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Product Information

74188 α(1→3)Galactosyltransferase Kit

Cat.-No.	Name	Amount
77038	**α(1→3) Galactosyltransferase, murine, recombinant from E. coli** ~ 0.5 U/ml[1] , E.C. 2.4.1.151	**5 x 0.6 ml**
40396	UDP-Galactose UDP-Gal; Uridine 5'-diphospho-α-D-galactose disodium salt *BioChemika*, ≥90% (HPLC)	**70 mg**
63536	**Manganese(II) chloride tetrahydrate** puriss. p.a., ACS, ≥99.0% (KT)	**500 mg**
93368	**Trizma®[2] hydrochloride** *BioChemika*, pH 7.0	**1 g**
05470	**Albumin from bovine serum** *BioChemika*, lyophilized, crystallized, ≥98% (HPCE)	**25 mg**
79385	**Phosphatase alkaline from bovine intestinal mucosa** *BioChemika*, solution (clear), >10000 U/ml[3], E.C. 3.1.3.1	**150 µl**

1) 1 U corresponds to the amount of enzyme which transfers 1 µmol of galactose from UDP-galactose to *N*-acetyllactosamine per minute at pH 7.0 and 37°C.
2) Trizma® is a registered trademark of Sigma-Aldrich Biotechnology, L.P.
3) 1 U corresponds to the amount of enzyme, which hydrolyzes 1 µmol 4-nitrophenyl phosphate per minute at pH 9.8 and 37 °C.

⇒ Glycosyltransferase Kits

As part of our commitment to the progress of biocatalysis in synthetic chemistry, Sigma-Aldrich has, in the past few years, developed and produced new glycosyltransferases for preparative carbohydrate synthesis. Several recombinant glycosyltransferases are now available from well-established fermentation processes and can be offered for synthetic applications.

Employing metabolic pathway engineering, researchers at Kyowa Hakko Kogyo Inc. (Tokyo, Japan) recently developed a large-scale production system for many nucleoside mono- and diphosphate sugar donors.[41-44] This technological breakthrough can be expected to enable industrial-scale economic synthesis of oligosaccharides and glycoconjugates in the near future.[7]

In order to support and stimulate scientific research in enzymatic carbohydrate synthesis, Sigma-Aldrich and Kyowa Hakko have agreed to cooperate in the development of various glycosyltransferase kits. Each kit is designed to offer the enzyme, the corresponding nucleotide sugar donor and further components for the transfer of a specific monosaccharide moiety to an acceptor substrate on a small preparative scale. To provide greater flexibility in research applications, each enzyme is supplied in aliquots for multiple reactions on a scale sufficient for product characterizations.

⇒ The unique Glycosyltransferase Kits include sufficient amounts of enzymes, nucleotide sugar donors, buffers and reagents, that are necessary for successful glycosylations on a preparative scale**!**

1.1 Glycosyltransferases - Introduction[1-7]

Oligosaccharides and polysaccharides are ubiquitous in nature as components of a broad range of molecular structures. They function as structural scaffolds, to regulate viscosity, for energy storage, and as key components of cell surfaces. Intense studies in recent years have revealed the vital role of carbohydrate moieties of cell surface glycoproteins and glycolipids in cellular communication processes and physiological responses.[8-11] Cell-surface glycoproteins and glycolipids act as protein ligands providing anchors for intercellular adhesion. They also provide points of attachment for antibodies and other proteins, and they function as receptor sites for bacteria and viral particles.[12,13] Altered cell surface glycosylation patterns are associated with cellular differentiation, development and viral infection and are diagnostic in certain cancers.[14] Oligosaccharides and glycoconjugates, which serve as competitive ligands, represent valuable tools in biological studies and potential drug targets in infectious deseases, inflammation and cancer. Glycosylation of proteins and other bioactive molecules may serve in site specific and controlled drug delivery, to increase solubility of hydrophobic molecules,[15,16] alter uptake and residency time *in vivo* [17,18] and decrease antigenicity.[19]

The growing recognition of the roles of carbohydrates in fundamental biological processes and their potential as new therapeutics has accentuated the requirement for a general availability of larger amounts of varying carbohydrate structures.

