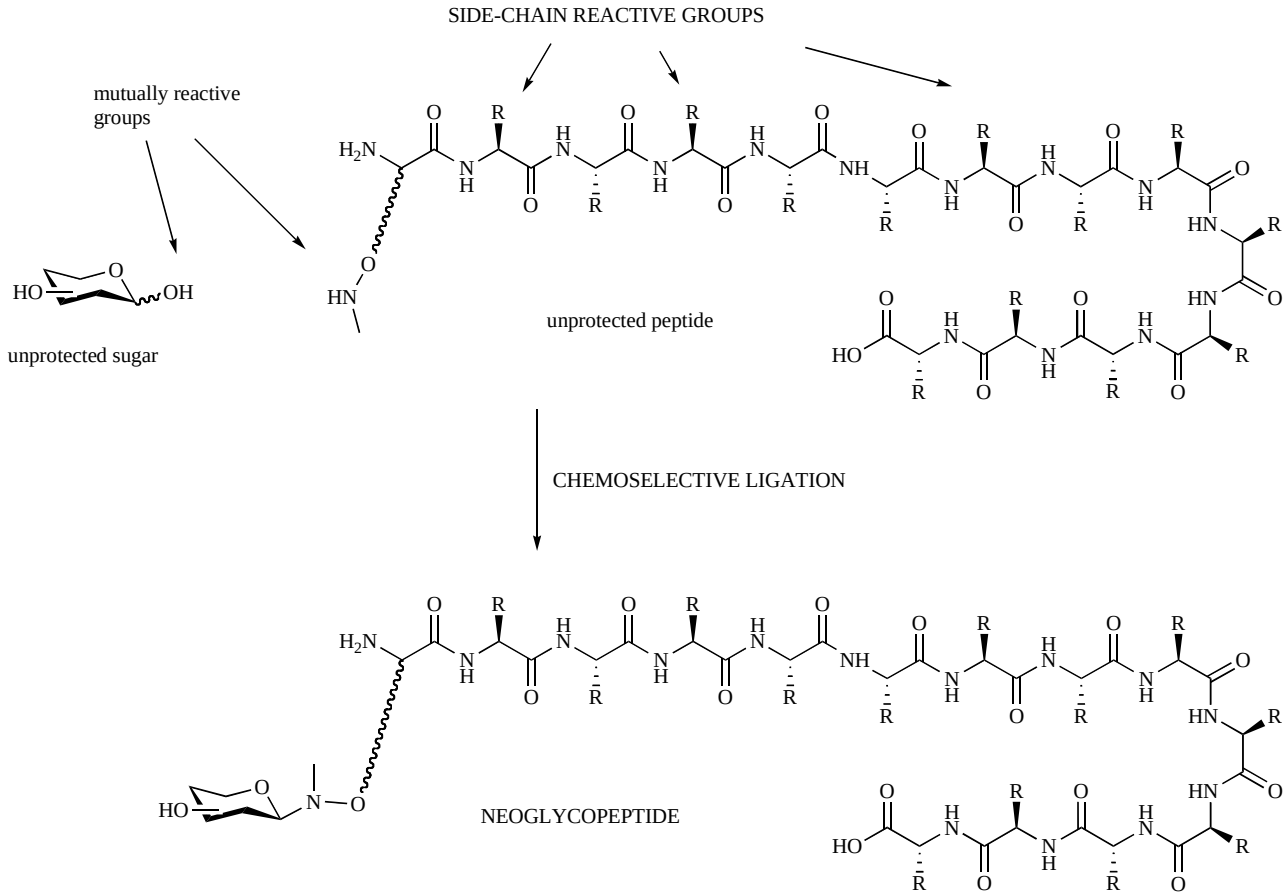
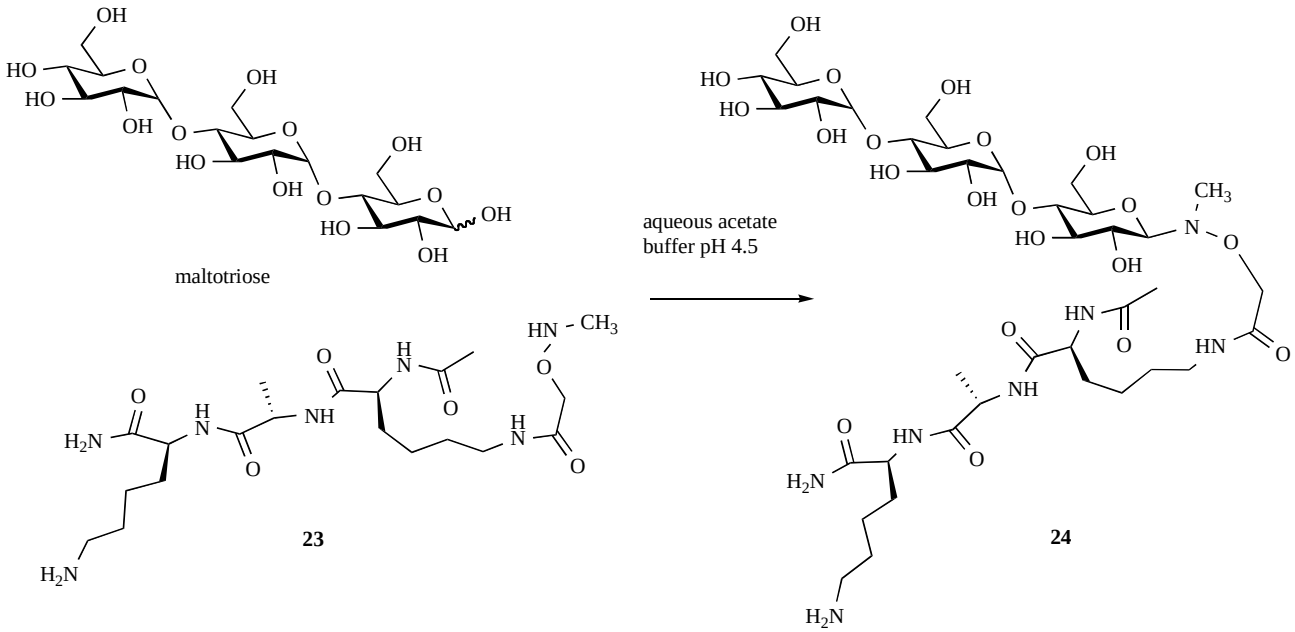


