Carbohydrates and Derivatives as Potential Drug Candidates with Emphasis on the Selectin and Linear-B Area

Reinhold Öhrlein*

NOVARTIS Pharma Ltd., CH-4002 Basle, Switzerland

Abstract: Enzymatic carbohydrate synthesis using glycosyltransferases is highly regio- and stereospecific and does not require extensive protecting group designs. Naturally occurring carbohydrates have been prepared by this biomimetic pathway successfully. As more and more transferases are isolated and get cloned and overexpressed, non-natural substrates were probed with these biocatalysts. Key-polar groups and non-essential residues of the substrates have been determined. Consequently, this technique was employed to generate natural and non-natural carbohydrate libraries for pharmaceutical purposes.

The synthesis of sialyl-Lewis^a- and sialyl-Lewis^x libraries and non-natural Linear-B derivatives applying glycosyltransferases is presented in this article. The respective transferases investigated are α(1-3)galactosyltransferase, β(1-3)galactosyltransferase, β(1-4)galactosyltransferase, α(2-3)sialyltransferase, α(1-3)fucosyltransferase III and α(1-3)fucosyltransferase VI. With respect to the natural acceptors, the aglycon part and the *N*-acetyl group of the glucosamide have been varied. All enzymes tolerate an unexpected wide range of non-natural acceptors, which is not yet exploited in its full scale. In addition, fucosyltransferase III and VI can be employed to convert also non-natural donors with non-natural acceptors at the same time. Thus sialyl-Lewis^a- and sialyl-Lewis^x-libraries which differ in three positions compared to the natural tetrasaccharides are generated very efficiently. Also a library of linear-B trisaccharides, a reactive xenoantigen, has been prepared enzymatically. The aglycon part and the natural *N*-acetyl group of the glucosamine which is a part of the acceptor substrate have been altered widely.

This convenient methodology is compared with the evolving solid-phase carbohydrate synthesis using conventional chemistry. The potential use of transferases in solid-phase carbohydrate chemistry is discussed together with the possibility to use these biocatalysts to synthesize carbohydrate mimetics. The presented findings may also be useful to design potential glycosyltransferase inhibitors.

Key Words: Glycosyltransferases, sialyl-Lewis^a, sialyl-Lewis^x, Linear-B trisaccharide, carbohydrate mimetics.

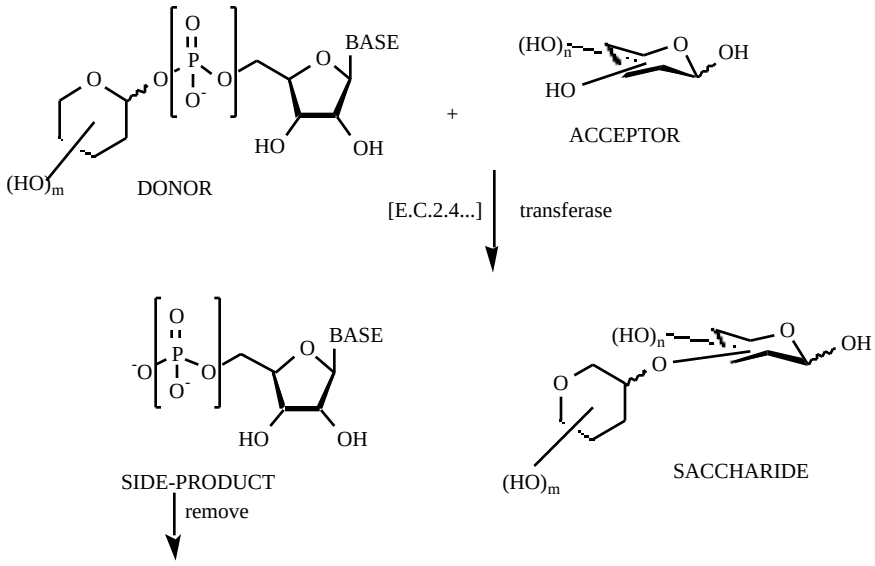
1 INTRODUCTION

Carbohydrates are a ubiquitous class of natural products. They are an evolutionary old and important class of compounds. Usually, they are attached to lipids or proteins and reveal an unsurmounted biochemical diversity and mediate numerous biological functions. Their essential participation in various healthy and pathological adhesion phenomena has now been widely recognized. But unless other natural compounds like peptides, nucleic acids or steroids, carbohydrates mainly remained a subject for specialists. They are highly homofunctional – mostly hydroxyl groups – and pose great analytical and synthetic problems. Conventional carbohydrate chemistry is therefore still plagued by tedious and inefficient protecting group manipulations accompanied by stereochemical imponderables. Hence high throughput screens with carbohydrates and carbohydrate derivatives are severely impeded. Nonetheless, they have experienced a lively renaissance in the pharmaceutical industry recently [1-5]. Carbohydrates are now acknowledged as key players in endogenous and exogenous recognition phenomena [4], e.g. in cell differentiation during ontogenesis [6], in malignant cell degeneration [7] and pathogenic adhesion events [8] to mention just a few. Carbohydrates involved in pathological inflammatory responses [9-11] and xenograft rejection [11-14] are worked on most intensively at present.

In order to evaluate the biological relevance of carbohydrate structures in these areas properly, large amounts of various natural and non-natural carbohydrates are demanded for high-through-put screens. To satisfy these needs adequately, a combinatorial technique for the preparation of carbohydrates is highly desirable. Although chemical routes towards solid and solution phase synthesis for carbohydrate libraries have been tried already a long time ago [15], there has not been made decisive progress in recent years. Some nice preparations have been published but they are not yet generally useable [16, 17]. The usual difficulties and shortages of conventional carbohydrate synthesis, e.g. excessive protecting group manipulations, sluggish reactivities of reagents, uncomplete stereospecificities, hazardous promotors etc. have still to be overcome in order

*Address correspondence to this author at the NOVARTIS Pharma Ltd., CH-4002 Basle, Switzerland;
E-mail: reinhold.oehrlein@pharma.novartis.com

1389-5575/00 $20.00+.00



Scheme 1. Example of an enzymatic glycosylation.

to develop an efficient and reliable combinatorial approach towards carbohydrate libraries [18].

