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Invited Review

Carbohydrate-Based Scaffolds for the Generation of Sortiments of Bioactive Compounds

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Summary. The polyfunctionality and conformational rigidity of carbohydrates make this class of compounds ideal scaffolds for the production of sortiments[1] of bioactive compounds. Examples of carbohydrate-derived peptidomimetics of biological interest, such as somatostatin agonists and integrin antagonists, are presented. In order to have access to solid phase supported sortiments of compounds, orthogonally protected or unprotected carbohydrates were linked to polymers and reacted in the solid phase employing different regioselective strategies. Original bicyclic and tricyclic glycidic scaffolds were easily obtained starting from natural sugars such as *D*-arabinose and *D*-fructose. Manipulation of these conformationally blocked compounds afforded different carbohydrate-based derivatives, among which azidoacids are useful precursors of β-turn peptidomimetics.

Keywords. Carbohydrates; Combinatorial chemistry; Scaffolds; Peptidomimetics.

Introduction

The relevant role played by carbohydrates in biological recognition phenomena lies in the unique feature of this class of natural compounds which combine high combinatorial potential and conformational rigidity. Monosaccharides, if compared to amino acids, contain more than one stereocentre (in general 5 for hexoses), are present in the pyranosidic or furanosidic form and, interestingly, the cyclic structure induces a unique conformational rigidity fundamental for biological interactions of oligosaccharides. It is now clear that the complex structure of oligosaccharides is only partially involved in the weak binding with their receptors; the remaining part appears to act as a scaffold that orients the binding determinants in the appropriate conformation and provides a connection to the aglycons. An interesting example is

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[1] Following the argumentation of *Roald Hoffmann* (Hoffmann R (2001) Angew Chem **113**: 3439), the terminus 'sortiment' is to be preferred over 'library' in this context

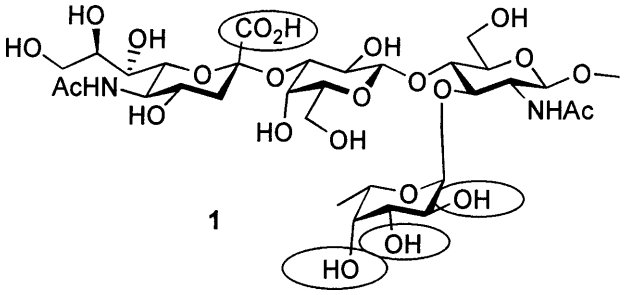

Fig. 1. The tetrasaccharide sialyl *Lewis*^{x}

represented by the tetrasaccharide sialyl *Lewis*^{x} (*sLe*^{x}, Fig. 1) that interacts with selectins, the cell adhesion molecules expressed at the surface of vascular endothelial cells after injury. This recognition triggers the anti-inflammatory response and the metastasis formation, *sLe*^{x} being a determinant of neutrophils and metastatic tumor cells. Extensive studies have shown that the structural requirements for the biological activity of this tetrasaccharide are mainly the presence of the carboxylic group of neuraminic acid and the hydroxyl groups of *L*-fucose (as highlighted in Fig. 1). This observation has prompted the synthesis of a variety of analogues in which the glycidic parts of the molecule not involved in the recognition are replaced by spacers with lower molecular, and hence synthetic, complexity and with improved metabolic stability. The studies have pointed out that the conformational rigidity induced by the glycidic rings is fundamental for the biological activity. The substitution of glycidic units with acyclic moieties causes a drop in the biological activity due to the gain of conformational freedom; on the contrary, the insertion of rigid scaffolds with limited conformational mobility restores an adequate biological activity. For instance, the 3,4-disubstitututed N-acetylglucosamine moiety of *sLe*^{x} can be substituted by 1,2-*trans*-cyclohexanediol (Fig. 2) in the synthesis of a highly effective mimic [1]. Furthermore, the orientation of the carboxylic group of neuraminic acid is quite important for an effective adhesion to E-selectins. Compound **2** (Fig. 2) lacks the pyranosidic ring of neuraminic acid and has a lower biological activity than compound **3** in which the methylcyclohexyl substituent decreases the mobility of the carboxylic group [2].

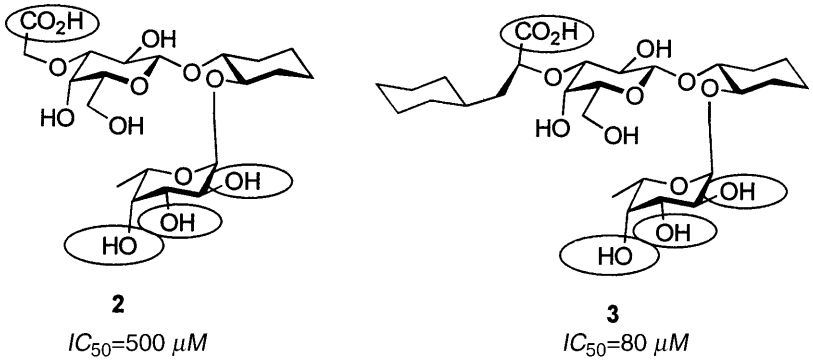

Fig. 2. Simplified mimic of sialyl *Lewis*^{x}

From these observation it is clear that the structural diversity and conformational rigidity of carbohydrates render these compounds interesting scaffolds capable to link to different pharmacophoric groups and orient them in a precise spatial arrangement.