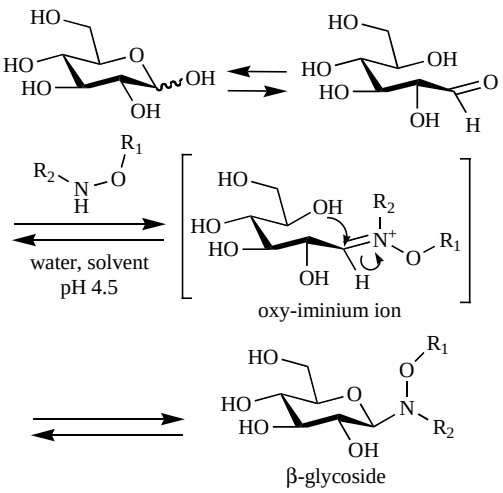

**Fig. (4).** Chemoselective glycosylation and neoglycopeptides formation.




**Scheme 3.** Chemoselective glycosylation reaction between maltotriose and tripeptide (23), bearing a methylamino-oxy function.

way. In this technique, two functional groups (generally an electrophile and a nucleophile) are introduced in two coupling fragments giving rise to the formation of a unique covalent bond even in the presence of an array of other unprotected functionalities. This reaction is exemplified in Fig. (**4**) in the case of the synthesis of a neoglycopeptide.

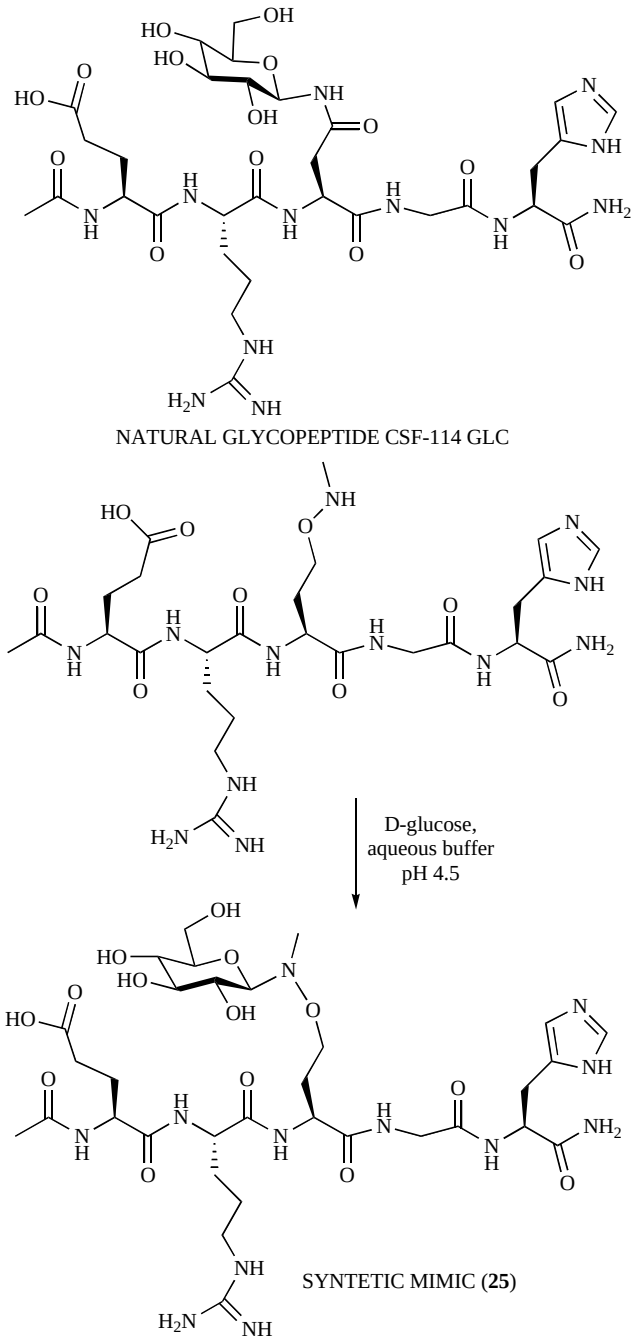
The chemoselective techniques so far developed for neoglycoconjugate synthesis fall into two broad categories: the first characterized by the reaction of a carbonyl group (ketone or aldehyde) with strong nucleophiles, and the second on the addition of sulphur nucleophiles to a variety of electrophiles [35]. The absence of aldehydes and ketones on the side chains of the naturally occurring amino acids inspired a set of chemoselective reactions based on the condensation of the anomeric carbon of a reducing sugar (aldehyde group in the cyclic hemiacetal form) with a variety of non-natural nucleophile groups introduced into the peptide chain. For example, the somatostatin analogue RC-160 bearing an oxyamino group was obtained by glycosylation in a chemoselective way after SPPS with a variety of unprotected mono- and oligosaccharides, obtaining oxime-linked neoglycopeptides with increased bioavailability [36]. The main drawback of these methods is that in the glycoconjugate the sugar is a linear oxime, quite different from the natural pyranose form that is the biologically active form. This problem was addressed with functionalization of the peptide moiety with an N-methylated oxyamino group. After condensation of peptide with unprotected mono and oligosaccharides, the cyclic pyranose form of the first attached sugar was maintained in the obtained neoglycopeptide. In Scheme (**3**) is depicted the first, prototypical application of this innovative reaction to the maltotriose neoglycosylation of a tripeptide containing Lys [37]. The glycosidic linkage of the first attached sugar was formed exclusively in the β-configuration in the case of D-glucose, D-galactose and N-acetylglucosamine, allowing the synthesis of mimetics structurally similar to their parent *N*-linked glycopeptides [38].




**Scheme 4.** Stereoselective formation of the β-oxyamino glycoside through the oxy-iminium intermediate.

The mechanism proposed to explain the diastereoselection in the glycosylation reaction is based on the formation of an intermediate oxy-iminium ion by reaction of *N*,*O*-disubstituted hydroxylamine group with the aldehyde of the open-chain sugar, Scheme (**4**) [37]. The preference for the β-anomer can be explained in terms of stabilization of the β conformation of the oxy-iminium intermediate through reverse anomeric effect that is particularly relevant when a positive charged nitrogen atom is linked to the anomeric position.