An alternative way to synthesize carbohydrates was pioneered by Whitesides' and Auge's groups [19, 20]. Both groups explored the use of biocatalysts – glycosyltransferases – to prepare carbohydrate chains. This technique lacks all the preparative inconveniences of the chemical synthesis and, in addition, excells from its absolute regio- and stereoselectivities [21]. Glycosyltransferases, also termed Leloir-transferases, are a class of biocatalysts which transfer a sugar unit from a nucleotide-activated donor sugar regio- and stereoselectively onto an OH-group of a growing oligosaccharide acceptor *in vivo* (scheme 1). Only one of the many OH-groups of the acceptor saccharide is glycosylated. The transfer occurs exclusively in an α-or β-mode. Next to the desired saccharide a nucleotide side product is produced which inhibits the transferase *in vivo* and *in vitro*. This compound has to be removed or recycled to achieve complete glycosylation.

However the enzymatic carbohydrate synthesis is not free of costs either. Two prerequsites have to be fulfilled: first the appropriate transferase [22] and second the respective nucleotide activated donor [23] have to available.

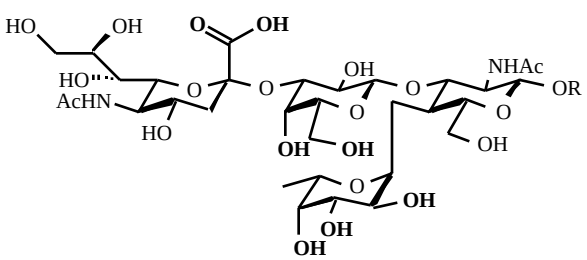
In this review we will focus on the use of transferases for the preparation of sialyl-Lewis^a-, sialyl-Lewis^x- and linear-B libraries.

2 TARGET STRUCTURES

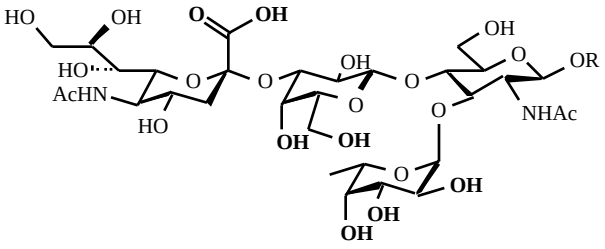
2.1 Selectin Ligands

Two isomeric tetrasaccharides, termed sialyl-Lewis^a and sialyl-Lewis^x, (see scheme 2) initiate the extravasation of leukocytes to inflamed tissues via interaction with selectin receptors [10]. Pathological inflammations and other selectin-mediated disorders like arthritis and asthma, are hoped to be cured by interfering with the carbohydrate selectin binding.

The X-ray structure of unliganded E-selectin has been obtained [24] and also NMR-studies of the ligand in solution and bound to E-, P- and L-selectins have been performed [25, 26]. Although these studies remained imprecise, some useful information could be retrieved. The conformation of bound and free sLe^x is quite similar and maximum binding is thought to be achieved by

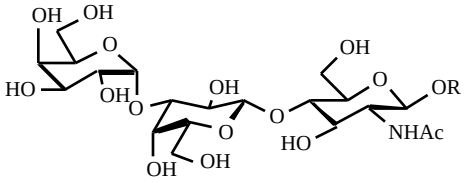


Sialy-Lewis^a (sLe^a)



Sialy-Lewis^x (sLe^x)

Scheme 2. Chemical structure of selectin ligands.



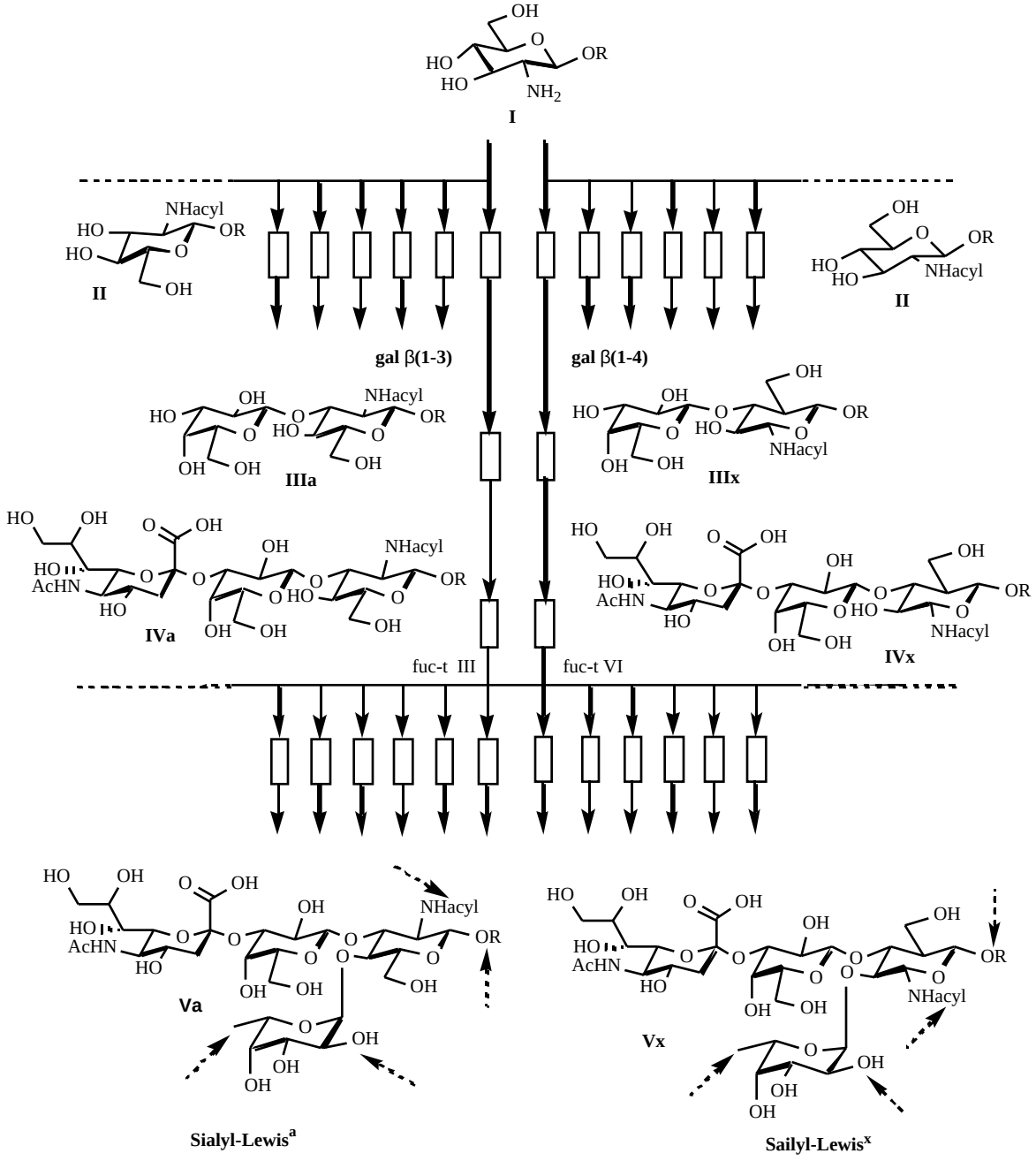
Scheme 3. Structure of Linear-B trisaccharide.