The isolation of glycoconjugates from natural sources provides only minute quantities, limiting carbohydrate structure and function studies to the characterization of glycan chains isolated from glycoproteins.[10] Moreover, it turned out to be nearly impossible to obtain homogeneous glycoproteins from overexpression systems.[20,21]

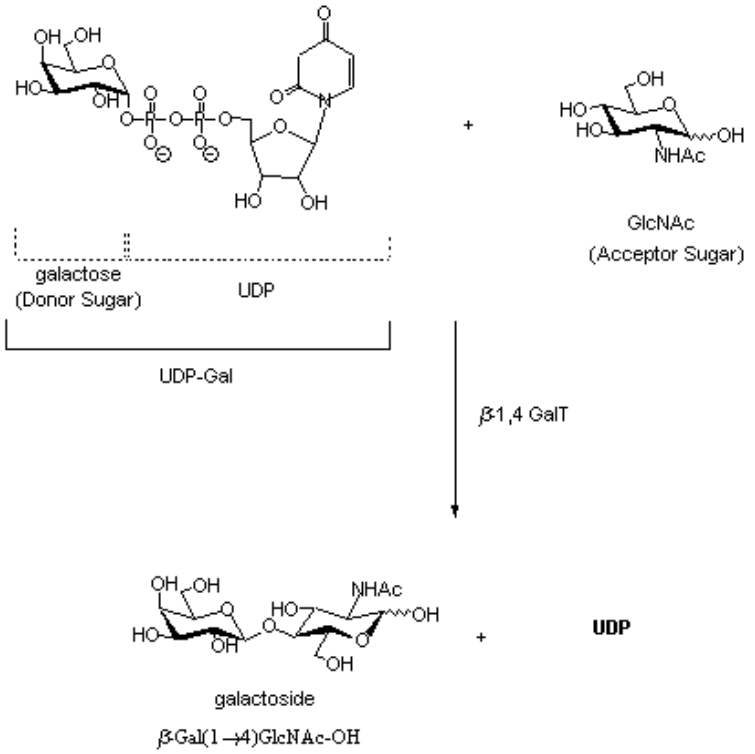
The presence of multiple functional groups and stereocenters in carbohydrates makes them challenging targets for the organic chemist. Decades of synthetic research have not yielded robust, automated protocols comparable to those developed for the preparation of peptides and oligonucleotides. Major issues for the economic, large-scale, chemical synthesis of carbohydrates and glycoconjugates are: [1,6,22-24]

- Multiple hydroxyl functionalities, which exhibit similar reactivities, must be suitably differentiated in order to obtain the desired glycosidic linkages with suitable levels of regioselectivity and stereospecificity. Therefore, laborious protecting group manipulations and complex synthetic schemes are required.
- The high diversity in linkages between specific monosaccharide units present in oligosaccharides and glycoconjugates still requires effective, regioselective, and stereospecific activation of either glycosyl donors or acceptors. This diversity in joints between monomer subunits even in simple oligosaccharides exceeds that of other biopolymers.
- Due to the fact that many carbohydrates are only soluble in water, their manipulation requires either an adaptation of organic reactions to aqueous media or a reversible modification of the carbohydrates to achieve solubility in non-aqueous solvents.

Biocatalysts, namely glycosyltransferases from the Leloir pathway,[25-27] responsible for the synthesis of most cell-surface glycoforms in mammalian systems, have been proven as viable alternatives in the preparation of oligosaccharides.[1-7] As more and more of these transferases are isolated or produced from recombinant sources, chemists have recognized enzymatic glycosidation as the method of choice to complement their classical synthetic techniques.

Leloir glycosyltransferases are highly regio- and stereospecific with respect to the glycosidic linkages formed. They use unprotected sugar precursors, thus avoiding tedious chemical elaborations, and provide products in high yields.