Glycidic Scaffolds

An interesting example of the use of monosaccharides as scaffolds for the synthesis of bioactive compounds is represented by the work of *Hirschmann et al.* [3] describing the synthesis of bioactive somatostatin mimics from the natural sugar *D*-glucose. The peptide hormone somatostatin (**4**, Fig. 3) is a cyclic tetradecapeptide which inhibits the release of several hormones including the growth hormone (GH). Since its biological activity is due to the interaction of the β-turn type I fragment *Phe-Trp-Lys-Thr* (highlighted in Fig. 3) with the receptor, the aminoacidic side chains responsible for the interaction have been attached to glycidic scaffolds in a proper spatial arrangement. Figure 4 describes a prototype of a carbohydrate-derived somatostatin mimic (**5**) prepared from commercially available 2,3,4,6-tetra-O-acetyl-α-*D*-glucopyranosyl bromide. Employing different glycidic starting materials, other analogues lacking the benzyloxy group alternatively in position 2, 3, or 4 of the sugar have also been synthesized [3]. More recently, this approach has been extended to a model study in solution for the preparation of solid-phase sortiments of compounds based on the α-*D*-glucose scaffold [4].

Another example of the use of carbohydrates to mimic non-carbohydrate bioactive compounds has been reported by *Nicolau et al.* who synthesized mimics such as compound **6** (Fig. 5) of the *Arg-Gly-Asp* (RGD) sequence [5]. The RGD sequence is responsible for the adhesion of extracellular matrix glycoproteins, such as fibronectin, fibrinogen, or vitronectin, to endothelial cells (Fig. 6), a phenomenon that promotes, *inter alia*, angiogenesis in the case of $\alpha_V\beta_3$-integrins and platelet

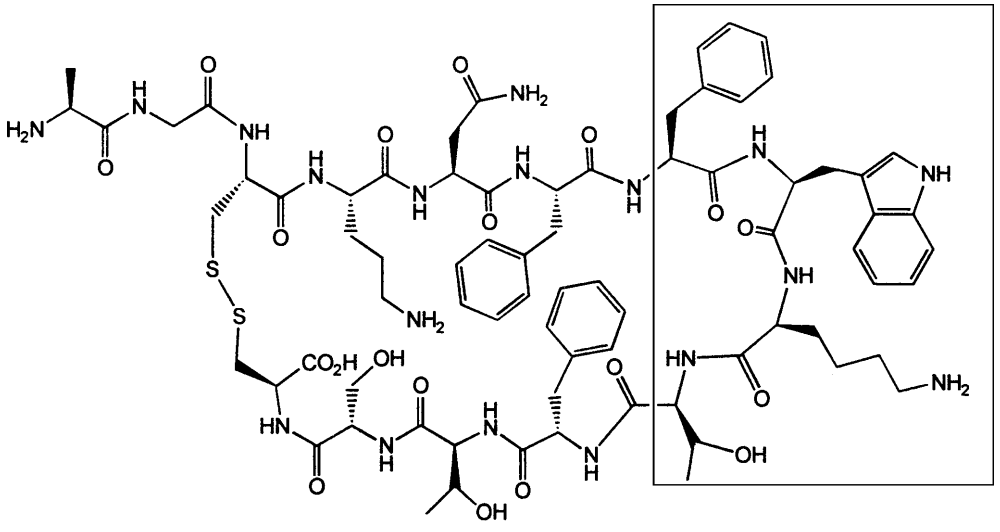

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Fig. 3. The hormone somatostatin

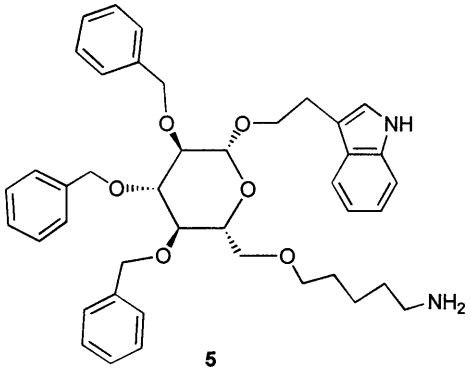


Fig. 4. A carbohydrate derived somatostatin mimic

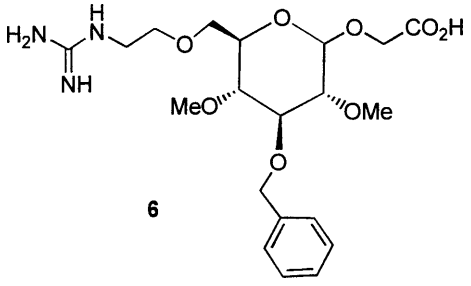


Fig. 5. Carbohydrate derived mimic of the RGD sequence

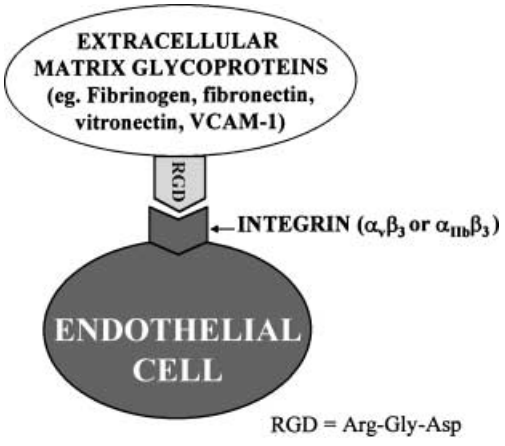


Fig. 6. Schematic representation of RGD-Integrin interaction

aggregation in the case of $\alpha_{\mathrm{IIb}}\beta_3$-integrins [6]. *Kessler* and co-workers have developed the synthesis of a series of amino acids derived from sugars (SAA) with different turn-inducing properties [7]. The SAA derived from glucosyluronic acid methylamine (*Gum*) was incorporated in cyclic RGD peptides that proved active as selective $\alpha_{\mathrm{V}}\beta_3$-integrin antagonists [8]. In all these examples, the potentials of the glycidic scaffold have only partially been exploited, since only a couple of hydroxyl