Compared to the classical glycosylation/conjugation methods, the chemoselective glycosylation presents several advantages: is high yielding, neither protecting groups nor activating agents are required, can be carried out in water or in aqueous buffered media, is stereoselective. The proto-




**Scheme 5.** Chemoselective synthesis of a neoglycopeptide mimetic (25) of the multiple sclerosis epitope glycopeptide CSF-114 Glc.

typical synthesis shown in the Scheme (**3**) suggested the creation of synthetic amino acids with hydroxylamino group side chain, that, once incorporated into peptides, should allow the chemoselective synthesis of neoglycopeptides. Several types of amino acids opportunely protected to be incorporated into the peptide sequence during solid phase peptide synthesis (SPPS) with the Fmoc protocol were synthesized, Fig. (**5**).

N-Fmoc or N-Boc amino acids with ONHMe or NH (OMe) groups on side chains protected as Boc or chloro-benzyl carbamates were prepared from L-homoserine by us and others and incorporated into peptide sequences during

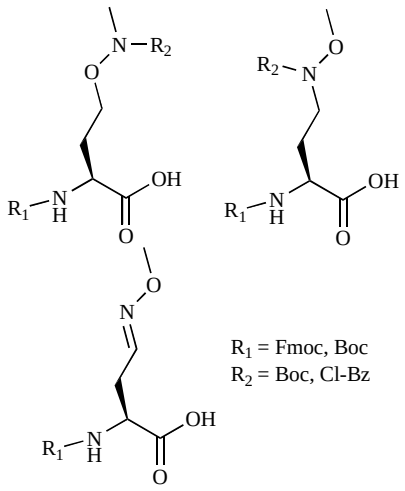


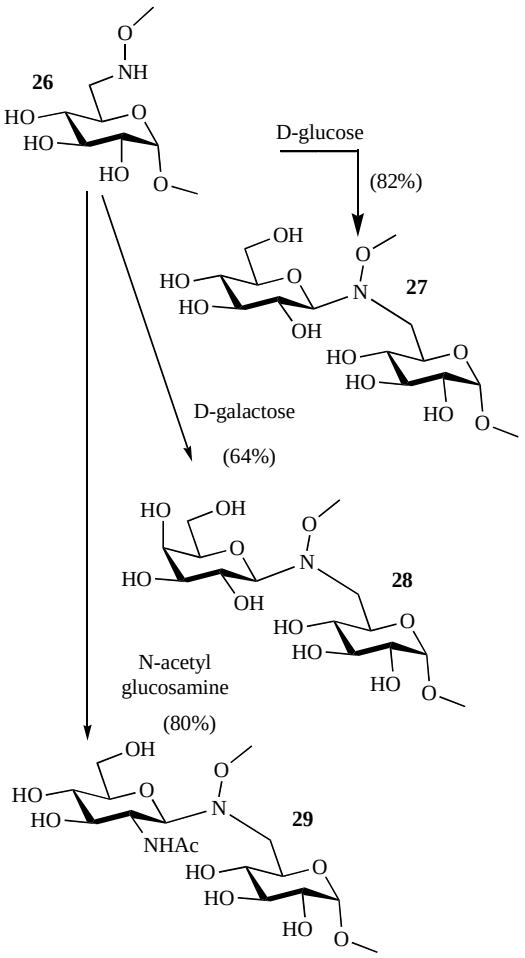

**Fig. (5).** Amino acids bearing protected oxyamino groups and oxime functionalities on side chains.

solid phase peptide synthesis. In the case of the NH(OMe) derivatives, the side chains precursor O-methyl oxime was directly incorporated during SPPS as hydroxylamine precursor and was converted into the hydroxylamine by $NaCNBH_3$ reduction after the cleavage of the peptide from resin and just before the chemoselective reaction with sugars. The oxime amino acid was used for the chemoselective assembly of an analogue of CSF114(Glc) glycopeptide that is an useful diagnostic probe to detect multiple sclerosis (MS) autoimmune syndrome because it reacts with high affinity with MS antibodies, Scheme (**5**) [39]. Unfortunately in this case the MS antibodies did not recognize and did not bind efficiently to neoglycopeptide (**25**). Despite our expectations, this negative result indicates that the glycosidic bond is likely to be part of the epitope recognized by the antibody and subtle modifications of this portion lower the affinity for antibodies dramatically.

### 2.4. Oligosaccharide Mimetics (Neooligosaccharides) by Chemoselective Ligation

The chemoselective ligation techniques can be used to assembly of oligosaccharide mimetics (neo-oligosaccharides). The synthesis of oligosaccharides and their analogues is laborious and challenging, requiring an extensive use of orthogonal protecting groups, anomeric activation and strictly anhydrous conditions in the glycosylation step; for these reasons it is still far from routine, both in solution [40] or on solid phase [41]. The use of chemoselective strategies could alleviate some of these challenges: anhydrous environment is not required, protecting groups and anomeric activating groups are not necessary. These are ideally optimal conditions for the development of solid phase synthesis and subsequent automation. In analogy with sugar-peptide ligation, reacting 6-deoxy-6-methoxyamino glycosides with reducing sugars, isosteric mimics of β-(1,6)-linked oligosaccharides could be obtained. To verify this hypothesis, monosaccharide (**26**), Scheme (**6**), bearing a methoxyamino group at C-6 [42] was first synthesised and subsequently reacted with unprotected reducing monosaccharides D-glucose, D-galactose and *N*-acetylglucosamine affording

stereoselectively the corresponding β-linked neodisaccharides (**27**-**29**), Scheme (**6**).