preorganized, rigid structures [27]. Although sLe^x and sLe^a are stereoisomers, the respective sugar units interacting with the selectins share similar spatial orientation. Thus, all three OH-groups of fucose, the 4- and 6-OH group of galactose and the carboxyl group of the sialic acid moiety are believed to interact with the selectin more closely [28, 29] (see highlighted groups in scheme 2). Here we include our results obtained for derivatives wherein polar and/or lipophilic residues have been introduced into the glucosamide moiety which is not involved in binding at first view.

2.2 Linear-B Trisaccharide

The linear-B trisaccharide is a potent epitope of xenoantigens (see scheme 3) which triggers the hyperacute rejection of e.g. pig to human transplants [12, 30]. The preformed anti-Linear-B antibodies in humans and Old World monkeys are polyclonal and comprise up to 1-2% of total human IgG and 3-8% IgM [31].

The binding pocket and the docking of linear-B has not yet been elucidated, but extensive NMR-studies in aqueous



Scheme 4. Enzymatic synthesis of sialyl-Lewis^a and sialyl-Lewis^x libraries.

solution revealed a u-shaped conformation of the trisaccharide. These investigations may help to better understand the binding of linear-B derivatives [31].

3 ENZYMATIC SYNTHESIS OF CARBOHYDRATE LIBRARIES

3.1 Sialyl-Lewis^a- and sialyl-Lewis^x-Libraries

Combinatorial carbohydrate synthesis is still in its infancy [32]. The chemical synthesis, either in solution or on solid-phase is plagued by the usual inadequacies of conventional carbohydrate synthesis. Although reports of enzymatic solid-phase carbohydrate synthesis date back to 1984 [33], only a limited number of papers have been published recently [34 - 36]. Only natural donor sugars have been used. The overall yields strongly depended on the resins and linkers used, the individual enzyme and the final cleavage methods. We applied the solution pathway. Scheme 4 illustrates the principal procedure how we prepared sLe^a- and sLe^x-libraries [37 - 39].

Table 1. Sialyl-Lewis^a-Library (Compare Scheme 4)

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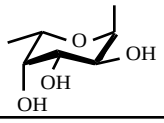
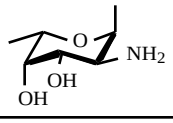
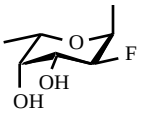
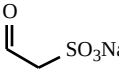
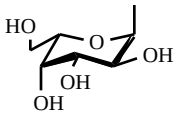
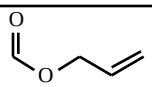
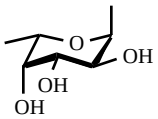
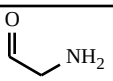
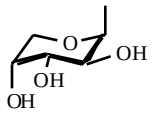
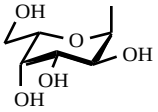
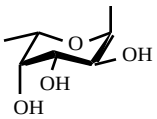
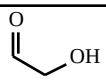
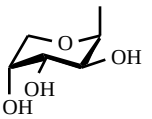
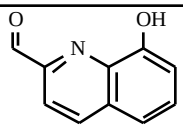
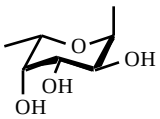
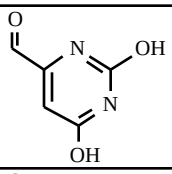
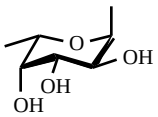
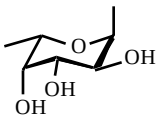
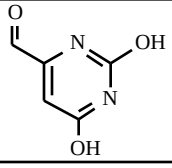
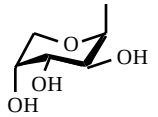
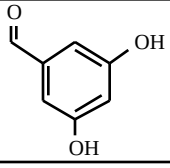
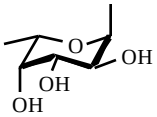
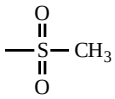
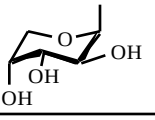
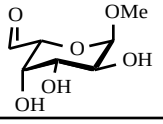
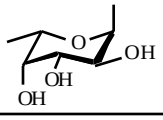
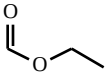
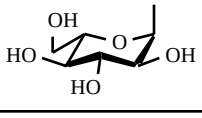
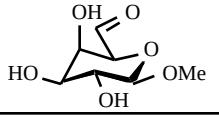
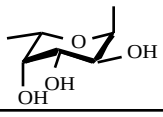
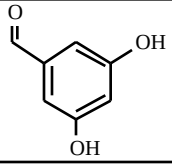
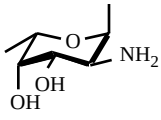
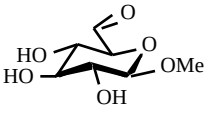
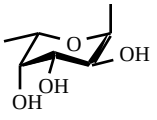
1			11		
2			12		
3			13		
4			14		
5			15		
6			16		
7			17		
8			18		
9			19		
10			20		