The biosynthesis of oligosaccharides, catalysed by glycosyltransferases from the Leloir pathway, resembles the corresponding chemical procedure (see Scheme 1). A donor sugar is activated in a first step, followed by the transfer of the activated moiety to an appropriate acceptor sugar. These enzymes utilize primarily eight different glycosyl esters of nucleoside mono- or diphosphates as activated monosaccharide donors to build a new glycosidic bond, such as UDP-Glc, UDP-GlcNAc, UDP-Gal, UDPGalNAc, GDP-Man, GDP-Fuc, UDP-GlcUA, and CMP-NeuAc.[26]



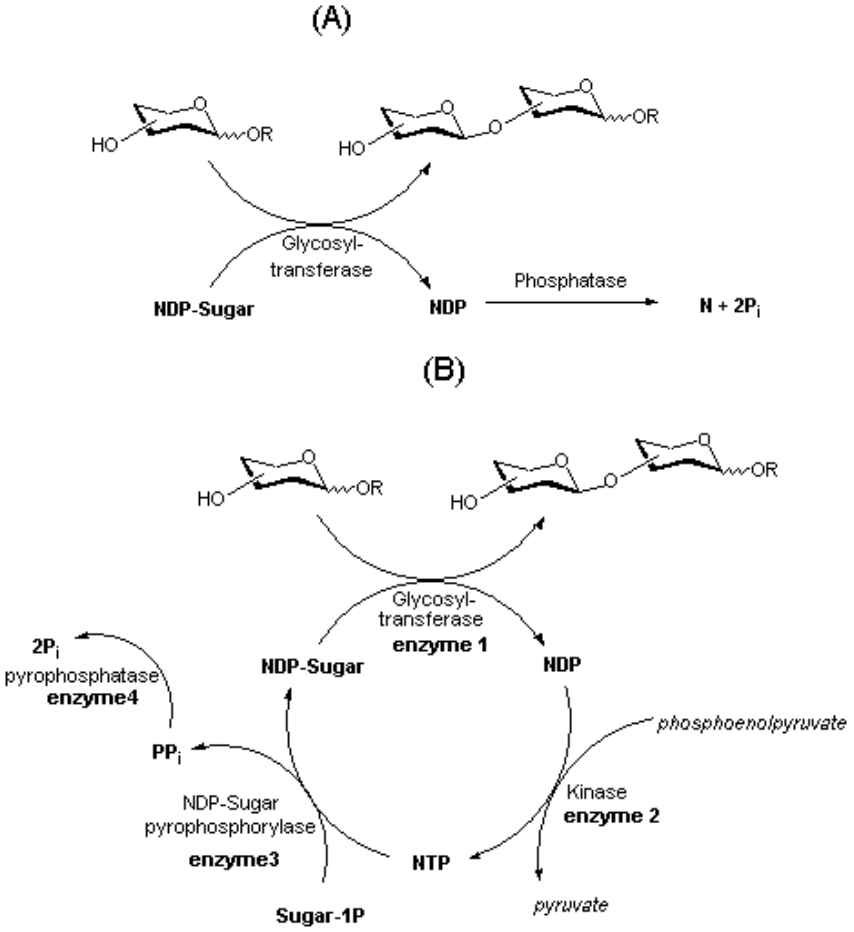
Scheme1. Glycosyltransferase-catalysed glycosidation using β(1→4) Galactosyltransferase {β(1→4) GalT}.

Glycosyltransferases are specific for the type of linkage (α or β), and the linkage position of the glycoside bond formed {*e.g.* α(1→3) or β(1→4)}. They had also been considered to be specific for a given glycosyl donor and acceptor, which led to the "one enzyme–one linkage" concept.[28,29] A number of recent observations have defeated the theory of absolute specificity regarding donors or acceptors:

- The transfer of analogues of some nucleoside mono- or diphosphate sugar donors by glycosyltransferases has been described.[30-36]
- The enzymes tolerate a certain range of modifications in the acceptor substrate, as long as specific structural requirements (*e.g.* appropriate stereochemistry and availability of the hydroxyl group involved in the glycosidic bond) are met in the acceptor molecule.[1-5] (For a comprehensive list of acceptor substrates of β(1→4)GalT-catalysed galactosidations, which have been described in the literature, please see Table 1 below.)