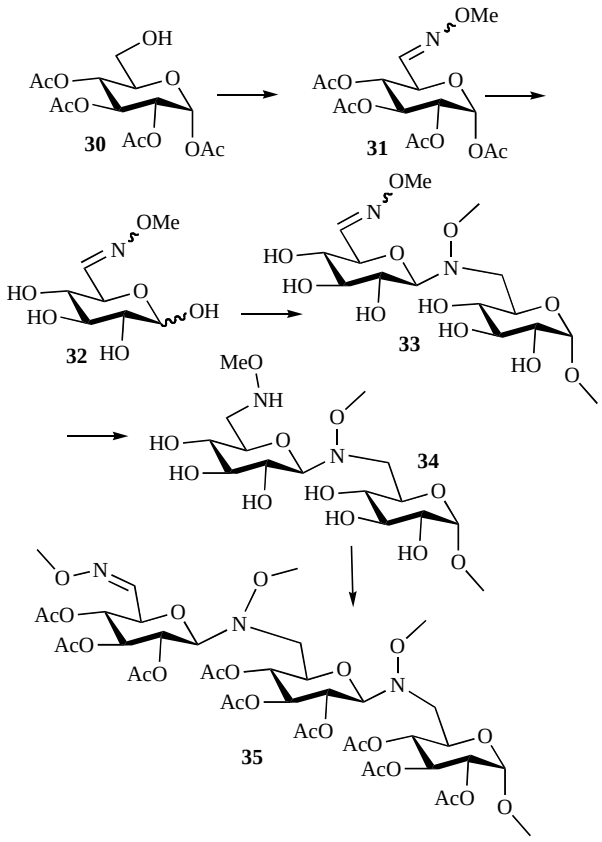

**Scheme 6.** Chemoselective glycosylation with formation of β-(1,6)-linked oxyamino disaccharides.

We also showed the possibility to use the chemoselective glycosylation approach in an iterative way for the preparation of oligosaccharide mimetics. For this purpose, the hydroxylamine function was masked as oxime in monosaccharide (**32**), that can first react chemoselectively as glycosyl donor with hydroxylamino monosaccharide giving disaccharide (**33**), as illustrate in Scheme (**7**). After $NaCNBH_3$ reduction of (**33**), the disaccharide (**34**) with an hydroxylamino group is obtained that can be reacted again with a reducing monosaccharide, giving trisaccharide (**35**); these reactions can be iterated for the preparation of linear, β-(1,6) oxyamino-linked polysaccharides.

Conformational analysis of β-(1,6)-linked oxyamino neodisaccharides was performed using NMR and computational methods [43], thus evidencing that these compounds, that have a *N*-*O*Me group at the pseudoglycosidic position have conformational behaviour similar to their natural counterparts with *O*-glycosidic bond.

### 2.5. Multivalent Neoglycopeptides as Synthetic Vaccines

It is now well known that altered glycosylation patterns either on glycoproteins or glycolipids are a hallmark of the




**Scheme 7.** Iterative, chemoselective synthesis of β-(1-6)-linked oxyamino oligosaccharides.

tumour phenotype, as described first by Meezan *et al.* in 1969 [44]. Since then, several Tumour-Associated Carbohydrate Antigens (TACAs) have been identified, such as the mucin-related epitopes Tn (GalNAcα-O-Ser/Thr), TF or T (Thomsen-Friedenreich, Galβ1→3GalNAcα-O-Ser/Thr) and STn (NeuAcα2→6GalNAcα-O-Ser/Thr) [45]. The TACAs can be used for the creation of anti-tumour vaccines, taking advantage from the known tendency of transformed cells to express selective carbohydrate motifs otherwise hidden in normal cells. The immunological response is elicited by the association in the same molecule of a carbohydrate, as B-cell antigen, and a peptide, or an entire protein, as T-cell epitope: thus, a bivalent antigen constituted by a saccharidic B epitope and a T epitope is desirable. Several studies with conjugate cancer vaccines containing natural or synthetic antigens have been reported [46] and many approaches have been explored to increase the immunogenicity of these molecules, such as multiple antigen glycopeptides (MAG) [47]. Since the antigens occur on the cell surface as multiple, not single, molecules, it is not surprising that the multiple antigen presentation on synthetic glycoconjugates results in their enhanced immunogenicity. As part of a wider project aimed at synthesizing glycopeptides by chemoselective ligation containing *C*-glycoside analogues of natural glycans [48], we focused our attention on the synthesis of neoglycopeptide **36**, Fig. (**6**) as potential small molecular weight antitumor vaccine [49], constructed by covalently linking a short peptide, as the T-epitope $H_2N$-AVHAAHAEINEAG-

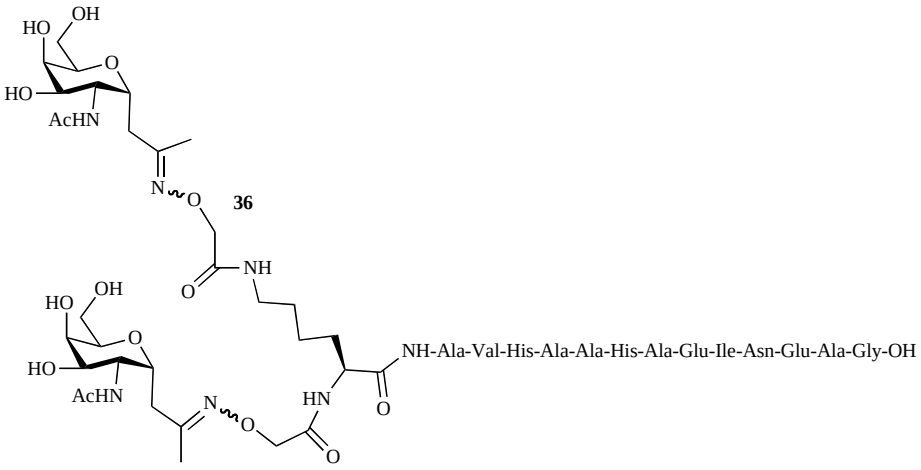



**Fig. (6).** Structure of a branched neoglycopeptide 36 as potential anticancer vaccine.

OH, to two copies of the carbohydrate epitope Tn, over expressed in various human carcinomas of epithelial origin [50, 51]. This tumour vaccine was obtained by using a convergent synthetic approach based on the chemoselective ligation of two units of the *C*-glycosyl analogue of the Tn antigen with a keto group in the anomeric appendage and a peptide functionalized with two aminoxy group at the *N*-terminal end. Tests *in vitro* showed that glycopeptide (**36)** is efficiently internalized by dendritic cells (DCs) and consequently presented to T cells indicating that the *C*-glycoside epitope GalNAc either acts as an internalization agent by binding specific DCs surface sugar receptors, or promotes receptor clustering that is essential for DCs activation [49]. *In vivo* assays on C57BL/6 mice indicated that specific T and B cell responses against the tumour were induced by immunization with neoglycopeptide (**36**).