Glucosamine derivative (I) was first acylated with a number of acid residues following standard protocols [40] to give the glucosamides (II). A series of these sugars is subsequently incubated with UDP-galactose and β(1-3)gal-t [41, 42]. This enzyme transfers a *D*-galactose unit from UDP-galactose exclusively onto the 3-OH group of a terminal glucosamide moiety in a β-mode. The resulting disaccharides (IIIa) (and a series of chemically synthesized compounds (IIIa)) were then incubated with CMP-sialic acid and recombinant α(2-3)sialyl transferase [41]. This enzyme transfers a sialic acid residue from CMP-sialic acid onto the 3-OH group of the previously introduced galactose unit in an α-mode to form trisaccharide (IVa). Both enzymatic reactions can be performed as a two step one pot reaction.

Table 2. Sialyl-Lewis^x-Library (Compare Scheme 4)

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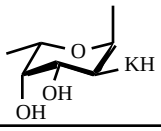
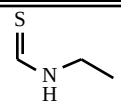
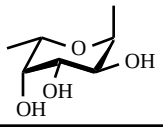
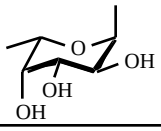
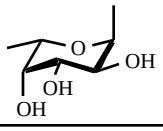
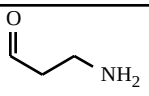
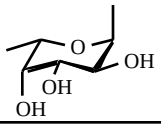
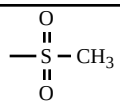
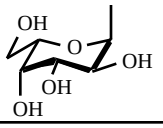
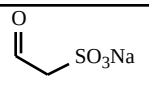
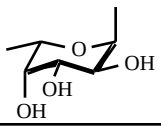
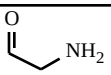
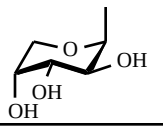
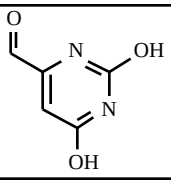
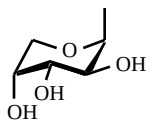
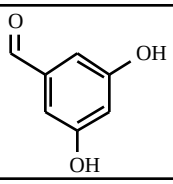
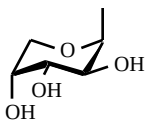
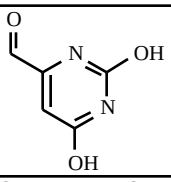
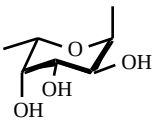
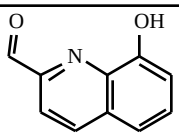
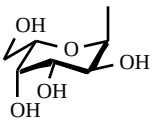
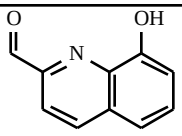
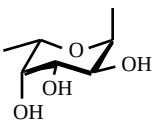
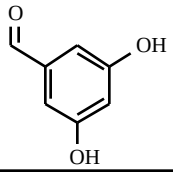
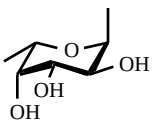
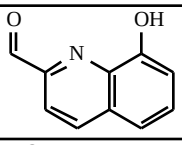
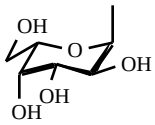
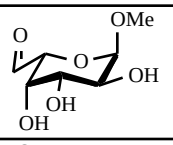
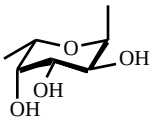
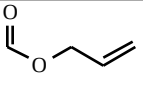
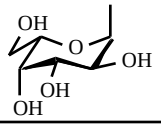
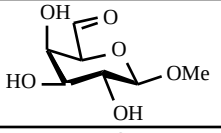
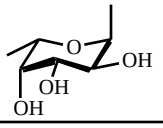
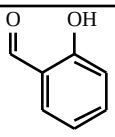
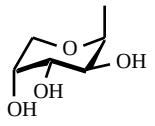
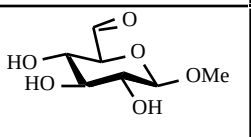
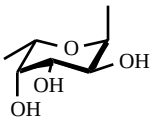
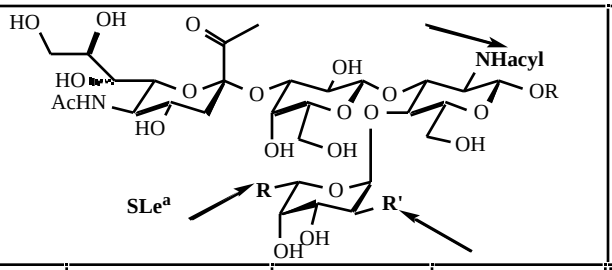
1			11		
2			12		
3			13		
4			14		
5			15		
6			16		
7			17		
8			18		
9			19		
10			20		

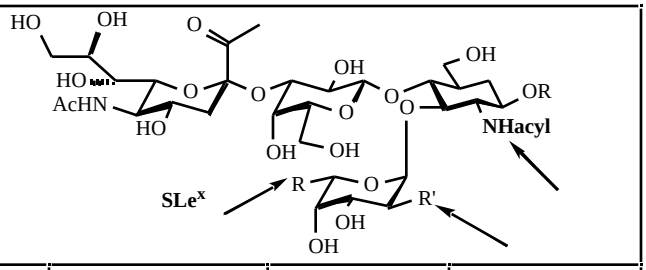
The single sugars (IVa) were then partitioned into a number of vials and separately incubated with GDP-activated donor sugars [44] and fucosyl-transferase III [39]. The enzyme transfers a sugar unit from the GDP-sugar donor onto the 4-OH group of the glucosamide residue in an α-mode to give the desired sLe^a-derivatives (Va).