A major issue in glycosyltransferase-catalysed glycosidations is the fact, that the nucleoside diphosphates generated during reaction are potent glycosyltransferase inhibitors. Two strategies have been described to prevent product inhibition:

(1) The addition of phosphatase to remove nucleoside diphosphates (Scheme 2 A).[37]
(2) Employing multienzyme regeneration systems, nucleoside diphosphates can be recycled to the appropriate nucleoside diphosphate sugars. Although several different enzymes and expensive cofactors are involved in these *in situ* regeneration systems, they are supposed to avoid the use of stoichiometric amounts of expensive sugar nucleotides. (Scheme 2 B). [38-40]



Scheme 2. Methods for avoiding product inhibition in glycosyltransferase-catalyzed synthesis: (A) Addition of phosphatase. (B) Recycling of sugar nucleotides (*NDP = nucleoside diphosphates, NTP= nucleoside triphosphates, N = nucleoside, P_i = phosphate*).

For more information about the complete range of glycosyltransferases and glycosyltransferase kits offered by Sigma-Aldrich, please visit our website at
www.sigma-aldrich.com/analytical-chromatography

Our fermentation and downstream processing unit can produce according to your requirements. For larger quantities, please contact Sigma-Aldrich Fine Chemicals at
www.sigma-aldrich.com/safc

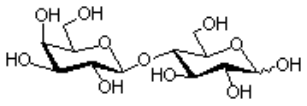
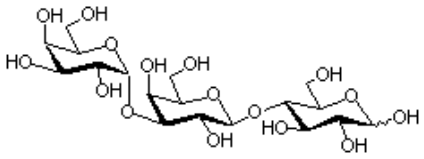
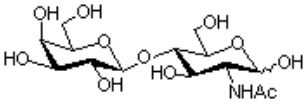
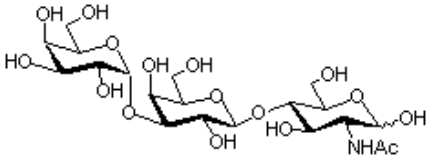
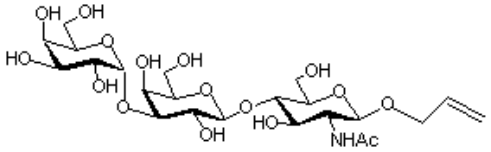
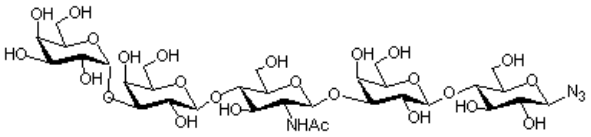
To learn more about our comprehensive portfolio of enzymes please have a look at the SIGMA-ALDRICH Enzyme Explorer – your research assistant on
www.sigma-aldrich.com/enzymexplorer

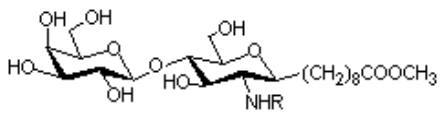
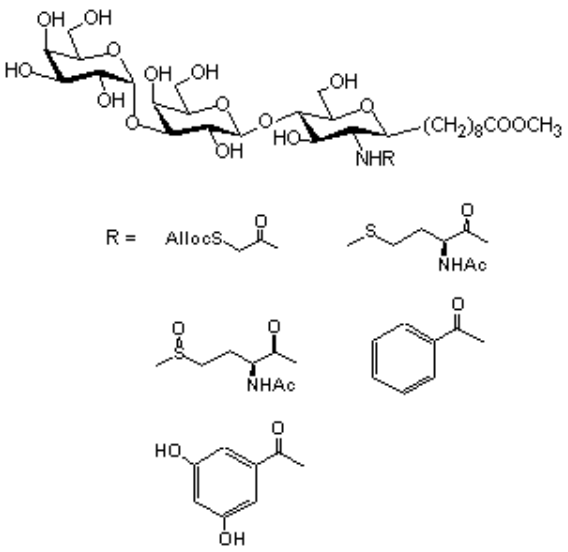
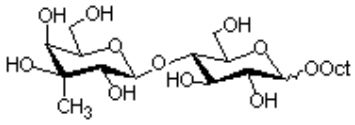
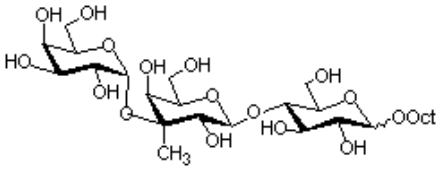
1.2. α(1→3) Galactosyltransferase