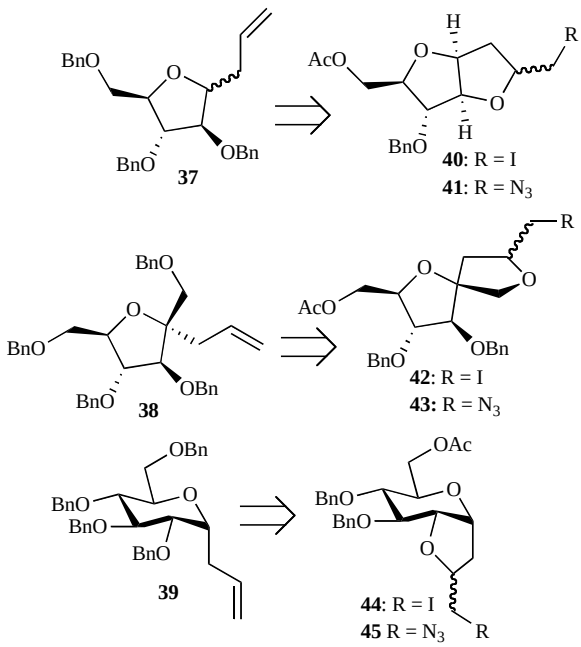
## 3. CARBOHYDRATE SCAFFOLDS

Carbohydrates are probably the most abundant chiral molecules available naturally. They are, in fact, constituents of all major structural molecules in living systems. The low cost, abundance, highest functional group density of any naturally occurring material, and the ease with which they can be obtained in a pure state are among the most important features that make carbohydrates prime candidates for the synthesis of chiral scaffolds [52] with application in the areas of pharmaceutical and medicinal chemistry. The key issue in this field is the development of better scaffolding structures which can be used as molecular templates to display pharmacophoric groups in well defined spatial orientations, allowing almost unlimited structural variations [53]. For example, in the area of protein-protein interactions, it is generally believed that the size and the rigidity of the structural elements within a small molecule, such as monosaccharides, tend to provide better binding/interfering agents. Turn mimetics for the inhibition of specific protein-protein interactions have been designed from carbohydrate cores; a β-D-glucose scaffold was synthesized by Hirschmann *et al.* [54] and included in a cyclic peptide, as a mimetic of the natural somatostatin. Sugar derived scaffolds, such as tetrahydrofuran based amino acid mimics [55], have also been used for the preparation of unnatural oligomers as peptidomimetics, referred to as foldamers [56] that is molecules that will adopt specific compact conformations. The benefits of such carbohydrate scaffolds include an attractive balance between rigidity and diversity of functional group orientation.

Our contribution in this field focussed on the use of monosaccharides D-glucose, D-galactose, D-arabinose and D-fructose for the construction of conformationally-locked scaffolds that have been exploited to synthesize libraries of bioactive compounds (sugar amino acids, GABA receptor ligands, Ras inhibitors).

### 3.1. New Bicyclic Scaffolds Derived from Natural Monosaccharides

Through similar reaction sequence we obtained bicyclic structures (**40**-**45**), Scheme (**8**), from allyl-C-glycosides of D-sugars arabinose (**37**), fructose (**38**), glucose (**39**).




**Scheme 8.** Bicyclic scaffolds from intramolecular iodocyclisation reaction on α-*C*-allyl glycosides, followed by nucleophylic displacement of iodine with azide.

The reaction sequence affording fused bicycles in the case of glucose and arabinose, or spiro-bicycles from fructose is based on the iodocylization of *C*-allyl glycosides. This reaction is the crucial step for the synthesis of the bicycle and its mechanism is based on the opening of the intermediate iodonium ion by attack of the γ-benzyloxy groups in the 5-exo-mode with formation of a cyclic iodoether with concomitant debenzylation. The bicyclic iodoether is obtained as a mixture of epimers at carbon C-2' (**40, 42, 44**). The iodocycles are then treated with tetrabutylamonium azide in toluene and subsequent debenzylation-acetylation of primary hydroxyls by treatment with acetic anhydride in acidic conditions, afforded azido-derivatives (**41, 43, 45**) that have been used as building blocks and scaffolds for the preparation of an array of bioactive compounds.

### *3.1.1. Sugar Amino Acids (SAAs)*

The conformational rigidity of the pyrane and furane rings makes sugar amino acids (SAAs) interesting building blocks in the induction of precise secondary structures in peptides and in the construction of peptidomimetics [57]. By varying the mutual positions of the amino and carboxylic groups in the glucopyranose scaffold, a set of SAAs was designed and found to be capable of inducing linear, β-turn and γ-turn conformations in peptides [58]. The same sugar amino acids have been used to induce the bioactive conformation in small RGD cyclic peptides, obtaining selective antagonists for the αvβ3 integrin [59]. In a slightly different conceptual approach, α-D-glucopyranose was employed as peptidomimetic scaffold devoid of an amide backbone to accommodate the side chain functionalities responsible of the biological activity of the peptide hormone somatostatin [60]. In this context, RGD mimics. and thrombin inhibitors were prepared by assembling amino acid side chain functionalities on D-glucose. Oligomers of pyranose sugar amino acids ("carbopeptoids") have been synthesised, and the furanose ring has also been exploited as building block for carbopeptoid assembling [61]. Homo-oligomers of tetrahydrofuran amino acids derived from the arabinofuranose were shown to adopt a novel repeating β-turn type secondary structure in tetrameric units stabilized by intramolecular hydrogen bond. In general, the synthesis of sugar amino acids can be accomplished in a few steps starting from commercially available or easily accessible monosaccharides. The amino functionality of the SAA can be introduced as an azide, cyanide or nitromethane equivalent, followed by subsequent reduction. The carboxylic function is introduced directly as $CO_2$, or as a hydrolyzable cyanide, by a Wittig reaction and subsequent oxidation or by selective oxidation of a primary alcohol. We then transformed the bicyclic compounds derived from D-arabinose and D-fructose, Scheme (**8**), into the sugar azido acids (**46**-**49)** depicted in Fig. (**7**), that can be readily converted in the corresponding SAAs.