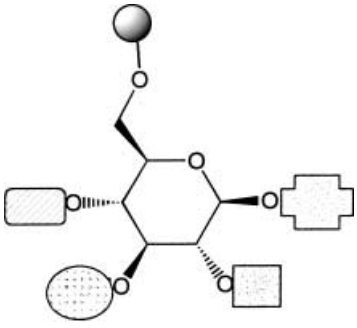


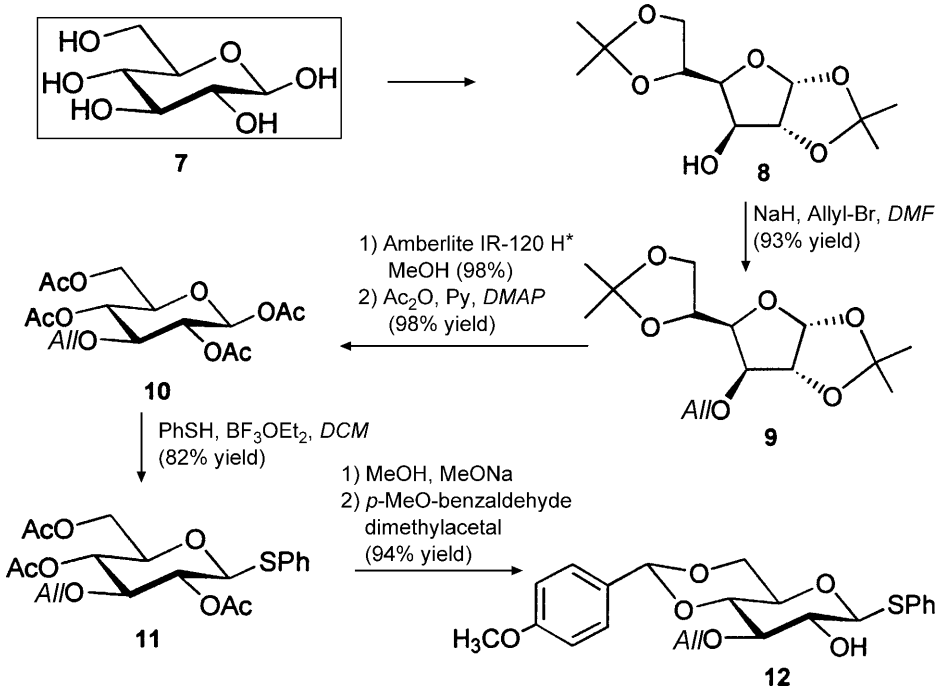
Fig. 7. Different pharmacophores linked to a solid phase supported carbohydrate

groups out of five were involved in the derivatization. Examples in which the whole potential diversity of the sugar is exploited in the preparation of glycidic scaffolds have been reported by *Kunz* [9], *Hirschmann* [4], and *Hanessian* [10] who were able to differentiate each hydroxyl group of the sugar. The problem consists mainly in the orthogonal protection of numerous hydroxyl groups; once this problem will be completely solved, orthogonally protected carbohydrates could be used in combinatorial chemistry, and compound sortiments in which the diversity consists in the different arrangement of pharmacophores linked to the hydroxyl groups of carbohydrates will be efficiently produced (Fig. 7). It is interesting to note that in addition to hydroxyl groups, natural sugars contain other functionalities such as the amino or the carboxylic group which further enhance the potential diversity.