α-(1→3) Galactosyltransferase (EC 2.4.1.151) has attracted much attention in the last recent years as a unique enzyme responsible for the formation of α-galactosyl epitopes bearing α-Gal(1→3)-β-Gal-OR termini. The interaction of such α-Gal epitopes (Galili antigens) on the surface of animal cells (e.g. porcine endothelial cells) with anti-galactosyl antibodies present in human serum is believed to be the main cause in antibody-mediated hyperacute rejection following xenotransplantation.[45-50] Experimental attempts to overcome hyperacute rejection revealed the need for substantial amounts of α-Gal oligosaccharides as well as synthetical α-Gal analogues and mimetics with high affinity to anti-Gal antibodies.
Earlier chemical syntheses of α-Gal trisaccharides were rather tedious,[51-54] while glycosidase-catalyzed transglycosylation reactions to form the desired α-Gal(1→3)-β-Gal-OR linkage resulted in poor yields and regioselectivities.[55-58]

Using recombinant α-(1→3) Galactosyltransferase, α-Gal epitopes and several derivatives were synthesized on preparative scale.[59-60] This approach provides an easy access to a wide variety of antigens for studies on xenotransplantation and also for other pharmaceutical research.[61]
The α-(1→3) Galactosyltransferase transfers a galactose unit from the activated donor UDP-galactose (UDP-Gal) onto the 3-OH group of a terminal β-linked galactose forming an α-linkage.
Several studies of α-(1→3) Galactosyltransferase substrate specificity have been carried out which showed a high acceptor promiscuity of the enzyme *in vitro*.[59,60,62,63] Acceptors, that were successfully used include lactose, β-lactosyl azide, β-thiophenyl lactoside, N-acetyllactosamine derivatives and lactosamine.[60] A wide range of N-acyl-derivatives of type II disaccharides are galactosylated by the enzyme. The natural occurring N-acetyl group can be replaced by carbamate, different protected amino acid residues and lipophilic as well as hydrophilic bulky aromatic residues.[59] α-(1→3) Galactosyltransferase is reported to be cabable of galactosyl transfer to an unnatural hindered tertiary hydroxyl of the acceptor sugar.[64] Such acetal formation reactions to a highly deactivated hydroxyl group are virtually impossible for the organic chemist.

