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Patents and Literature

Utilization of Glycosyltransferases to Change Oligosaccharide Structures

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ABSTRACT

Carbohydrates on cell surfaces are important biomolecules in various biological recognition processes. Elucidation of the biological roles of complex oligosaccharides necessitates an efficient methodology to synthesize these compounds and their analogs. Enzymatic synthesis renders itself to be useful in the construction of an oligosaccharide structure owing to its mild reaction condition, high regio- and stereoselectivity. This review article focuses on the recent progress in oligosaccharide syntheses catalyzed by glycosyltransferases, namely sialyltransferase, galactosyltransferase, fucosyltransferase, and *N*-acetylglucosaminyltransferase. A survey of the latest patent and literature related to this field is also included.

Index Entries: Glycosyltransferase; sialyltransferase; galactosyltransferase; fucosyltransferase; *N*-acetylglucosaminyltransferase, oligosaccharide.

Abbreviations: Glc, glucose; Gal, galactose; GalNAc, *N*-acetylgalactosamine; Fuc, fucose; GlcNAc, *N*-acetylglucosamine; UDP-Glc, uridine 5′-diphosphate glucose; UDP-GlcNAc, uridine 5′-diphosphate *N*-acetylglucosamine; UDP-Gal, uridine 5′-diphosphate galactose; UDP-GalNAc, uridine 5′-diphosphate *N*-acetylgalactosamine; GDP-Man, guanosine 5′-diphosphate mannose; GDP-Fuc, guanosine 5′-diphosphate fucose;

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UDP-GlcUA, uridine 5′-diphosphate glucuronic acid; CMP-Sialic acid, cytidine 5′-monophosphate sialic acid.

INTRODUCTION

Carbohydrates are among the most important biomolecules *(1)*. Aside from serving as energy sources and structural materials, the large repertoire of oligosaccharide moieties of the cell-surface glycoproteins and glycolipids play key roles in cell–cell interaction processes *(2–6)*. Recognition of carbohydrates by proteins and nucleic acids is highly specific owing to the biologically critical information embedded within the complex oligosaccharides. However, the exact contributions of the sugar moieties to glycoprotein and glycolipid function remain largely unknown. The studies on structure–function relationship of cell-surface oligosaccharides require an efficient methodology for the syntheses of novel oligosaccharide structures. The stereocontrolled oligosaccharide synthesis can be achieved through conventional chemical methods based on sophisticated protection-deprotection, activation, and coupling strategies. The inherent problems in the classical synthetic chemistry have been the major impediments to the development of carbohydrate chemistry *(7)*. An alternative approach is to use enzymes, especially glycosyltransferases *(8,9)*, to synthesize complex oligosaccharides in vitro. This approach obviates the demand for the cumbersome protection/deprotection and chemical glycosylation steps that are essential to a classic chemical approach. It has been so far the most promising route for the synthesis of complex oligosaccharides and their analogs. There have already been several excellent reviews in this field *(7,10–12,14,15)*. In this review, recent developments in glycosyltransferase-catalyzed oligosaccharide synthesis are discussed.

Generally speaking, the glycosyltransferase-catalyzed oligosaccharide synthesis calls for the following three components in a reaction system (Fig. 1):

1. an acceptor oligosaccharide structure;
2. a sugar donor that is usually activated as glycosyl esters of nucleoside mono- or diphosphates *(16)*; and
3. a suitable glycosyltransferase in the light of the "one enzyme, one linkage" concept *(17)*.

For any particular glycosidic linkage, in principle there should exist a specific glycosyltransferase responsible for the highly stereo- and regioselective formation of the glycosidic bond. The Leloir glycosyltransferases employ eight activated monosaccharide donors in the practical synthesis: UDP-Glc, UDP-GlcNAc, UDP-Gal, UDP-GalNAc, GDP-Man, GDP-Fuc, UDP-GlcUA, and CMP-sialic acid.

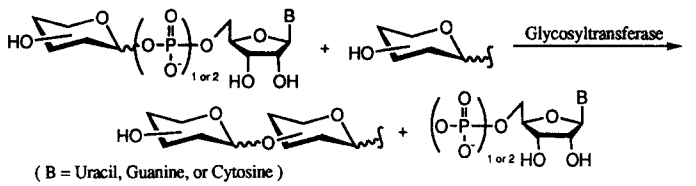


Fig. 1. The glycosidic bond formation catalyzed by Leloir glycosyltransferase.

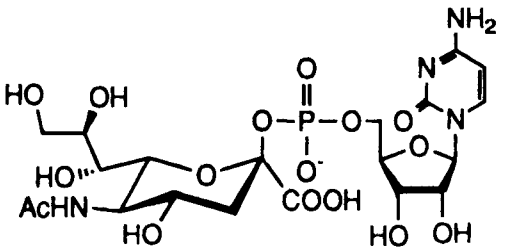


Fig. 2. CMP-Sialic acid.

Despite many advantages, such as straightforwardness and speed, the utilization of glycosyltransferases is still suffering several intrinsic weaknesses. Of the most detrimental is the limited availability of the glycosyltransferases. It remains difficult to isolate the enzymes on a large scale from their natural sources. However, a new era of enzyme-catalyzed oligosaccharide synthesis has been opened by the advent of the modern cloning techniques. It is now possible to make several glycosyltransferases readily available in sufficient quality and quantity. More than 30 glycosyltransferases from various sources have been cloned and are available for enzymatic synthesis of oligosaccharides *(8,10)*. It is conceivable that the increasing availability of glycosyltransferase through cloning techniques will have a strong impact on the oligosaccharide synthesis in the foreseeable future. Another problem is that the activated donor monosaccharide nucleotide is too expensive to be used in stoichiometric amounts and its released product, nucleoside mono- or diphosphate, could terminate the reaction by inhibiting the glycosyltransferase. One way to solve this problem is to utilize the *in situ* sugar nucleotide regeneration system in which the glycosyltransferase-catalyzed reactions are coupled with the regeneration of sugar nucleotides from nucleoside phosphates *(12)*.