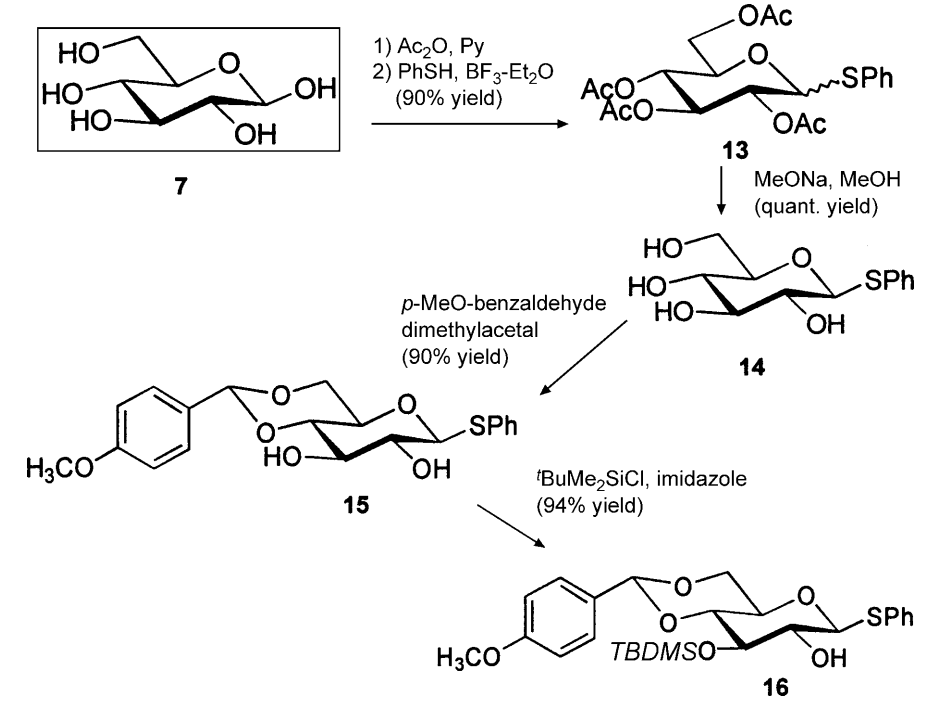
The development of orthogonalities among the numerous hydroxyl protecting groups of sugars is a particularly tedious task. Furthermore, if the combinatorial approach is developed in the solid phase, the linkage of the sugar to the solid support must be retained throughout the synthesis and cleaved selectively at the end of the process. As each derivatization step requires two reactions (deprotection and derivatization), a hexose linked to the resin *via* a hydroxyl group has still four positions to be manipulated, which means eight reactions. In oligosaccharide synthesis the protective groups are classified as temporary and permanent protections, the latter being removed at the end of the synthesis without affecting the glycosidic linkages. A permanent protection should link the glycidic scaffold to the resin and ensure complete orthogonality to the commonly used temporary protecting groups and to the derivatization conditions.

A variety of groups including silanes [11], thioethers [12], benzylidene acetals [13], succinamides [14], photolabile esters [15], *p*-acylaminobenzyl esters [16], alkenes [17], *tris*-(alkoxy)-benzyl amines (*BAL*) [18], and phenoxyacetates [19] have been employed to anchor monosaccharides to polymers in the first step of the solid-phase oligosaccharide synthesis. Most of these linkers interfere with some common deprotection or derivatization conditions, thus limiting the versatility and flexibility in synthetic planning.

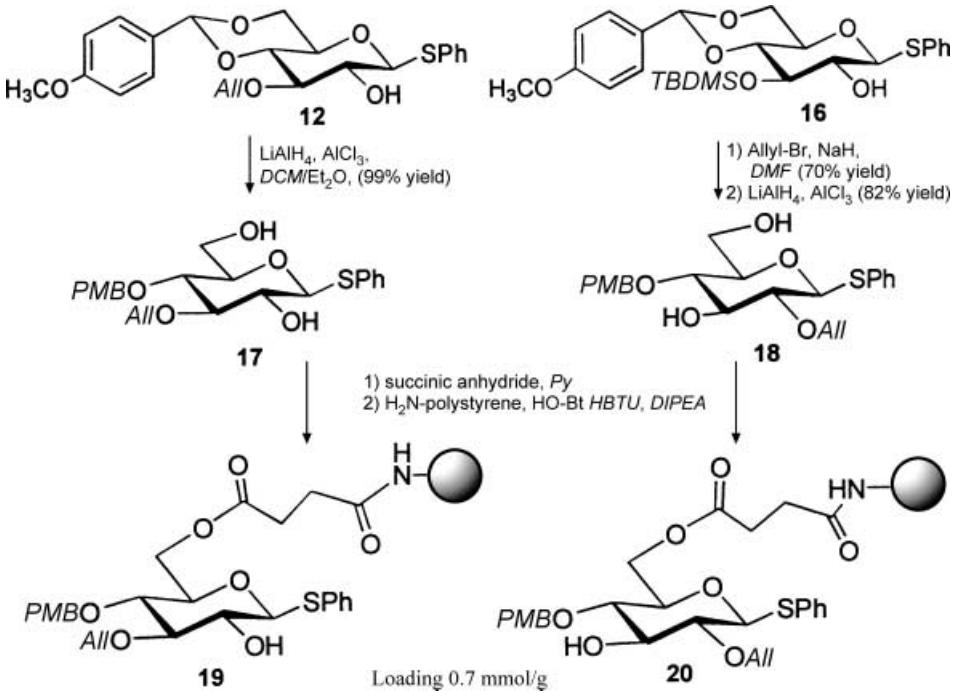
Examples of solid phase supported orthogonally protected glucoderivatives are reported in Schemes 1–3. Starting from *D*-glucose (**7**) and exploiting different protections as shown in the Schemes, phenylthio 3-O-allyl-4-O-*p*-methoxybenzyl-β-*D*-glucopyranoside (**19**) and phenylthio 2-O-allyl-4-O-*p*-methoxybenzyl-β-*D*-glucopyranoside (**20**) linked to a polystyrene-divinylbenzene-NH_2 resin through a