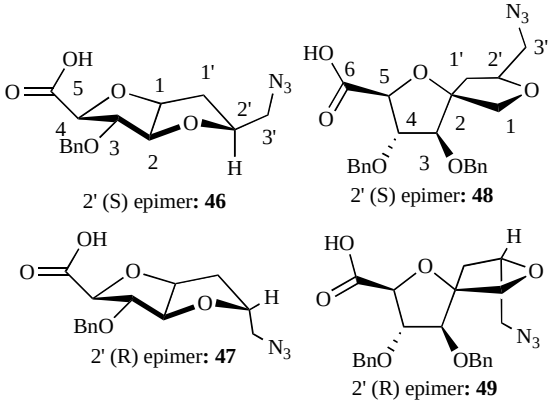
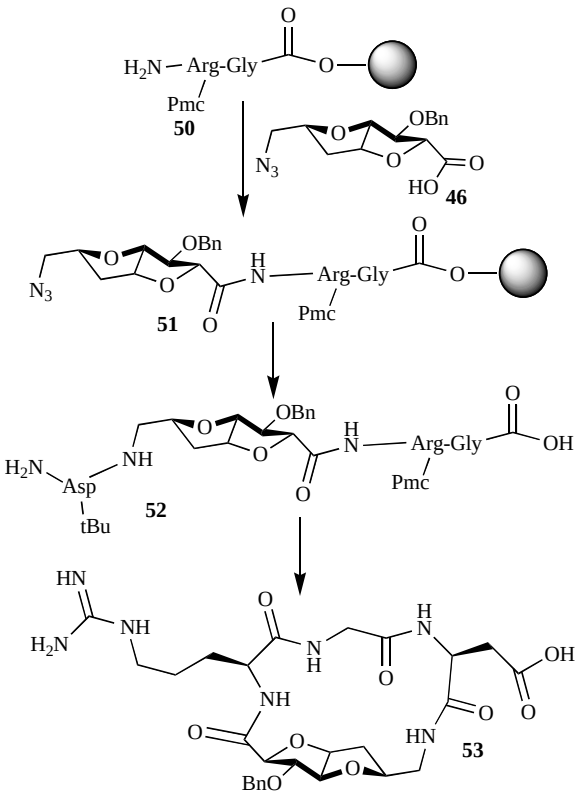



**Fig. (7).** Diastereomeric couples of arabinose- and fructose-derived bicyclic sugar azido acids.

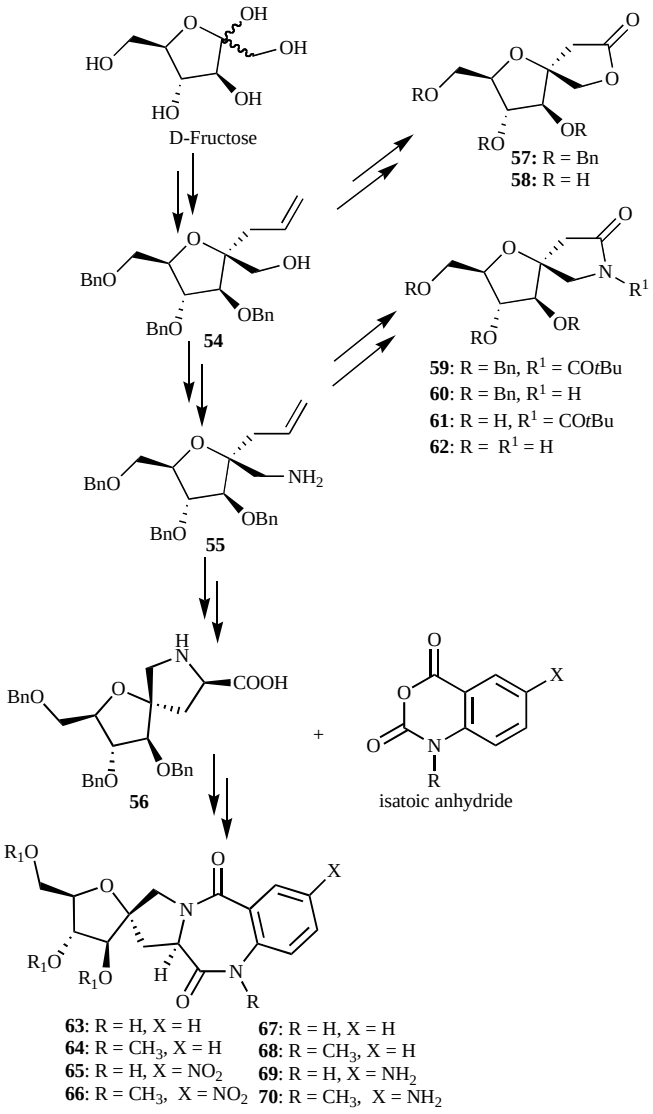



**Scheme 9.** Synthesis of cyclic RGD cyclic peptide (53) incorporating the arabinose-derived SAA.

Arabinose-derived SAA **46** was used in the synthesis of cyclic peptides containing the Arg-Gly-Asp (RGD) tripeptide **53** as mimetics of the correspondent integrin loop, according to synthetic Scheme (**9**). These RGD loop mimetics have great potential as anti-integrin and anti-adhesion agents for tumour therapy. SAA was incorporated into a synthetic cyclic peptide containing the RGD sequence. The cyclic peptide **53** was assayed for its ability to inhibit the adhesion of foetal bovine aortic endothelial GM7373 cells expressing the αvβ5 integrin to their ligands: fibroblast growth factor 2 (FGF-2), vitronectin (VN) and fibronectin (FN). A selective inhibition of cells adhesion to FGF-2 and VN was observed, indicating that the synthetic RGD loop mimetic is a specific antagonist of the αvβ5 integrin [62].

## 3.2. Glycidic Scaffolds for the Construction of new GABA Receptor Ligands

D-Fructose was the scaffold of choice for the synthesis of fructose-fused γ-butyrolactones and lactams (**57**-**62**), Scheme




**Scheme 10.** Synthesis of GABA analogues 57-62 and chimeric benzodiazepines **63-70**.

(**10**) [63] and novel, conformationally constrained chimeric 1,4-benzodiazepine-2,5-diones (**63-70**) [64] as $GABA_A$ receptor ligands.