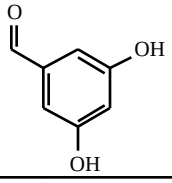
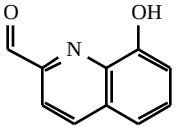
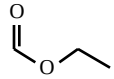
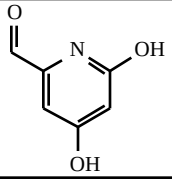
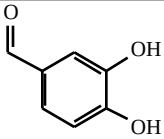
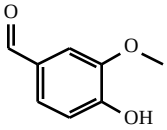
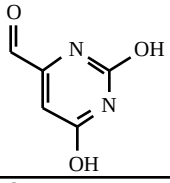
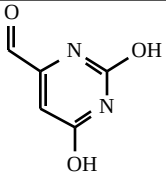
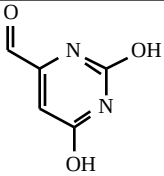
It was quite surprising that all the enzymes tolerated wide variations of non-natural NH-acyl residues of the glucosamide moiety (see table 1). The natural NHAc group could, e.g., be replaced by positively or negatively charged residues (entries 12, 13) as well as lipophilic (entry 4) and bulky aromatic moieties (entries 6, 10 15). Even sulfonamides were accepted (entry 8) [37]. In addition, the natural GDP-fucose donor could be substituted by non-natural donors like 2-amino-fucose (entries 10, 11), 2-fluoro-fucose (entry 2), *L*-galactose (entries 4, 12), *L*-glucose (entry 9) and *D*-arabinose (entries 5, 16).

SLe^x-libraries have been obtained in an analoguous manner (see scheme 4) [45]. In a first step the sugars (II) were incubated with UDP-galactose and bovine β(1-4)gal-t [40, 46]. This enzyme transfers a galactose unit from UDP-gal onto the 4-OH group of a terminal N-acetyl glucosamide residue in a β-fashion exclusively (IIIx). Sialylation is then performed as decribed above. The resulting trisaccharides (IVx) were subsequently 'fucosylated' with cloned fucosyl-transferase VI [39, 47]. This biocatalyst transfers a fucose-unit from GDP-fucose onto the 3-OH group of an internal glucosamide in an α-mode to give tetrasaccharide (Vx). Also in this case (see table 2), it is quite surprising that all three enzymes tolerated non-natural acceptors which have the natural N-acetyl residue of the glucosamide moiety replaced by e.g. positively (entry 3, 14) or negatively charged (entry 4) residues, lipophilic bulky aromatic residues (entries, 5, 8, 10), thiocarbonyl groups (entry 11) or even sulfonamides (entry 13). This could not have been predicted in advance as

Table 3. E-selectin Binding Data; R = $(CH_2)_8COOCH_3$)





entry	acyl	sugar: R, R'	RIC (μmol)	entry	acyl	sugar: R, R'	RIC (μmol)
1		CH_3, OH	580	7		CH_3, OH	1000
2		CH_3, OH	3834	8		CH_3, OH	13
3		$HOCH_2$, OH	581	9		$HOCH_2$, OH	376
4		H, OH	5256	10		CH_3, OH	40
5		CH_3, OH	2031	11		H, OH	1137
6		CH_3, OH	5256	12		CH_3, OH	25

the 'fucosylations' took place in the very neighborhood of those non-natural residues. In addition, fucosyl-transferaseVI accepted GDP-galactose and GDP-arabinose and transferred the respective sugars in the expected manner.

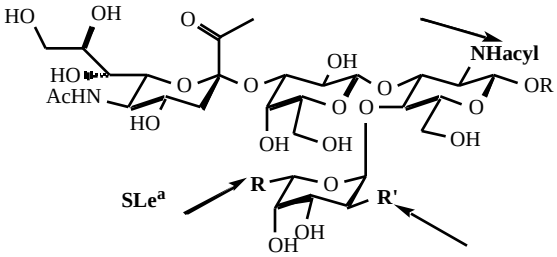
Even more surprising were the enzymatic transformations of the 'saccharopeptide' structures (see tables 1 and 2, entries 18-20). In these compounds the glucosamine (I) was acylated with a series of *D*- and *L*-glycuronic acids [48, 49]. The bulky polar residues were tolerated by β(1-4)gal-t, α(2-3)sia-t, fuc-t III and fuc-t VI. Fuc-t VI had to approach those bulky moieties quite closely in order to transfer the fucose onto the 3-OH group properly. Furthermore, none of those glycuronic amides did inhibit any of the transferases. In addition to these variations, the aglycon part of the sugars (I) could be dropped or replaced by a number of synthetically useful residues, which allows further elaboration of the desired carbohydrates [50-52].

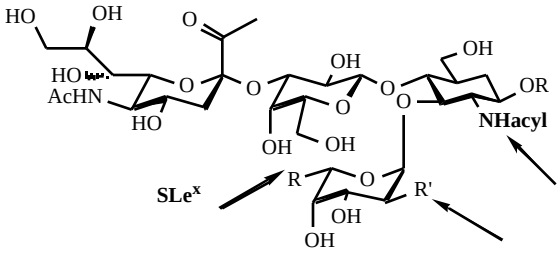
The glycosylations described above did not need any tedious deconvolution procedures as is often the case in combinatorial chemistry. Each single compound was obtained from the reaction mixture by simple reversed phase filtration [53] to remove protein and salt contaminants. Unreacted starting sugars were not deleterious in the ensuing bio-assays but were removed by a short silica column.