Scheme 1



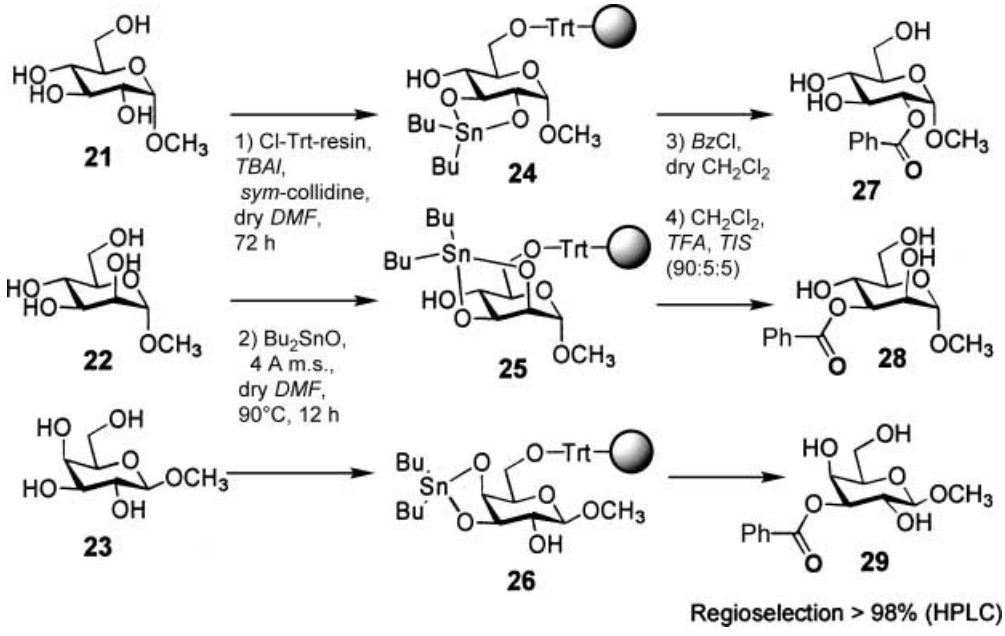
Scheme 2



Scheme 3

succinamide linker have been synthesized [20]. In these compounds, a hydroxyl group (in position 2 or 3) is deprotected and a second one (3 or 2) is protected as allyl ether which was selectively hydrolyzed on solid phase by transition metal catalyzed isomerization of the double bond followed by acid treatment. The third OH (at position 4) is protected as *p*-methoxybenzyl ether that was cleaved selectively on the solid phase either with oxidants such as *DDQ* or under acidic conditions (50% *TFA* in CH_2Cl_2). Finally, the anomeric position was selectively functionalized on the solid phase by reaction of the thiophenyl group activated as sulfoxide with an alcohol in the presence of triflic anhydride and a base. After regioselective decoration of the four independent positions, the succinamide linker, which turned out to be stable under the deprotection and functionalization conditions, was hydrolyzed with bases.

Schemes 1–3 clearly indicate the complexity underlying the preparation of orthogonally protected substrates to be linked to a solid support. It is clear that the possibility of a regioselective derivatization of the free hydroxyl groups of unprotected sugars bound to solid supports would be much more interesting and effective. In order to explore this opportunity, we investigated the behaviour of the monosaccharides methyl α-*D*-glucopyranoside (**21**), methyl α-*D*-mannopyranoside (**22**), and methyl β-*D*-galactopyranoside (**23**) linked to a trityl resin by the primary hydroxyl group. When treated with Bu_2SnO and then with benzoyl chloride, the benzoylation of the stannylidene intermediates (Scheme 4) occurred in positions 2, 3, and again 3, respectively, with a regioselectivity above 98% [21]. These results are promising in the perspective of decorating polymer-bound monosaccharides regioselectively with a series of functional groups (including other monosaccharides in the stannylene-assisted regioselective glycosylation). In this context, new