Sialyltransferase

Sialyltransferase is a family of enzymes used in the biosynthetic pathway to catalyze the attachment of sialic acid from CMP-sialic acid, which is an activated sialyl ester of cytidine 5′-monophosphate (Fig. 2) to the terminal position of cell-surface glycoprotein and glycolipid oligosaccharide, which is either *N*-linked to asparagine or *O*-linked to threonine or serine.

Table 1
Acceptor Substrates Used in the Sialyltransferase Catalyzed Reactions

Acceptor substrates[a]	Amount[b]	References
α-2,6 Sialyltransferase		
Gal β OMe	A	*37*
Gal β $OPhNO_2$	A	*81*
Gal β 1,4Glc β OMe	A	*37*
Gal β 1,4 GlcNAc	B	*82,25,28,83*
Gal β 1,4 GlcNAc β OMe	B	*37*
Gal β 1,4 GlcNAc β 1,3 Gal β 1,4 Glc	B	*37*
Gal β 1,4 GlcNAc β-N-Asn	B	*84*
Gal β 1,4 GlcNAc β 1,2 Man α OMe	B	*85*
Gal β 1,4 GlcNAc β 1,3 (Gal β 1,4 GlcNAc β 1,6) Gal β 1,4 Glc β OMe	B	*85*
Gal β 1,4 GlcNAc β R; (R=OH, N3, GlyGlyAsnGlyGly or N-Alloc-PheAsnSerThrIle)	B	*26*
Gal β 1,4 GlcNAc β 1,3 Gal β 1,4 Glc β-sphingosine	B	*86*
Gal β 1,4 GlcNAc β 1,6 (*Gal* β 1,4 GlcNAc β 1,3) Gal β-$O(CH_2)_5COOMe$	A	*87*
Gal β 1,4 GlcNAc β 1,4 (*Gal* β 1,4 GlcNAc β 1,2) Gal β-$O(CH_2)_5COOMe$	A	*87*
α-2,3 Sialyltransferase		
Gal β $OPhNO_2$	A	*81*
Gal β 1,4 Glc β OMe	A	*37*
Gal β 1,4 Glc β ceramide	C	*88*
Gal β 1,4 GlcNAc β OMe	A	*37*
Gal β 1,3 GalNAc β OR (R=CH_3CH_2, $BrCH_3CH_2$)	B	*89*
Gal β 1,3 GalNAc β OR (R=H, $(CH_2)_5CO_2CH_3$)	A	*37,35*
Gal β 1,3 GlcNAc β OR (R=Me, Ph, $(CH_2)_5CO_2CH_3$)	A	*37*
Gal β 1,3 GlcNAcOMe	B	*89*
Gal β 1,3 GlcNAc β 1,3 Gal β $O(CH_2)_8CO_2Me$	A	*65*
Gal β 1,3 GlcNAc β 1,6 Gal β $O(CH_2)_8CO_2Me$	A	*65*
Gal β 1,3 GlcNAc β 1,4 Gal β 1,4 Glc, LSTa	A	*37*
Gal β 1,4 GlcNAc β 1,4 Gal β 1,4 Glc, LSTd	A	*37*
Gal β 1,4 GlcNAc β 1,3 Gal β 1,4 Glc β-sphingosine	B	*86*
Gal β 1,3 (NeuAc α 2,6) GalNAcOPh	A	*90*
3Me-O-Gal β 1,4 Glc β 1,6 (*Gal* β 1,4) GlcNAc β 1,3 Gal β 1,4 Glc β 1,6 (*Gal* β 1,4) GlcNAc β Me	A	*91*

[a] The donor substrate is CMP-sialic acid; the acceptor residue is italicized.
[b] A, 1–10 mg; B, 10–100 mg; C, 0.1–1 g.

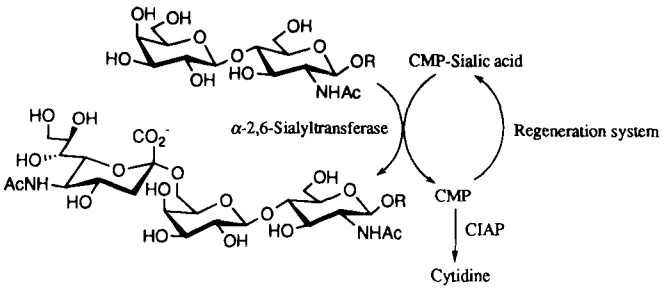
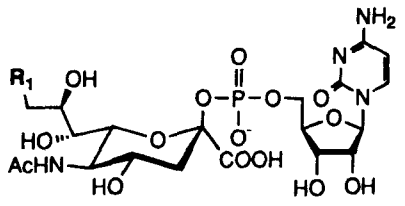


Fig. 3. Regeneration of CMP-Sialic acid or decomposition of CMP by CIAP improves sialylation efficiency.

The importance of sialosides in the biological processes stems from their contributions to the enormous diversity of natural oligosaccharide sequences generated from a limited number of core structures *(18)*. They are the receptor determinants for animal viruses, mycoplasma, animal and plant lectins, bacterial toxins, and certain tumor-specific antibodies *(5,13,19)*. In addition, sialic acids often function in the masking of carbohydrate groups that can be recognized by certain receptors.