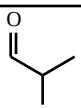
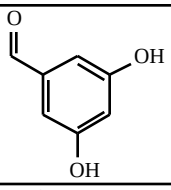
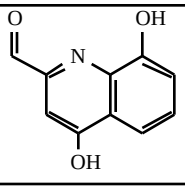
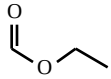
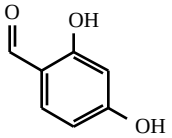
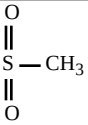
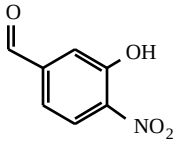
The sLe^a- and sLe^x-derivatives have been tested in a cell free competitive E- and P-selectin binding assay (see table 3 and 4) [54, 55]. A few of them were investigated in an adhesion assay under flow conditions [56].

Let's first look into the E-selectin binding data (table 3). In the sLe^a-case, no derivative could be obtained with a higher affinity towards E-selectin than the parent sLe^a (entry 1). In the sLe^x-case on the other hand, the aromatic acyl groups at the glucosamine exhibited an improved binding

Table 4. P-Selectin Binding Data; R = $(CH_2)_8COOCH_3$)





entry	Acyl	sugar: R, R'	RIC (μmol)	entry	Acyl	sugar: R, R'	RIC (μmol)
1		CH_3, OH	1267	8		CH_3, OH	1198
2		CH_3, F	272	9		CH_3, OH	65
3		$HOCH_2$, OH	99	10		CH_3, OH	4
4		CH_3, OH	103	11		CH_3, OH	63
5		CH_3, NH_2	54	12		CH_3, OH	92
6		$HOCH_2$, OH	899	13		H, OH	8
7		H, OH	775	14		CH_3, OH	43

(entries 8, 10, 12) compared to the parent compound (entry 7). This gave rise to the assumption that additional binding pockets close to the sugar binding sites were hit by those residues. One could also speculate that E-selectin discriminates between sLe^a- and sLe^x-derivatives when comparing the data of entries 6 and 12 or 4 and 12.

Looking at the P-selectin data (table 4), in the sLe^a-case, a number of derivatives have been prepared with a more pronounced binding to P-selectin than the parent sLe^a (entry 1). Especially interesting are the entries 2 and 5 which have the 2-OH group of fucose replaced by fluorine or amine, respectively. These findings are somewhat contradictory to previous assumptions which say that all of the fucose hydroxyl groups are required for tight binding. But the overall picture remains inconclusive regarding entries 3 to 7!

Also in the sLe^x-case, derivatives (entries 9-14) were obtained with better binding to P-selectin than the parent sLe^x (entry 8). The aromatic compounds (entries 12-14) gave again conspicuous data. Previously, the glucosamide sugar was regarded not necessary for the binding of sLe^a and sLe^x to the selectins (compare above) and has therefore been replaced by simplified structures [57 - 59]. We equipped this moiety with non-natural residues. This strategy was easily accomplished by a chemo-enzymatic synthesis (see tables 3, 4). We did not examine the use of non-natural UDP-gal and CMP-sia donors yet. A lot of those donors have been prepared recently and investigated with the respective transferases [21, 23]. These results have been nicely compiled by Palcic et al. [60] who determined key polar groups of both the acceptor and donors. Applying these results even more varied sLe^a- and sLe^x-structures should be accessible enzymatically.

3.2 Linear-B Trisaccharides

Also the linear-B trisaccharide (III) is easily accessible by the enzymatic route (scheme 5).

In a first step glucosamide (I) was incubated with UDP-gal and β(1-4)gal-t (compare scheme 5) to give a series of lactosamides (II). These disaccharides were subsequently incubated with UDP-gal and α(1-3)gal-t [61-63]. This enzyme transfers a galactose unit from UDP-gal onto the 3-OH group of a terminal β-linked galactose in an α-mode exclusively. A number of lactosamide acceptors, bearing non-natural replacements of the natural NH-acetyl residue and various non-natural aglycons, were accepted by α(1-3)gal-t [61, 64]. Selected examples are listed in table 5. These examples show that the penultimate sugar moiety of the acceptor was not fully recognized by the α(1-3)gal-t *in vitro*. Instead of the natural NH-acetyl residue, amino- or hydroxyl groups (entries 9, 7, 8, 11), lipophilic residues (entries 2, 3) or even bulky aromatic moieties (entries 4, 5, 6, 12) were tolerated. The aglycon part of the acceptor could be varied widely (entries 8, 11, 12) which allows a further elaboration of the linear-B structure.

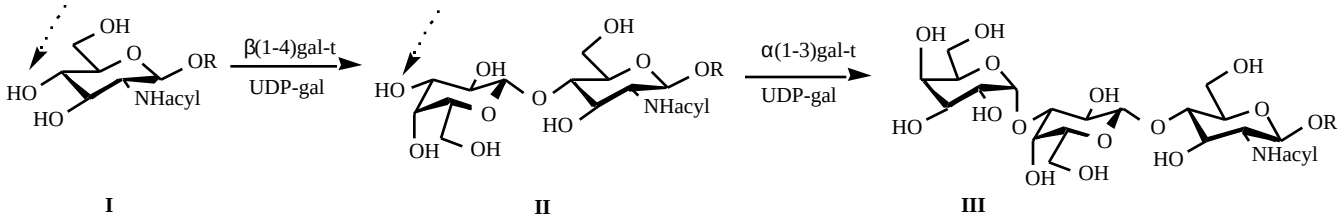
The key polar groups of the substrates, necessary for recognition of the enzyme, have been elucidated recently [65]. This allows the enzymatic preparation of linear-B derivatives using combinedly non-natural donors and acceptors. In addition, both enzymatic steps (scheme 5) have been carried out in one pot [64, 66], even with *in situ* generation of the UDP-gal donor. Taking the Hindsgaul findings [65] into consideration, it would be interesting to investigate if a one pot reaction with both enzymes in the presence of two different donors would lead to defined or scrambled linear-B derivatives. It has been found that the donor preferences and the respective kinetics of both enzymes differed.