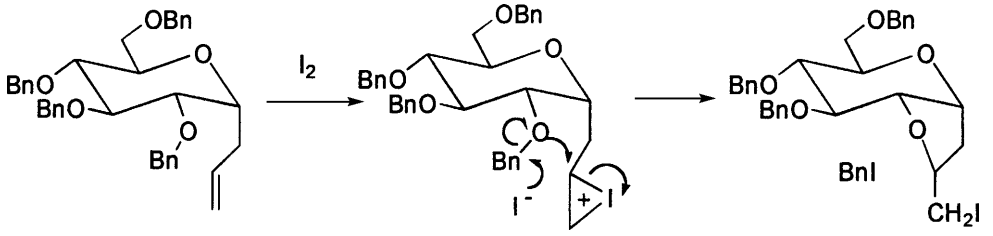


Scheme 4

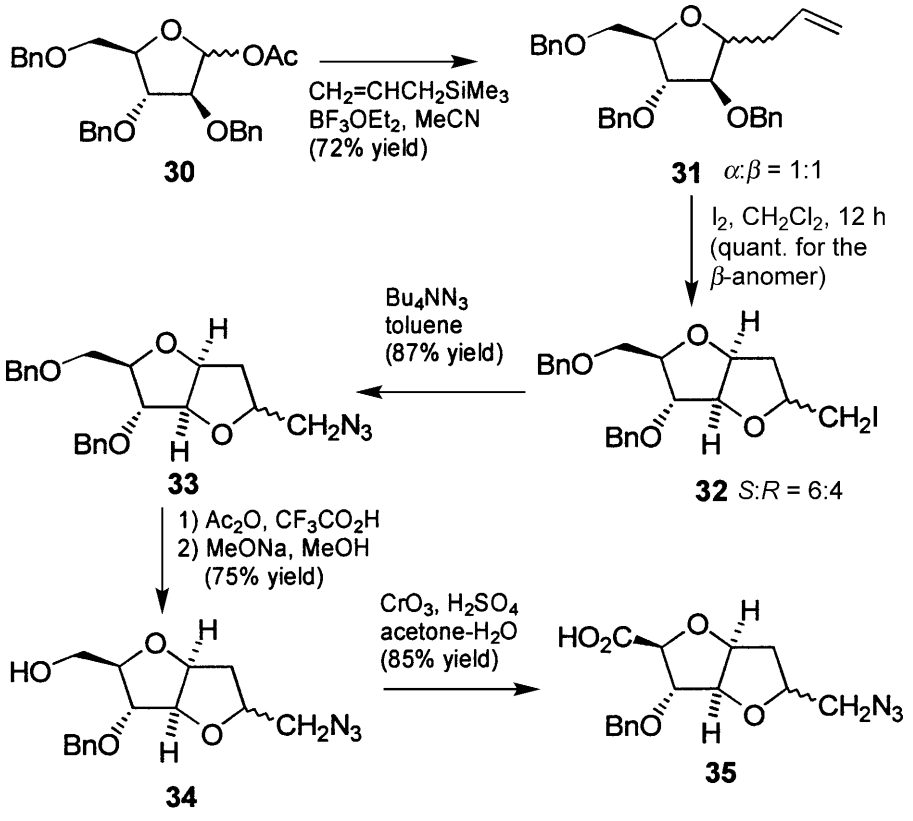
opportunities can arise from lipase-catalyzed acylation of sugars on solid phase, once the problems related to the permeability of the resin to the enzymes and to solid-phase kinetics are solved.

Conformationally Constrained Glycidic Scaffolds

In the development of glycidic scaffolds for the production of sortiments of compounds of pharmaceutical interest, two main aspects must be taken into account. First of all, the originality of the scaffold is important in order to allow patenting, and natural sugars are of course not ideal substrates from this point of view. Secondly, the conformational rigidity should be ideally enhanced; this strategy affords much more active compounds once the correct conformation has been achieved. Having both of these aspects in mind, we studied the possibility to synthesize original polycyclic scaffolds starting from perbenzylated sugars by an easy debenzylation-iodocyclization procedure [22]. Starting from polybenzylated sugars with an anomeric appendage with a C=C double bond, a cyclic iodoether was obtained by treatment with iodine. The mechanism of this iodocyclization consists in the attack of the intermediate iodonium ion by the benzyloxy group in γ-position with respect to the double bond with loss of the benzyl group (Scheme 5). Two examples of application of this reaction are reported in Schemes 6 [23] and 7 [24]. The allylic substituent has been introduced at the anomeric centre of polybenzylated acetyl arabinofuranoside **30** and methyl fructofuranoside **36** affording the corresponding allyl C-glycosides **31** and **37**. Treatment of **31** and **37** with iodine results in the iodocyclization. In the case of the arabinoderivative **31**, in which a mixture of α- and β-C-glycosides is obtained, it is possible to effect a kinetic resolution due to the easier formation of the *cis*-fused bicyclic structure **32**. Besides the formation of a second cycle, the iodocyclization