So far the cDNA clones of eight distinct sialyltransferases have been obtained, which account for the following eight different sialic-acid linkages: Sia α 2,3 Gal; Sia α 2,4 Gal; Sia α 2,4 GlcNAc; Sia α 2,6 Gal; Sia α 2,6 GalNAc; Sia α 2,6 GlcNAc; Sia α 2,6 Man; and Sia α 2,8 Sia *(23)*. Each enzyme is extremely specific for not only the sugar acceptor but also the penultimate sugar and the connecting linkage. Among them, α 2,6 Gal and α 2,3 Gal sialyltransferases have been purified to homogeneity and applied to the in vitro enzymatic oligosaccharide synthesis *(19–22)*.

The Sia α 2,6 Gal linkage is a common component of glycoprotein oligosaccharides. The α-2,6-sialyltransferase (EC 2.4.99.1) purified from rat liver is very specific for the acceptor containing a Gal β 1,4 GlcNAc structure at the terminus. The problem of low reaction efficiency could be circumvented by either applying the CMP-sialic acid *in situ* regeneration system *(12,24,25)* or using CIAP (calf intestinal alkaline phosphatase) to decompose the released CMP and UDP to cytidine and uridine during enzymatic reaction (Fig. 3) *(26)*. Both CMP and UDP are known to be inhibitors of sialylation and galactosylation reactions. Many efficient multienzyme cofactors' *in situ* regeneration systems have been developed and summarized in recent reviews *(9,11)*. In the design of potential α-sialoside inhibitors of viral adhesion, the binding affinity could be greatly enhanced through the application of the multivalency concept. Recently, multivalent sialoglycopeptides were synthesized by using recombinant α-2,6-sialyltransferase and tested as inhibitors against influenza virus *(27)*. The results revealed that the sialosides' multivalency increased their binding to the influenza virus.



	R_1	References
1	$C_6H_5C(O)NH$	28, 29
2	$CH_3C(O)NH$	28, 29
3	N_3	28, 29
4	NH_2	28, 29
5	F	11

Fig. 4. CMP-Sialic acid analogs.

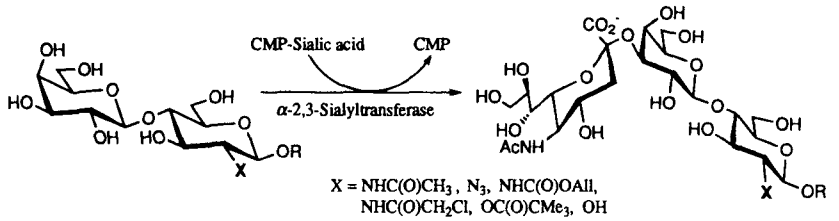


Fig. 5. Acceptor substrate analogs in the α-2,3-sialyltransferase catalyzed reaction.

Unlike its strict acceptor-substrate specificity, α-2,6-sialyltransferase has been shown to accommodate a number of donor substrate analogs with modifications at the 9-position of the sialic-acid moiety of CMP-sialic acid (Fig. 4) *(28–32)*. These analogs are usually transferred onto the acceptors at a lower rate than the corresponding natural donors. Further insight into the biological functions of sialic acids could be gained through studying these synthetic sialoside analogs.

The α-2,3-sialyltransferase (EC 2.4.99.5) is responsible for the Sia α 2,3 Gal β 1,3 GlcNAc structure which is often found in the *N*-linked oligosaccharides of glycoproteins. The enzyme is highly specific for the acceptor substrate terminating with Gal β 1,3 GlcNAc, as well as Gal β 1,4 GlcNAc *(33)*. However, substitutions with azide, carbamate, phthalimide, or pivaloyl group on the 2-position of the reducing terminal residue have made the analogs effective, or even better acceptor substrates for the enzyme (Fig. 5) *(34)*. Complex sialosides such as ganglioside GM_3 have been synthesized through the utilization of sialyltransferase products as glycosyl donors.

In addition to the synthesis of α-2,3-sialic acid containing oligosaccharides *(35–37)*, a number of bivalent sialoglycosides anchored on different linkers have been synthesized to study the inhibition and ligand recognition *(38)*.

Another α-2,3-sialyltransferase (EC 2.4.99.4) is responsible for the α-2,3-sialic acid linkage found in the *O*-linked oligosaccharides. Its acceptor substrate is disaccharide Gal β 1,3 GalNAc *(39)*.

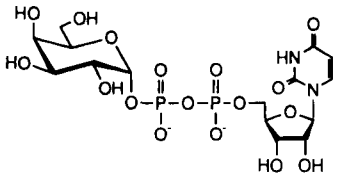


Fig. 6. UDP-Galactose.

Galactosyltransferase

One of the best studied glycosyltransferases with respect to synthesis and substrate specificity is β-1,4-galactosyltransferase (EC 2.4.1.22) *(40)*, which catalyzes the transfer of galactose from UDP-galactose (Fig. 6) to β-glycoside of *N*-acetylglucosamine to form the substructure Gal β 1,4 GlcNAc.

Both GlcNAc and Glc are acceptor substrates of β-1,4-galactosyltransferase. However, when α-glycoside of Glc is used as acceptor, the presence of α-lactalbumin α-LA) in the reaction becomes necessary for the transfer of Gal to the α-linked Glc residue. The K_m of β-1,4-galactosyltransferase for α-linked Glc is reduced about a 1000-fold in the presence of α-LA from 2 *M* down to 2 m*M* *(41)*. Besides the two acceptors previously mentioned, many other acceptor substrates have been used in the enzymatic-oligosaccharides synthesis *(42)*. The reaction rates substantially decrease for the acceptors GlcNAc modified at the 3- or 6-position, but they are still useful for synthetic purpose *(44,45)*. Some other substrates, such as 2-deoxyglucose, 5-thioglucose and D-xylose, are good substrates each with a relative rate >60% that of glucose *(45)*. Monosaccharides with negative charge such as glucuronic acid and α-glucose-1-phosphate are not acceptable acceptor substrates. Nevertheless, the acceptor specificity of β-1,4-galactosyltransferase can be loosened by lowering the pH of the reaction system, and this methodology has been applied to the rapid synthesis of some novel anti-inflammatory compounds in which disaccharides cellobiose, laminaribiose, gentiobiose, and maltose acted as acceptors (*see* Laine and Yoon in Patent Section). Another noteworthy example is the galactosylation of xylose by β-1,4-galactosyltransferase in which xylose is recognized by the enzyme both in the normal and in the reverse orientations *(45)*. Owing to the high symmetry of xylose, the frame-shifted recognition of modified acceptor substrates resulted in the formation of β,β-1,1 linked disaccharides *(46–48)* instead of the expected formation of pure β-1,4-galactosyl xylopyranoside.