4 POTENTIAL FUTURE DEVELOPMENTS AND CONCLUSIONS

Several important points have to be included when dealing with carbohydrates or carbohydrate derivatives as potential future drug candidates.

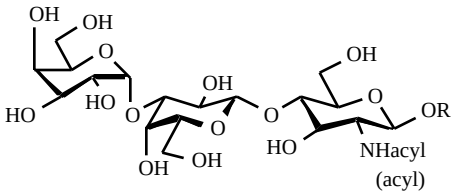
4.1 Multivalency

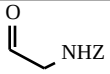
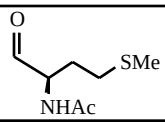
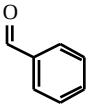
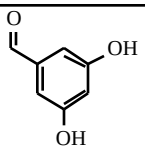
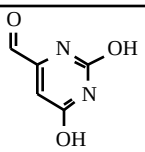
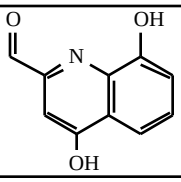
The interaction of a single carbohydrate or oligosaccharide molecule with its receptor is often very weak and the binding constants are in the millimolar range [67]. Carbohydrate-based adhesion phenomena are of a oligo- or polyvalent nature. Thus the binding is enhanced by entropic and steric factors [68]. So sLe^a- and sLe^x-tetrasaccharides have been integrated into various peptides or liposomes to form oligovalent carbohydrate structures [69-72]. Significant improvements of the binding constants were reported. An elegant approach was to start with a preassembled backbone equipped with a number of uniform carbohydrate stumps. The desired oligosaccharide was then elongated using biocatalytic techniques [69, 73, 74]. That way, both the backbone and the carbohydrate ligand might be varied in wide ranges. This technique has the additional advantage that the final aglycon is already linked to the desired sugar. Another approach recently reported is to assemble the sugar residue onto an aglycon which is subsequently oligo- or polymerized [72]. Either way, alterations in the backbone



Scheme 5. Enzymatic synthesis of linear-B trisaccharide.

Table 5: Enzymatically Synthesized Linear-B Derivatives; * lit. [61]; ** lit. [64]



entry	acyl	aglycon	entry	acyl	aglycon
1*		$O(CH_2)_8COOMe$	7**	OH	H
2*		$O(CH_2)_8COOMe$	8**	OH	N_3
3*		$O(CH_2)_8COOMe$	9**	NH_2	*H*
4*		$O(CH_2)_8COOMe$	10**		$OCH_2CH{=}CH_2$
5*		$O(CH_2)_8COOMe$	11**	OH	SPh
6*		$O(CH_2)_8COOMe$	12*		$O(CH_2)_8COOMe$

and/or the sugar units lead to combinatorial carbohydrate-based ligands.

4.2 Solid Phase Synthesis

Compared to solid-phase peptide synthesis, solid-phase carbohydrate synthesis is far less developed [32]. As presented above several groups tackled this task applying transferases [33-36]. Some technical problems encountered were e.g. low transfer rates because of heterogenous reaction mixtures, uncomplete conversions and the final cleavage of the carbohydrate. Elegant solutions have been proposed to solve this problem. For example, light sensitive linkers [33, 75], peptidase-cleavable linkers or dithiothreitol labile linkers were investigated [36]. These approaches are promising although the released sugars still need to be attached to a proper aglycon in a final step.