γ-amino butyric acid is the primary inhibitory neurotransmitter in the mammalian central nervous system (CNS) [65]. GABA operates through multiple receptors subdivided into the ionotropic $GABA_A$ and $GABA_C$ receptors and the metabotropic $GABA_B$ receptor [66]. These receptors are the target for many endogenous and exogenous modulators that regulate normal and pathological brain mechanisms, such as sleep, memory, epilepsy and emotions [67], and for a number of drugs [68], including benzodiazepines, pyrrolo[2,1-*c*][1,4] benzodiazepines, barbiturates and neurosteroids. Also, γ-butyrolactones and γ-butyrolactams are of biological relevance as GABA receptor ligands. Consequently, compounds that act at GABA receptors have considerable therapeutic interest for use in a variety of neurological disorders, such as epilepsy, anxiety, schizophrenia. For GABA receptor ligand action, penetration of the blood-brain barrier (BBB) is required, and lipophilicity is the most important parameter that crucially influences its penetration. Hence additional hydroxyl derivatization of the fructose scaffold may be used to increase lipophilicity, as well as to modulate the activity of pharmacophores or the receptor specificity. Interesting feature of this work is that the same key intermediates derived from fructose have been used as scaffolds to target two different binding sites on the same receptor.

The synthesis of fructose-derived GABA analogues (**57**–**62**) and chimeric benzodiazepines (**63**–**70**) requires access to key intermediates (**54**) and (**55**), Scheme (**10**), where the pharmacophore is engineered into the carbohydrate scaffold in the form of a *C*-fructoside.

Preliminary biological evaluation for receptor binding studies of $GABA_A$ receptors β-disubstituted γ-butyrolactones, γ-butyrolactams and conformationally constrained 1,4-benzodiazepine- 2,5-diones were performed. The data showed that both butyrolactones (**57**) and (**58**), and *N*-substituted lactams (**59**) and (**61**), are ligand for GABA binding site, demonstrating that the sugar scaffold does not seem to hinder the binding, as both benzylated and deprotected

lactones and lactams had comparable activity. Furthermore, benzodiazepine derivatives (**66**, **67**) and (**70**) showed a significant affinity for their $GABA_A$ receptor. Hence, the carbohydrate moiety can effectively be used to modulate drug pharmacokinetic properties and lipophilicity.

### 3.3. Sugar-Derived Compounds that Inhibit Ras Activation and Ras Signalling

D-glucose and arabinose-derived bicyclic compounds were then used as rigid scaffolds for the synthesis of compounds that bind human Ras protein and inhibit its activation. Ras proteins are intracellular small GTPases that function as molecular switches that couple membrane receptors to intracellular signalling pathways regulating cell growth and differentiation [69]. Ras-GTP is the active form, whose intracellular level derives from the balance between GTP hydrolysis (mediated by GTPase Activating Proteins, GAPs) and GDP to GTP exchange (catalyzed by Guanine nucleotide Exchange Factors, GEFs). Mutation of one of the *ras* genes, usually *k-ras*, is a very common event in human cancer [70]. The GAP-promoted hydrolysis of GTP to GDP is defective in oncogenic Ras mutants that are constitutively active, their abnormal activity causes uncontrolled cell proliferation leading to tumour insurgence and diffusion.

A number of different approaches aimed at abrogating oncogenic Ras activity have been explored in clinical trials; however, the search for small molecules with anticancer properties directly targeting Ras proteins is still open. Rational design and combinatorial approaches allowed the identification of low-molecular weight active compounds targeting the Ras activation process at different levels: inhibitors of farnesyl, geranylgeranyl and palmitoyltransferases; [69] GTP analogues activated to spontaneous phosphate hydrolysis that should compensate the very low