On the aspect of donor substrate, UDP-Gal is the only preferred sugar nucleotide donor; galactose analogs such as glucose *(9)*, 2-deoxygalactose *(49,50)*, 4- and 6-deoxygalactose *(48,52)*, and 5′-thiogalactose *(53)* can also

Table 2
Relative Transfer Rates of Acceptor Substrate Analogs in the β-1,4-Glactosyltransferase Catalyzed Reactions (Donor Substrate Is UDP-Gal) *(104)*.

Acceptor substrate	Product	α-LA	Rel. rate (%)
GlcNAc	Galβ1,4GlcNAc	–	100
6-*R* GlcNAc ($R = CH_3CO$)	Galβ1,4 6-*R* GlcNAc ($R = CH_3CO$)	–	4
3-*R* GlcNAc ($R = CH_3(CH_2)_2O$)	Galβ1,4 3-*R* GlcNAc ($R = CH_3(CH_2)_2O$)	–	0.5
Glc	Galβ1, 4Glc	+	100
3-*R* Glc ($R = CH_3O$)	Galβ1,4 3-*R* Glc ($R = CH_3O$)	+	10

be transferred from their corresponding UDP derivatives to provide an enzymatic route to oligosaccharides that terminate in β-1,4-linked residues other than galactose, but the reaction rate is very slow at only 0.09–5.5% of the rate at which UDP-Gal is used. Interestingly, when in the presence of α-LA, the β-1,4-galactosyltransferase transfers GalNAc to free GlcNAc to produce GalNAcβ 1,4 GlcNAc at a rate 55% of that compared to the rate when UDP-Gal is the donor in the absence of α-LA. However, β-1,4-galactosyltransferase is unable to transfer GalNAc to Glc with or without α-LA *(41)*. This finding is contrary to our earlier understanding that the major effect of α-LA on β-1,4-galactosyltransferase is to cause a switch in monosaccharide-acceptor specificity from GlcNAc to Glc.

Despite the fact that the galactosyltransferase-catalyzed reaction with unnatural substrates is quite slow, a milligram-scale synthesis of oligosaccharides can be achieved with β-1,4-galactosyltransferase *(54)*.

Fucosyltransferase

Fucosylation is the terminal and critical step in the in vivo synthesis of many biologically important oligosaccharides *(49)*. The importance of fucosyltransferases originated from the presence of fucose in antigenic determinants of several human-erythrocyte blood groups. Fucose is activated as guanosine 5′-diphosphate β-L-fucose (Fig. 7) in the fucosyltransferase-catalyzed reaction.

Several fucosyltransferases have been cloned and their properties studied in the past several years *(56–61)*. Among them, three fucosyltransferases have already been used in the in vitro oligosaccharide synthesis. These are α-1,2-fucosyltransferase (EC 2.4.1.69), α-1,3-fucosyltransferase, and α-1,3/4-fucosyltransferase (EC 2.4.1.65).

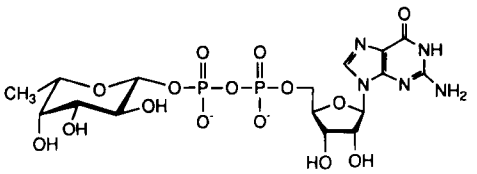


Fig. 7. GDP-Fucose.

Table 3
Acceptor Substrates Used in the Fucosyltransferase-Catalyzed Reactions

Acceptor substrates[a]	Amount[b]	References
α-1,2 Fucosyltransferase		
Gal β OCH_2CH_3	B	*62*
Gal β 1,4 GlcNAc	B	*62*
Gal β hexanolamine	B	*62*
Gal β 1,4 GlcNAc hexanolamine	B	*62*
α-1,3 Fucosyltransferase		
NeuAc α 2,3 Gal β 1,4 *GlcNAc* β $O(CH_2)_5CO_2Me$	A, B	*62,92*
Gal β 1,4 5 *thio-Glc*	B	*92*
NeuAc α 2,3 Gal β 1,4 *GlcNHR* β $O(CH_2)_8CO_2Me$ ($R=CH_2COCH_3$, $CH_2COCH(CH_3)_2$, $CH_2COCH_2NH_2$, CH_2CSCH_3, $CH_2CO_2CH_2CH=CH_2$, $CH_2CONHCH_2CH_3$, $CH_2CO(CH_2)_2NO_2^-$, $CH_2COCH_2SO_3Na$, CH_2COPh, $CH_2SO_2CH_3$, $CH_2CO(CH_2)_2NHC(NH)NH_2$)	B	*69*
NeuAc α 2,3 Gal β 1,4 2-*R Glc* β $O(CH_2)_8CO_2Me$ ($R=N_3$, NH_2, NHPr, NHAc)	B	*93*
NeuAc α 2,3 Gal β 1,3 2-*R Glc* β $O(CH_2)_8CO_2Me$ ($R=N_3$, NH_2, NHPr, NHAc)	B	*93*
Gal β 1,4 *R* (*R=GlcNAc, Glc, 5-S-Glc, Glucal,*)	B	*94*
Gal β 1,3 *GlcNAc*	B	*94*
NeuAc α 2,3 Gal β 1,4 *R* (*R=GlcNAc, Glucal*)	B	*94*
NeuAc α 2,6 Gal β 1,4 *GlcNAc*	B	*94*
α-1,4 Fucosyltransferase		
Gal β 1,3 *GlcNAc*	B	*92*
NeuAc α 2,3 Gal β 1,3 *GlcNAc* β 1,6 Gal β $O(CH_2)_5CO_2Me$	C	*93*
NeuAc α 2,3 Gal β 1,3 *GlcNAc* β 1,3 Gal β $O(CH_2)_5CO_2Me$	C	*93*
Gal β 1,3 *GlcNAc* β $O(CH_2)_8CO_2Me$	C	*95,71*