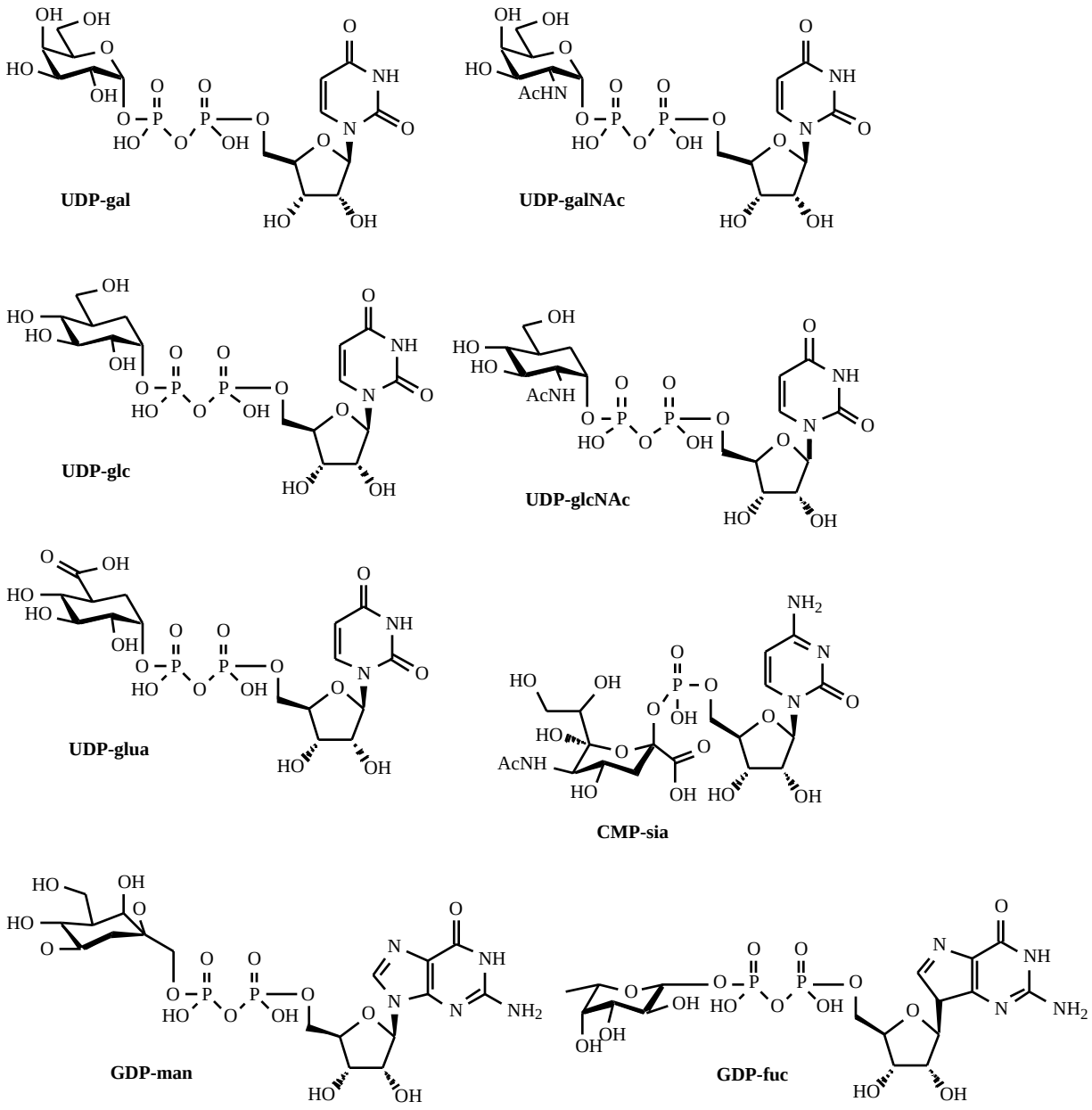
4.3 Sugar Nucleotides

Interestingly, it has been found that mammalian cells use only eight nucleotide-activated donors to assemble their highly diverse cell surface carbohydrate structures [21, 76] (scheme 6). Additionally, posttranslational enzymatic modifications are wide spread.

These donors and their non-natural variations thereof can be introduced *in vitro* into the carbohydrate chain applying transferases. This may be beneficial for future carbohydrate research. A number of non-natural donors have been described and their syntheses reviewed [21, 23]. Unfortunately, only a few natural donors are currently available on a large scale. This somewhat impedes enzymatic carbohydrate synthesis.

4.4 Glycosyltransferases

It is assumed that mammalian cells use a few hundred different glycosyltransferases to assemble their individual carbohydrate structures. A series of those enzymes have already been cloned and overexpressed [22, 77-80]. Thus more glycosyltransferases are now available to the glycochemists. Furthermore, attempts to crystalize the catalytic domain of these enzymes have recently been initiated and reported [81]. This gives new insights into the binding mode of both the donor and acceptor. By this way tailor-made glycosyltransferases might be produced with enhanced tolerance and improved kinetics towards non-



Scheme 6. Nucleotide-activated donor sugars of mammalian cells.

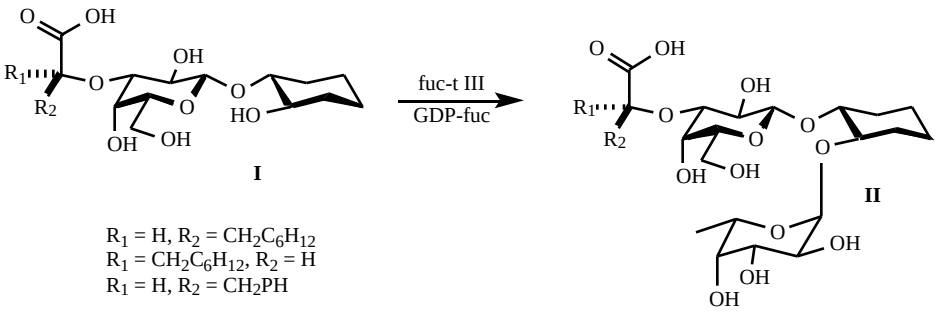
natural substrates [21, 60, 82]. Thus artificial carbohydrate libraries could be 'routinely' accessible for high-through-put screens.

4.5 Carbohydrate Mimetics and Glycosyltransferase Inhibitors

The synthesis of carbohydrate mimetics using natural transferases has recently been reported [83]. Scheme 7 illustrates the unexpected synthetic versatility of fuc-t III.

In this example the natural acceptor of fuc-t III – sialyl *N*-acetyl lactosamide – has been exchanged by a pseudo trisaccharide (I) (scheme 7). The sialic acid residue was replaced by a *D*- or *L*-lactic acid derivatives and the *N*-acetyl glucosamide sugar by *R,R*-trans-1,2-cyclohexanediol. Surprisingly, fuc-t III accepts this non-natural compound and transfers a fucose unit from GDP-fucose onto the only available OH-group of the cyclohexanediol ring with the desired α-selectivity. These findings, the observation presented above and further investigations [84-88] demonstrate the high promiscuity of glycosyltransferases *in vitro*.

On the other hand, this makes it quite difficult to design efficient glycosyltransferase inhibitors. There is an interest in carbohydrate-related inhibitors because enhanced amounts of certain transferases and their glycosylation products, respectively, were detected in malignant cells [6, 89, 90]. Inhibition of the involved transferases with selective drugs might block malignant aberrations and related areas [91, 92], which run with the enhanced expression of specific transferases [93 - 95].



I

$R_1 = H, R_2 = CH_2C_6H_{12}$
$R_1 = CH_2C_6H_{12}, R_2 = H$
$R_1 = H, R_2 = CH_2PH$

II

Scheme 7. Enzymatic synthesis of mimetics.

In conclusion, the synthetic chemist appreciates the substrate flexibility of the glycosyltransferases in combination with their reliable regio- and stereospecific activity. On the other hand, this wide tolerance makes it difficult to specifically design inhibitors. Overall, glycosyltransferases still remain an elusive class of enzymes as do the majority of the glycan structures in nature [4]. Carbohydrate-related diseases remain to challenge biologists and medicinal chemists alike.

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