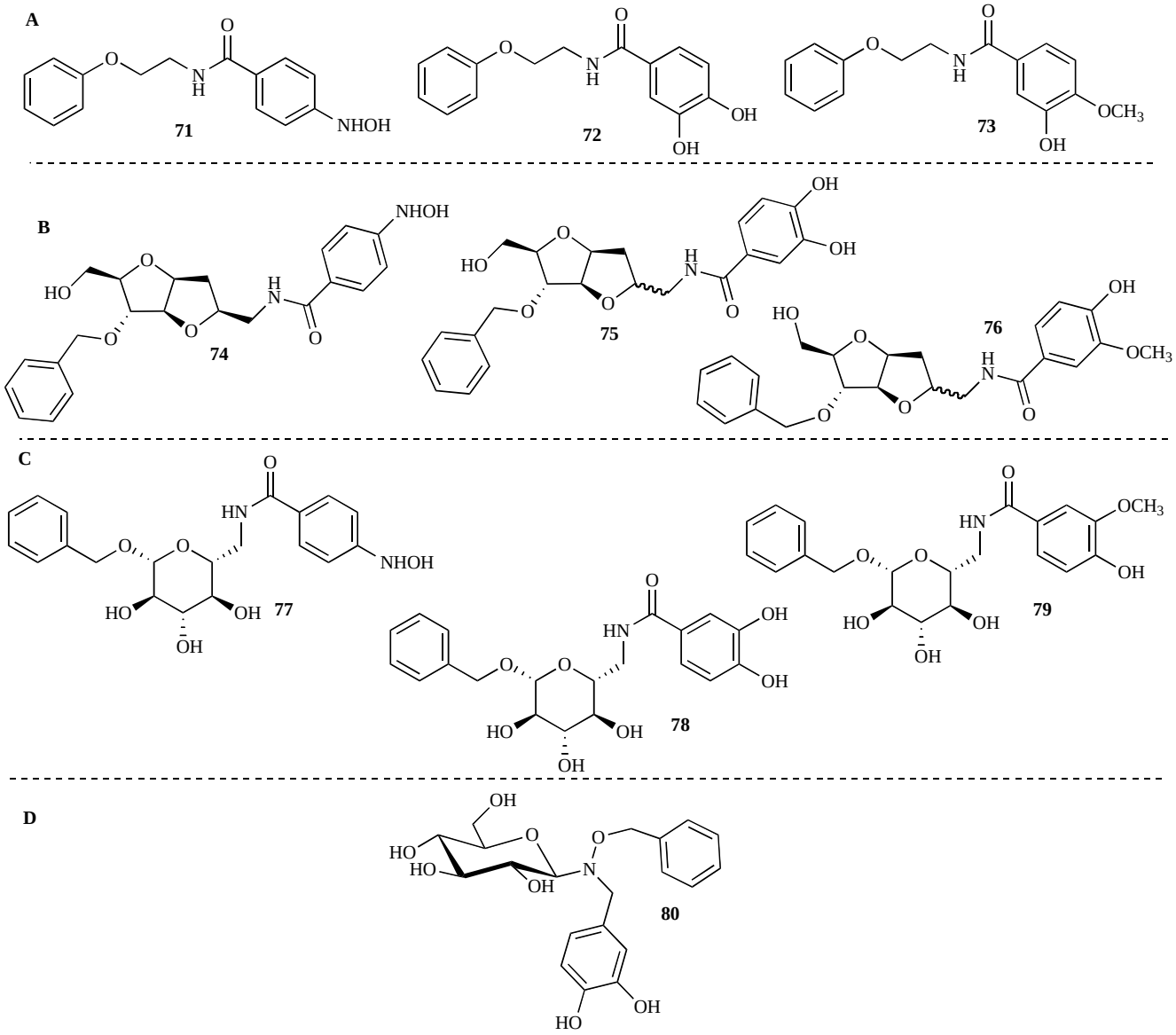



**Fig. (8).** Different generations of Ras ligands, **A**) Compounds with a linear linked between aromatic units; **B**) compounds with a D-arabinose-derived scaffold; **C**) compounds with a D-glucose-derived scaffold; **D**) compounds formed by an aromatic moiety conjugated to a sugar.

GTPase activity of oncogenic Ras mutants, and inhibitors of the interaction of Ras with the downstream effector Raf.

Inspired by the pioneering work of Schering-Plough researchers [71], we proposed an alternative approach to anticancer drug development based on small organic molecules that bind to Ras-GDP complex, and inhibit the GEF-promoted nucleotide exchange on Ras. The design of these Ras ligands was leaded by the use of the same pharmacophoric groups that are present into the compounds previously developed by Schering-Plough. These group are constituted by an aromatic moiety and an aromatic hydroxylamine, and were linked to different scaffolds giving different generations of compounds depicted in Fig. (**8**). In the first panel of compounds, a linear linker was inserted between the aromatic groups [72], in the second group a bicyclic structure derived from D-arabinose was used as scaffold [73], in the third generation a D-glucose is present as scaffold [74]. In the last generation of compounds, a natural monosaccharide (glucose, galactose, *N*-acetylglucosamine) or a disaccharide (lactose) is conjugated to the aromatic pharmacopohore [75].

The chemical structure of the pharmacophore groups has also been varied and the active but toxic hydroxylamine was replaced with a catechol moiety with retention of the biological activity.

All compounds were active in inhibiting the GDP-GTP exchange in vitro on purified H-Ras, with a potency in the micromolar range. The more active compound was molecule [75] with an arabinose-derived scaffold [73]. These compounds were also more soluble in aqueous buffers used for biological experimentation that the compounds of the first panel, similar to that developed by Schering-Plough. Binding experiments with purified Ras were performed by using the Saturation Transfer Difference (STD) NMR technique [76].

NMR experiments showed that compounds of the first and second generation bind to Ras and that the aromatic moieties play a prominent role in the interaction with the protein.

In contrast with the first two generations of compounds, glucose-derived compounds of the third group were almost inactive in inhibiting nucleotide exchange in vitro and gave very weak interaction with Ras in STD experiments [74]. Surface Plasmon Resonance (SPR) experiments with immobilized His-tagged Ras, showed that arabinose-derived molecules interfere with Ras-GEF (Cdc25) interaction, and this would very likely have the effect to lower the rate of the GEF-catalyzed nucleotide exchange.

A detailed analysis of the binding contacts between active inhibitors and human H-Ras was performed by docking calculations [75]. We obtained structures in which the ligands bind in a cleft in the vicinity of the Switch II region, in good agreement with NMR data obtained by STD and tr-NOE experiments. For all compounds it is evident that the interaction of the two aromatic groups is the driving force for Ras binding. In particular, the phenylhydroxylamine group or the aromatic catechol are involved in hydrophobic interactions with the aromatic ring of Tyr96, and form hydrogen bonds with the hydroxyl group of Tyr96 and some amino acids of the Switch II region such as Gly60, Gln61 and Glu62. hydrophobic contacts with Tyr96.

The selective toxicity of Ras inhibitors for colorectal cancer cells HCT-116 was also investigated, and it was found that arabinose-derived Ras inhibitors are more toxic for cell clones expressing oncogenic Ras (G13D mutant) that for cells with normal (wild-type) Ras [77].

The molecules belonging to the third group, formed by a natural sugar conjugated to an aromatic pharmacophore, besides being active as inhibitors of nucleotide exchange of Ras, were also the most water-soluble inhibitors of the all series. With this last group of compound $^{15}$N-edited NMR binding experiments were done and the residues of Ras involved in the ligand binding were determined [75]. This was the first determination of Ras-inhibitor binding interface.

The resolution of the Ras-GDP-inhibitor complex at an atomic level will allow the design and synthesis of more potent Ras ligands with improved potency and selectivity. These molecules, together with inhibitors described in this paragraph, are promising lead compounds for the development of anticancer drugs based on oncogenic Ras targeting.

## CONCLUSION AND PERSPECTIVES

Despite the difficult chemistry lying beyond carbohydrates that hampered the development of glycomedicine, the last few years chemists have strongly improved their capacity to manipulate this class of compounds. The richness of carbohydrates structures found in nature and the massive involvement of carbohydrates in molecular recognition events and signalling processes found in most physiological and pathological functions of organisms, fuelled the design of new drugs based on glycomimetics, and the use of carbohydrate as scaffolds with low toxicity for drug development. Our contribution to both these field has been reviewed here. Several carbohydrate-based therapeutics are already on the market and we can expect that novel sugar-derived drugs will be developed in the next years targeting enzymes, receptors, or generally modulating signal pathway by interfering with multiple targets.

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