[a] The donor substrate is GDP-Fuc; the acceptor residue is italicized.
[b] A, 1–10 mg; B, 10–100 mg; C, 0.1–1 g.

The enzyme α-1,2 fucosyltransferase is involved in the formation of the H-blood group antigen which is a precursor of the human ABO blood-group antigens. The H antigen is a fucosylated structure in the form of Fuc α-1,2 Gal β-R whose expression is normally restricted to the surfaces of human erythrocytes and a variety of epithelial cells *(62)*. The trans-glycosylation reaction catalyzed by α-1,2-fucosyltransferase transfers fucose from GDP-Fuc onto the 2-OH group of the terminal Gal residue of the acceptor Galβ1,3 GlcNAc- (type I) or Gal β 1,4 GlcNAc- (type II). However, there is no absolute specificity for the penultimate-sugar residue or the glycosidic linkage between the galactose and the penultimate residue *(63)*. The type-II blood group with specific ^{13}C enrichment of the Gal and Fuc residues has been synthesized and used to evaluate both intra- and interresidue conformations in solution *(62)*. All α-1,2-fucosyltransferases are stimulated from two- to tenfold by divalent metal cofactor such as Mn^{2+} and Mg^{2+}, but show some activity even in the presence of metal chelators such as EDTA *(63)*.

The α-1,3-fucosyltransferase is responsible for the fucosylation of the 3-OH of the GlcNAc moiety in both *N*-acetyllactosamine and sialyl α-2,3-*N*-acetyllactosamine *(64,65)*. A variety of the cDNAs coding for α-1,3-fucosyltransferase *(66)* have been cloned and expressed in cell lines in recent *(56,57,59–61)*. They are discriminated by differences in substrate specificities, cation requirement, sensitivity to inhibitors, and tissue distribution *(67)*. These enzymes include FucTIII (human α-1,3/4-fucosyltransferase), FucTIV (myeloid-expressed α-1,3 FucT), FucTV, and VI (plasma type enzyme), and FucTVII (leukocyte-expressed α-1,3 FucT). A systematic study and comparison of the acceptor specificity has been carried out among the recombinant forms of FucTIII, FucTIV, and FucTV *(68)*. Several acceptor substrates with modifications in the GlcNAc residue can also be fucosylated *(64)*. Most recently, the cloned-fucosyltransferase VI was used in the enzymatic fucosylation of nonnatural sialyl Lewis^x derivatives and exhibited to tolerate a wide range of acceptors spanning from small and bulky aliphatic as well as charged and sulfonamide replacements of the natural *N*-acetyl residue of GlcNAc *(69)*.

The Lewis α-1,3/4-fucosyltransferase (EC 2.4.1.65) purified from human milk has been shown to fucosylate either 4-OH of GlcNAc in type-I terminal sequence (Gal β 1,3 GlcNAc β-OR) to afford Lewis^x or 3-OH of GlcNAc in type-II terminal sequence (Gal β 1,4 GlcNAc β-OR) to produce Lewis^a *(66)*. The Lewis fucosyltransferase is specific for these structures even when they are sialylated at 3-OH or fucosylated at 2-OH of the terminal Gal residue *(70)*. Besides the natural acceptor sequences ubiquitous on mammalian cell-surface glycoproteins and glycolipids, several nonnatural fucose analogs such as 3-deoxyfucose and arabinose have been synthesized and found to be active donors for the synthesis of Le^a-active trisaccharide analogs (Fig. 8) *(22)*. These analogs are invaluable probes in studies on the molecular

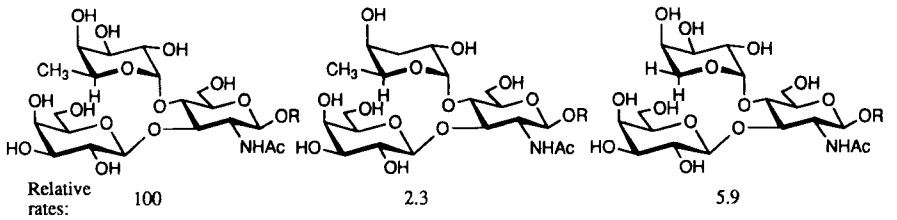


Fig. 8. Le^a trisaccharide analogs and the comparison of their fucosylation rates (R=$(CH_2)_8CO_2Me$).