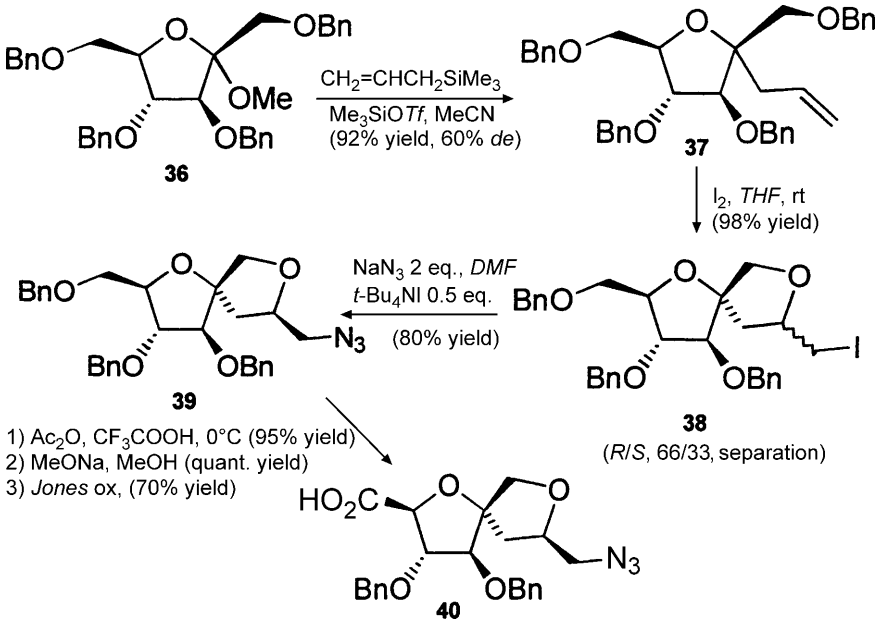
Scheme 5



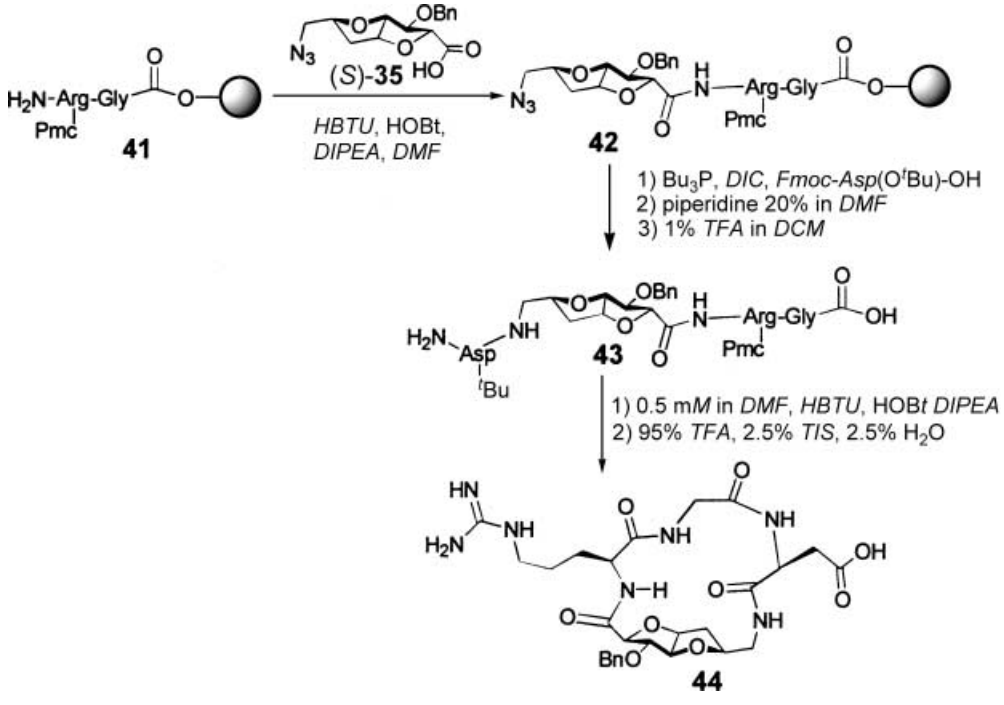
Scheme 6

reaction gives rise to a new useful functional group, the alkyl iodide, which can be exploited for further functionalizations. In the examples reported in Schemes 6 and 7, treatment of the iodides **32** and **38** with tetrabutyl ammonium azide afforded the azides **33** or **39**, precursors of the corresponding amines. A carboxylic function was subsequently introduced in these glycidic scaffolds in order to obtain sugar-aminoacids. The primary hydroxyl group of **33** and **39** was selectively deprotected by acetolysis of the benzyl ether followed by basic hydrolysis of the acetate. The primary hydroxyl group of the obtained compounds was oxidized using the *Jones* reagent affording the azidoacids **35** and **40**.

The conformational analysis of azidoacidic scaffold (*S*)-**35** and (*R*)-**35** was performed by NMR spectroscopy and molecular modelling. Both compounds



