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Transferase-catalyzed synthesis of non-natural oligosaccharide-libraries (SLe^a- and SLe^x-analogues)

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Abstract

A number of non-natural glucosamine derivatives with various *N*-acyl and aglycon residues are prepared chemically. The *N*-acyl residues comprise aliphatic, polar, charged, aromatic and heteroaromatic replacements of the natural *N*-acetyl group. Both $\beta(1-4)$- and cloned $\beta(1-3)$-galactosyl-transferase tolerate a wide range of these replacements and yield the corresponding type II and type I disaccharides when incubated together with UDP-galactose. These disaccharides are subsequently sialylated with cloned $\alpha(2-3)$-sialyl-transferase and CMP-sialic acid to give trisaccharides, which are sialylated at the 3-OH group of the terminal galactose. The sialylated type I compounds are finally incubated with cloned fucosyl-transferase III and type II compounds with cloned fucosyl-transferase VI, respectively. Thus, sialyl-Lewis^a and sialyl-Lewis^x libraries are generated. Additionally, both fucosyl-transferases accept donor substrates which have either the natural fucose-moiety or the guanosine-unit of the natural GDP-fucose donor replaced by non-natural congeners.

Keywords: Glycosyl-transferases; Guanosine-diphosphofucose analogues; Oligosaccharide libraries; Sialyl-Lewis^x; Sialyl-Lewis^a; Enzymatic synthesis

Glycoconjugates play key roles in various adhesion and cell–cell recognition phenomena [1,2]. In order to probe carbohydrates as potential drug candidates [3], a proper understanding of carbohydrate–protein interaction is mandatory. This necessitates a rapid access to a wide range of natural and non-natural carbohydrate epitopes [4]. An alternative to the tedious chemical procedures for oligosaccharide synthesis is given by protocols based on the use of glycosyl-transferases for carbohydrate assemblage [5]. Glycosyl-transferases are a class of enzymes, which transfer a monosaccharide unit highly regio- and stereospecifically onto a growing oligosaccharide chain in vivo. The monosaccharide sources are nucleotide mono- or diphosphate sugars.

Recently, improved protocols make available these activated donor substrates and non-natural congeners thereof in large quantities, e.g., UDP-gal, [1] CMP-sia [6] and GDP-fuc [7]. In addition, a number of glycosyl-transferases have been

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[1] For example, UDP-galactose is offered and produced by Yamasa Jpn, in kilogram amounts.

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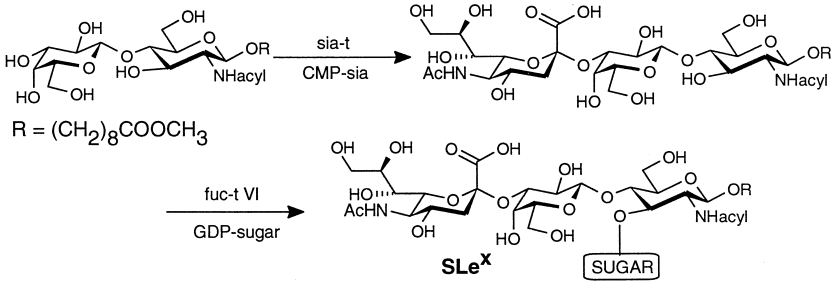
produced by cloning and microbiol overproduction for preparative use [8]. We have recently shown that commercial $\beta(1–4)$-galactosyl-transferase [9], cloned $\alpha(2–3)$-sialyl-transferase [10] and cloned fucosyl-transferase VI [11] can be used successfully to prepare SLe^x-derivatives, which have the natural *N*-acetyl-group of the glucosamine-moiety replaced by a broad array of aliphatic, aromatic and charged groups. The versatility of this approach has been extended further. We incubated non-natural acceptor substrates with non-natural 'fucose'-donors [12] (confer Scheme 1 and Table 1). Surprisingly, also these 'fucoses' are transferred to the 4-OH group of the *N*-acyl glucosamine units3 in the expected α-mode.

Accordingly, a sialyl-Lewis^a-library has been produced. First, a series of type I disaccharides has been synthesized chemically or via enzymatic $\beta(1–3)$-galactosylation [13]. These disaccharides are subsequently treated with cloned $\alpha(2–3)$-sialyl-transferase and CMP-sia to α-sialylate, the terminal galactose at the 3-OH group. In a final incubation with cloned fucosyl-transferase III and various GDP-'fucoses' (see Scheme 2 and Table 2), the 'fucose'-

Table 1
Enzymatic preparation of SLe^x derivatives



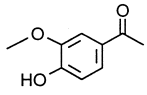
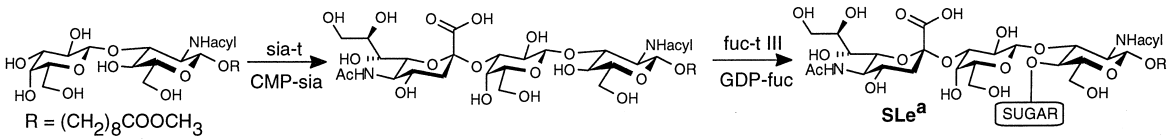
acyl	sia %	sugar	'fuc' %
-C(O)H	95	fuc	100
		L-gal	71
-C(O)$CH_2CH{=}CH_2$	72	fuc	88
		L-gal	66
		ara	66
-C(O)CH_2NH_2	49	fuc	61
-C(O)CH_2SO_3Na	92	fuc	45
-C(S)CH_3	77	fuc	82
	86	fuc	73
		L-gal	68
		ara	81

Table 2
Enzymatic preparation of SLe[a] derivatives



acyl	sia %	sugar	%	acyl	sia %	sugar	%	acyl	sia %	sugar	%
										fuc	78
	55	ara	89		70	ara	60		71	ara	82
		2F-fuc	73			fuc	49			L-gal	77
										fuc	87
	78	L-gal	94		94	fuc	76		87	$2NH_2$-fuc	26
		L-glc	48							ara	72
	59	ara	96		60	ara	67		53	L-gal	81

moieties are linked to the 4-OH group of the *N*-acyl-glucosamine units in an α-mode.

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