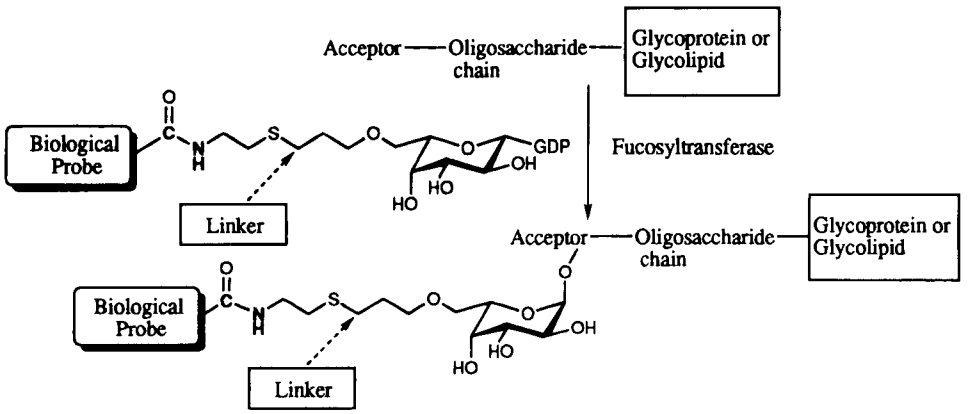


Fig. 9. General method for labeling the cell-surface sugar chain with a biological probe.

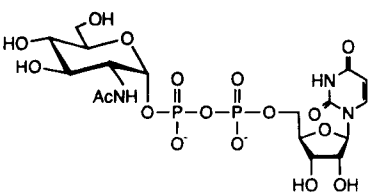


Fig. 10. UDP-*N*-Acetylglucosamine.

recognition between proteins and complex carbohydrates. Moreover, the α-1,4-fucosyltransferase has been demonstrated to be able to modify the glycoprotein by transferring chemically synthesized fucose analog substituted at C-6 with a very bulky human blood group B trisaccharide antigen to the acceptor oligosaccharide chains of type O erythrocytes *(72)*. This work was extended by a recent synthesis of an aminated GDP-Fucose analog in which the amino group can be acylated with an *N*-hydroxysuccinimide ester of biotin (Fig. 9) *(73)*. Thus prepared GDP-Fucose analog modified at 6-OH of L-galactose with biotin is an active donor for the fucosyltransferase catalyzed reaction with *N*-acetyllactosamine. As a large number of the carbohydrate structures commonly found on cell-surface mammalian

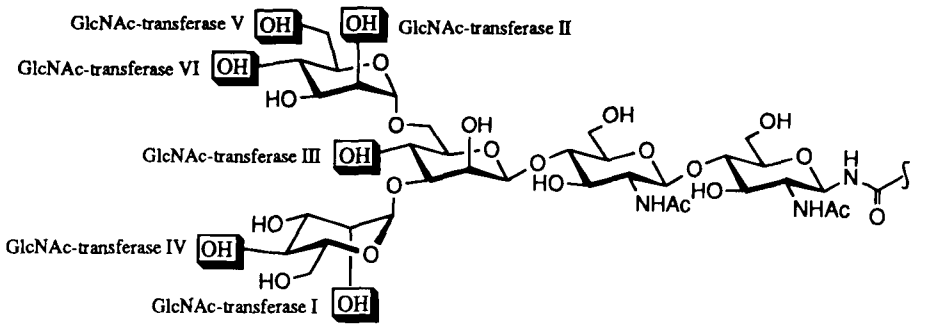


Fig. 11. Six *N*-acetylglucosaminyltransferases responsible for the *N*-glycan branching.

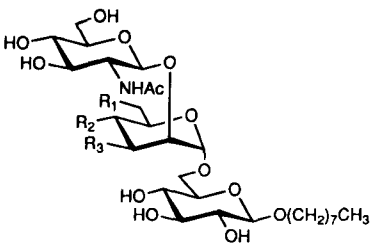


Fig. 12. Trisaccharide substrate of *N*-acetylglycosaminyltransferase-V.

glycoproteins and glycolipids can serve as acceptors for the Lewis enzyme, this enzyme is therefore a potential candidate as a universal reagent to label the sugar chains of cell surfaces with biological probes via a fucose "spacer".

N-Acetylglucosaminyltransferase

N-Acetylglucosaminyltransferases (EC 2.4.1) are involved in the control of *N*-glycan branching *(74)*. They catalyze the addition of GlcNAc residues from UDP-GlcNAc (Fig. 10) to the mannose residues of the cell-surface Asn-linked oligosaccharides. The enzyme activity changes have been correlated with several disease processes such as anemia, cancer, oncogenic transformation, and metastasis *(75)*.

The specificities of *N*-acetylglucosaminyltransferases I-VI are illustrated in Fig. 11. These enzymes have been applied to the synthesis of GlcNAc containing oligosaccharides. Several nonnatural analogs of UDP-GlcNAc have been used as donor substrates of *N*-acetylglucosaminyltransferases *(76)*. *N*-acetylglucosaminyltransferase-V has been studied with respect to its acceptor substrate specificity *(78,79)*. A series of analogs of a trisaccharide substrate GlcNAc β-1,2 Man α-1,6 Glc β-OR (Fig. 12) with sequential substitutions on R_1, R_2, R_3 positions of the central mannose residue can be recognized by the enzyme *(79)*, so was its nonpyranosidic

analog where the ring oxygen on the mannose was replaced with a methylene group to yield the carba-sugar *(80)*. Nevertheless, all three OH groups on the terminal GlcNAc residues are essential to the acceptor recognition by *N*-acetylglucosaminyltransferase-V. These results will be used in the rational design of inhibitors for this tumor-associated enzyme.

PATENTS AND LITERATURE

Introduction

In this Patents and Literature section is summarized the latest development achieved by both industrial and academic research groups in the area of glycosyltransferase catalyzed oligosaccharide synthesis. The subject of the following section is Utilization of Glycosyltransferases to Change Oligosaccharide Structures. Patents reported cover the time period of January 1993 to August 1996. The literature part lists the articles referenced in the review section and emphasis is placed on the review articles and research publications in this field from 1990 to 1996.