Scheme 7

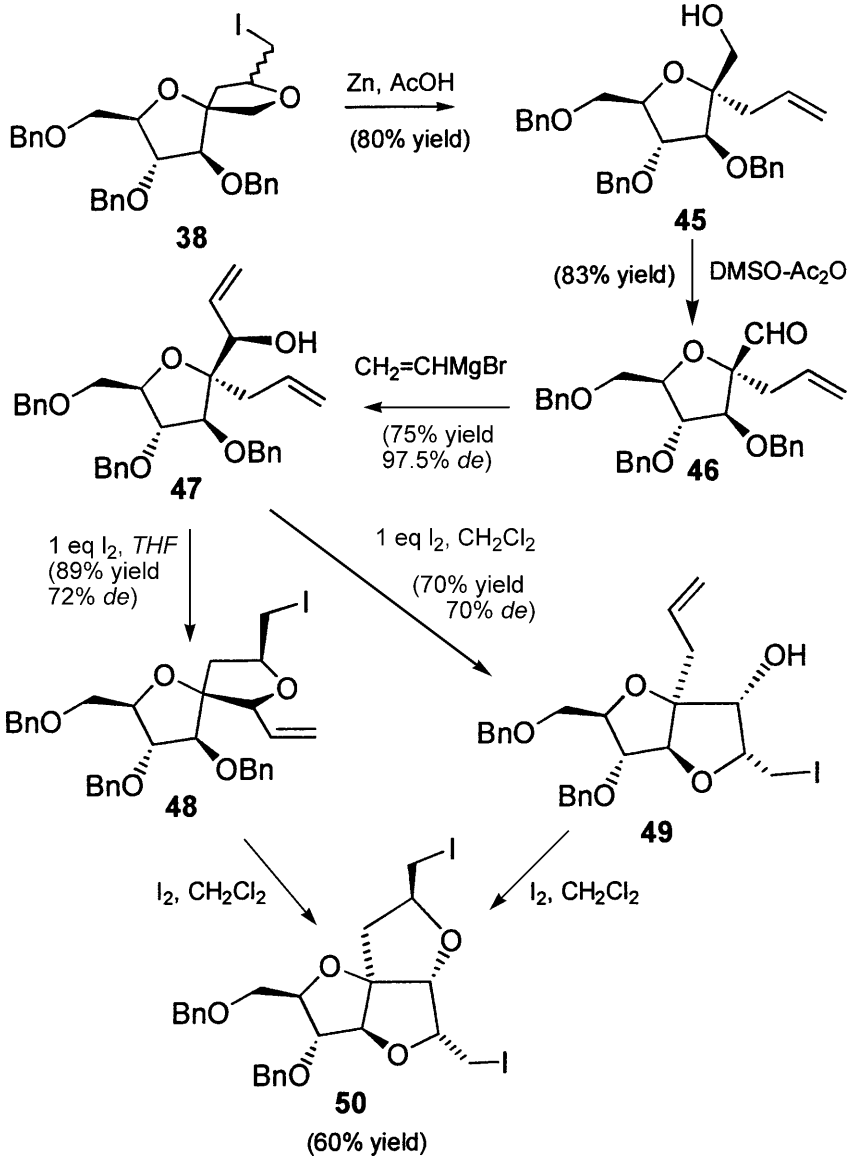


Scheme 8

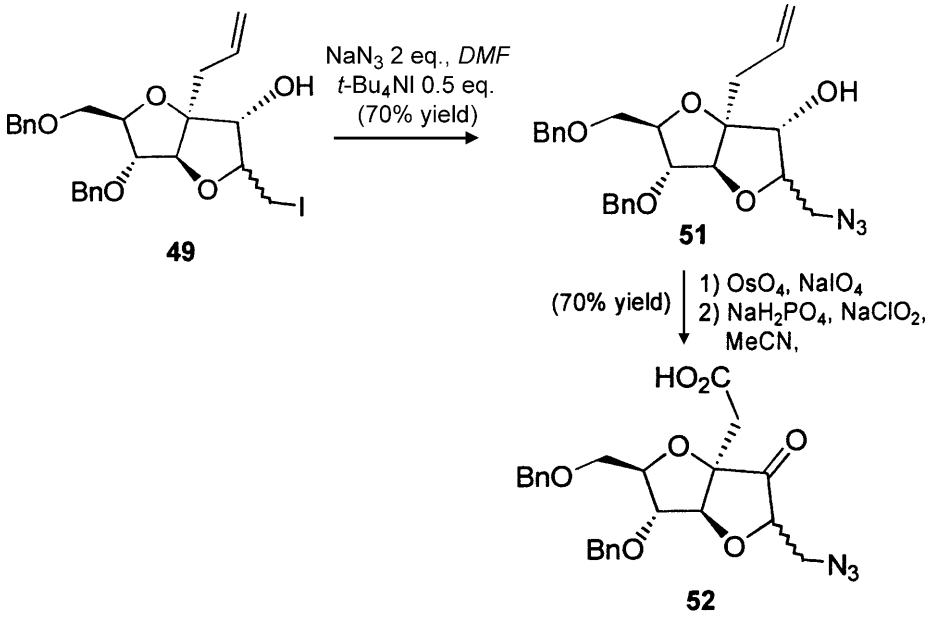
turned out to be good isosters of the dipeptide in position $i+1$ and $i+2$ of a β-turn. Compound (*S*)-**35** was incorporated as amino acid in a cyclic RGD peptide according to the synthetic procedure shown in Scheme 8 [23]. Compound **44** was

tested as inhibitor of the adhesion of fetal bovine aortic endothelial GM7373 cells to their ligands: fibroblast growth factor 2 (FGF-2), vitronectin (VN), and fibronectin (FN). A selective inhibition of the adhesion of FGF-2 and VN was observed, indicating a role of **44** as $\alpha_V\beta_3$ integrin antagonist [25].

Alternatively, the cyclic iodoethers obtained by iodocyclization-debenzylation undergo reductive elimination if treated with Zn and acids, restoring the original double bond (Scheme 9). The final result in this case is the selective debenzylation on the hydroxyl group in γ-position to the double bond. The free hydroxyl group can therefore be manipulated in order to perform further transformations. In the case reported in Scheme 9, the free hydroxyl group of compound **45** has



Scheme 9



Scheme 10

been oxidized with *Swern* reagent, and the aldehyde **46** was treated with vinyl magnesium bromide in order to introduce a further C=C double bond. Compound **47** is obtained in 97.5% *de*, the attack of the *Grignard* reagent occurring from the *re*-face of the carbonyl group. The two C=C double bonds of compound **47** are in the correct position to be involved in two different iodocyclizations; in fact, the double bond of the allylic appendage at the anomeric centre can generate a cyclic iodoether with the free hydroxyl group, whereas the double bond of the vinyl group can originate a cycle with the oxygen of the benzyl group in position 3 of the parent fructoside. Interestingly, the stereochemistry of the vinylation is correct for two cyclization in the same molecule. We have been able to obtain alternatively each bicyclic scaffold **48** or **49** depending on the experimental conditions. For short reaction times in *THF* with 1 equivalent of iodine, compound **48** is formed chemo- and stereoselectively, whereas in dichloromethane compound **49** was obtained in 70% yield and 71% *de*. By treatment with an excess of iodine in dichloromethane, as expected both **48** and **49** afforded the tricyclic scaffold **50**. Alternatively, the polyfunctional glycidic scaffold **52**, bearing five different functionalities (a carboxylic acid, a ketone, an azide, a primary and a secondary protected hydroxyl group), has been obtained (Scheme 10).

Conclusions

The present review focuses on the use of carbohydrates or carbohydrate-derived compounds as scaffolds. Carbohydrates present an attractive option for non-peptide scaffolding as they contain readily convertible functional groups on a pyran or furan ring with a well-defined spatial orientation. Playing with carbohydrates, it is possible to produce an unexpected variety of new conformationally rigid

polyfunctional molecules. Searching for new pharmacologically active compounds, sortiments of molecules have been obtained in solid phase from regioselective decoration of carbohydrates with pharmacophores. The bioactive compounds containing a carbohydrate or carbohydrate-derived core have generally a reduced conformational mobility. Consequently, they are also excellent candidates for structure-activity investigations.

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