PATENTS

Gotschlich, E. C.
GLYCOSYLTRANSFERASES FOR BIOSYNTHESIS
OF OLIGOSACCHARIDES, AND GENES ENCODING THEM
US 05,545,553; August 13, 1996
Assignee: The Rockefeller University (New York, NY)

The present invention is directed to nucleic acids encoding glycosyltransferases, the proteins encoded thereby, and to methods for synthesizing oligosaccharides using the glycosyltransferases of the invention. The enzymes are characterized by catalyzing reactions such as adding Gal β 1→4 to GlcNAc or Glc; adding GalNAc or GlcNAc β 1→3 to Gal; and adding Gal α 1→4 to Gal. This enzyme are particularly suited to the synthesis of Galβ1, 4GlcNAcβ1,3Galβ1,4Glc (a mimic of lacto-*N*-neotetraose), GalNAcβ1,3Galβ1,4GlcNAcβ1,3Galβ1,4Glcβ1,4 (a mimic of ganglioside), and Galα1,4Galβ1,4Glcβ1,4HepR (a mimic of the saccharide portion of globo-glycolipids).

Ito, Y. and Paulson, J. C.
USE OF TRANS-SIALIDASE AND SIALYLTRANSFERASE
FOR SYNTHESIS OF SIALYL α 2→3 β GALACTOSIDES
US 05,409,817; April 25, 1995
Assignee: Cytel, Inc. (San Diego, CA)

A single vessel cyclic synthesis process for preparation of a sialyl α 2→3 β galactoside is disclosed. A sialyltransferase acceptor is sialylated in an aqueous reaction medium by an α-2,3-sialyltransferase and CMP-sialic acid to form a sialyl-donor substrate and CMP. In the presence of the trans-sialidase of Trypanosoma crusi, that sialyl-donor substrate provides a sialyl group for a trans-sialidase acceptor, thereby preparing the sialyl α 2→3 β galactoside. The α-2-3-sialyltransferase acceptor is reformed upon trans-sialidation of the latter acceptor, and the sialyl-donor substrate is reformed using the α-2,3-sialyltransferase and a CMP-sialic-acid recycling system that combines CMP with sialic acid that is also present in the vessel. The K_m/V_{max} value for the α-2,3-sialyltransferase acceptor is less than one-tenth the value of K_m/V_{max} of the trans-sialidase acceptor for the α-2,3-sialyltransferase.

Kashem, M., Venot, A.P., and Smith; R.
METHODS FOR THE SYNTHESIS OF MONOFUCOSYLATED OLIGOSACCHARIDES TERMINATING IN DI-*N*-ACETYLLACTOSAMINYL STRUCTURES
US 05,374,655; December 20, 1994
Assignee: Alberta Research Council (Edmonton, CA)

Disclosed are methods for the preparation of monofucosylated and sialylated derivatives of the compound Galβ1,4GlcNAcβ1,3Galβ1, 4GlcNAcβOR. In particular, the methods of this invention provide for a multistep synthesis, wherein selective monofucosylation is accomplished on the 3-OH group on only one of the GlcNAc units found in the Galβ1,4GlcNAcβ1,3Galβ1,4-GlcNAcβOR compound. In this step, monofucosylation is achieved by use of the α-1,3-fucosyltransferase.

Laine, R. A. and Yoon, E.
SYNTHESIS OF ANTI-INFLAMMATORY COMPOUNDS, AND NOVEL TRISACCHARIDES USEFUL IN THE SYNTHESIS OF ANTI-INFLAMMATORY COMPOUNDS
US 05,426,178; June 20, 1995
Assignee: Board of Supervisors of Louisiana State University and Agricultural and Mechanical College (Baton Rouge, LA)

Novel oligosaccharides useful in the rapid synthesis of certain anti-inflammatory compounds are disclosed, as is a rapid method of synthesizing the oligosaccharides. Low pH can loosen the acceptor specificity of β-1,4-galactosyltransferase, allowing the rapid synthesis of novel oligosaccharides. The disaccharides cellobiose (β 1,4), laminaribiose (β 1,3), gentiobiose (β 1,6), and maltose (α 1,4) acted as acceptors for β-1,4-galactosyltransferase under low-pH conditions. From these four acceptors, the following four novel trisaccharides were synthesized: Galβ1,4Glcβ1,3Glc,

Galβ1,4Glcβ1,4Glc, Galβ1,4Glcβ1,6Glc, and Galβ1,4Glcα1,4 Glc. These trisaccharides, and other oligosaccharides, may be synthesized in a few days with the disclosed technique, as opposed to the several mo with organic-synthetic methods. These trisaccharides may be used as intermediates in the rapid synthesis of anti-inflammatory compounds.

Nudelman, E., Sadozai, K. K., Clausen, H., Hakomori, S.-I., and Stroud, M.
SYNTHESIS OF Le^x, DIMERIC Le^x (DIFUCOSYL Y2; III 3 FUCV3 FUCNLC6CER), SIALYLATED FORMS THEREOF; AND ANALOGUES THEREOF.
US 05,421,733; June 6, 1995
Assignee: The Biomembrane Institute (Seattle, WA)

A process for preparing difucosyl Y2 antigen (dimeric Le^x), said process comprising: 1. preparing a lactonorhexaosylceramide backbone or a lactonorhexaosylsaccharide backbone linked to a carrier molecule; and 2. enzymatically focusylating said backbone at the III 3 and V 3 positions through an α-1,3 linkage. A process for preparing Le^y antigen analogs, said process comprising: 1. preparing a lactonorhexaosyl-ceramide backbone or a lactonorhexaosylsaccharide backbone linked to a carrier molecule; and 2. enzymatically fucosylating said backbone at the terminal β-Gal through an α-1,2 linkage; and 3. enzymatically fucosylating said backbone at one or more positions through an α-1,3 linkage, provided that steps 2, and 3, can be conducted simultaneously or in any order.

Paulson, J. C., Ujita-Lee, E., Colley, K. J., Adler, B., Browne, J. K., and Weinstein, J.
METHOD FOR PRODUCING SECRETABLE GLYCOSYLTRANSFERASES AND OTHER GOLGI PROCESSING ENZYMES
US 05,541,083; July 30, 1996
Assignee: The Regents of the University of California (Oakland, CA) and Amgen (Thousand Oaks, CA)

A method for genetically engineering cells to produce soluble and secretable Golgi processing enzymes instead of naturally occurring membrane-bound enzymes. Cells are genetically engineered to express glycosyltransferases, which lack both a membrane anchor and a retention signal. The resulting altered enzyme becomes soluble and secretable by the cell without losing its catalytic activity. Secretion of the soluble glycosyltransferase by the cell provides for increased production and simplified recovery of glycosyltransferase.--

Roth, S.
SACCHARIDE COMPOSITIONS, METHODS AND APPARATUS FOR THEIR SYNTHESIS
US 05,180,674; January 19, 1993
Assignee: The Trustees of the University of Pennsylvania (Philadelphia, PA)

A method and an apparatus for preparing saccharide compositions is disclosed. The method is reiterative and comprises the following three steps: 1. A glycosyltransferase capable of transferring a preselected saccharide unit to an acceptor moiety is isolated by contacting the acceptor moiety with a mixture suspected of containing the glycosyltransferase under conditions effective to bind the acceptor moiety and the glycosyltransferase and thereby isolate the glycosyltransferase. The acceptor moiety is a protein, a glycoprotein, a lipid, a glycolipid, or a carbohydrate. 2. The isolated glycosyltransferase is then used to catalyze the bond between the acceptor moiety and the preselected saccharide unit. 3. Steps 1. and 2. are repeated a plurality of times with the intermediate product obtained in the first iteration of the method being used as the acceptor moiety of the second iteration.

Thiem, J., and Wiemann, T.
PROCESS FOR THE ENZYMATIC SYNTHESIS OF 2-DEOXY-β-D-GALACTOSIDES
US 05,264,352; November 23, 1993
Assignee: Hoechst Aktiengesellschaft (Frankfurt Am Main, Germany)

The invention relates to a process for the enzymatic synthesis of 2-deoxy-β-D-galactosides. The enzyme galactosyltransferase is able to catalyze the transfer of 2-deoxygalactose residues from the donor substrate uridine 5′-diphospho-2-deoxy-D-galactose to *N*-acetylglucosamine or aspartyl-*N*-acetylglucosamine or, in the presence of lactose, to terminal glucose. The reaction was carried out with the aid of the coenzyme lactalbumin.

Venot, A. P., Unger, F. M., Kashem, M. A., Bird, P., and Mazid, M. A.
METHODS FOR THE ENZYMATIC SYNTHESIS OF α-SIALYLATED OLIGOSACCHARIDE GLYCOSIDES
US 05,352,670; October 4, 1994
Assignee: Alberta Research Council (Edmonton, Canada)

Disclosed are methods for the enzymatic synthesis of α-sialylated oligosaccharide glycosides containing an analog of sialic acid. Specifically, in the disclosed methods, sialyltransferase is activated to transfer an analog of sialic acid, employed as its CMP-nucleotide derivative, to the nonreducing sugar terminus of the oligosaccharide glycoside acceptor

which has from 2 to 10 saccharide units. The analog of sialic acid and the oligosaccharide employed in this method are selected to be compatible with the sialyltransferase employed.

Wong, C.-H. and Gaeta; F. C. A.
COMBINED USE OF β-GALACTOSIDASE AND SIALYTRANSFERASE COUPLED WITH IN SITU REGENERATION OF CMP-SIALIC ACID FOR ONE POT SYNTHESIS OF OLIGOSACCHARIDES
US 05,374,541; December 20, 1994
Assignee: The Scripps Research Institute (La Jolla, CA) and Cytel Corporation (San Diego, CA)

A single reaction vessel process for the synthesis of a sialylated galactoside is disclosed. The synthesis utilizes a β-galactosidase to catalyze the reaction of a galactose-containing substrate and an acceptor to form a new galactosyl glycoside that is then sialylated using a cyclic multi-enzyme-synthesis system to form CMP-sialic acid that sialylates the formed galactosyl glycoside in the presence of an α-sialytransferase. The value of K_m/V_{max} for the formed galactosyl glycoside as a substrate for the α-sialyltransferase is less than one-third the K_m/V_{max} value for the galactose-containing substrate for that α-sialyltransferase.

Wong, C.-H., Ichikawa, Y., and Shen, G.-J.
METHOD AND COMPOSITION FOR SYNTHESIZING SIALYLATED GLYCOSYL COMPOUNDS
US 05,278,299; January 11, 1994
Assignee: Scripps Clinic and Research Foundation (La Jolla, CA)

The present invention provides a method for synthesizing a sialylated glycosyl compound comprising reacting in the presence of each other a sialic acid, a glycosyl compound, a CMP-sialic acid regenerating system, a pyrophosphate scavenger, and catalytic amounts of a CMP-sialic acid synthetase and a sialytransferase having substrate specificity for the glycosyl compound. The composition for sialylating glycosyl compounds comprising a sialic acid, CMP-sialic acid regenerating system, a pyrophosphate scavenger, and a catalytic amount of CMP-sialic acid synthetase. The composition can further comprise an aqueous solvent having a suitable buffer and enzyme cofactors as well as a catalytic amount of a sialyltransferase having substrate specificity for the glycosyl compound.

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