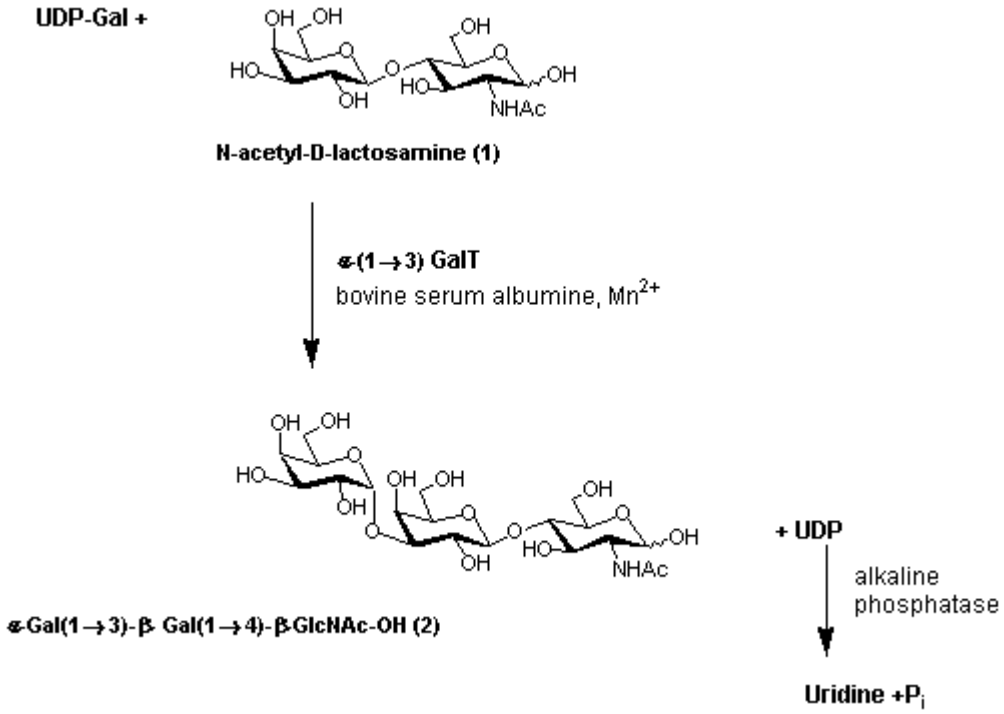
Table 1. Acceptors and products of α(1→3)GalT catalyzed transfer of galactose.

Acceptor Substrate	→	Product	Ref.
	→		[60]
β-Gal(1→4)-β-Glc-N_3	→	α-Gal(1→3)-β-Gal(1→4)-β-Glc-N_3	[60]
β-Gal(1→4)-β-Glc-S-C_6H_5	→	α-Gal(1→3)-β-Gal(1→4)-β-Glc-S-C_6H_5	[60]
Acceptor Substrate	**→**	**Product**	**Ref.**
	→		[60]
β-Gal(1→4)-β-GlcNAc-OAll	→		[60]
β-Gal(1→4)-β-GlcN-OH	→	α-Gal(1→3)-β-Gal(1→4)-β-GlcN-OH	[60]
β-Gal(1→4)-β-GlcNAc(1→3)-β-Gal(1→4)-β-Glc-N_3	→		[60]
β-Gal(1→4)-β-GlcNAc-$O(CH_2)_8COOCH_3$	→	α-Gal(1→3)-β-Gal(1→4)-β-GlcNAc-$O(CH_2)_8COOCH_3$	[59]

	→		[59]
	→		[64]

1.3. Practical Notes

Procedure 1:



Scheme 4. Enzymatic galactosidation of LacNAc.

For the galactosylation of *N*-acetyl-D-lactosamine (**1**) to yield the trisaccharide α-Gal(1→3)-β-Gal(1→4)-β-GlcNAc-OH (**2**), the following procedure, which was calculated for one aliquot of α(1→3)GalT (**77038**, , ~0.3 U/aliquot), may serve as a representative experimental protocol:
388 mg Trizma® hydrochloride buffer pH 7.0 (**93368**) and 198 mg $MnCl_2 \cdot 4H_2O$ (**63536**) are dissolved in 50 ml in MilliQ water to prepare the reaction buffer pH 7.0.
To 3.0 ml of the reaction buffer is added 11.5 mg LacNAc (No **01240**, preferably 10 mM or higher. *This concentration corresponds to the K_M of the acceptor. Maximum velocity is achieved at acceptor concentrations 3 times higher than K_M*), 22 mg UDP-galactose (**40396,** at least 1.2 equiv.), 3 mg bovine serum albumine (No **05470**), 3 µl alkaline phosphatase (**79385**,10 U/ml) and 0.3 U of α(1→3)GalT (**77038,** 0.5 ml). The reaction mixture is incubated at 37°C and the conversion is monitored regularly by TLC[65] (Figure 1). The non UV-active sugars are visualized using either anisaldehyde (No **10440**) spray reagent[66] or 2´,7´-dichlorofluorescein (No **35848**) dipping solution.[67]

Following this protocol, enzymatic conversion should almost be completed after 4 hours. However, poorer acceptors may require much longer reaction times and/or an excess of α(1→3)GalT.

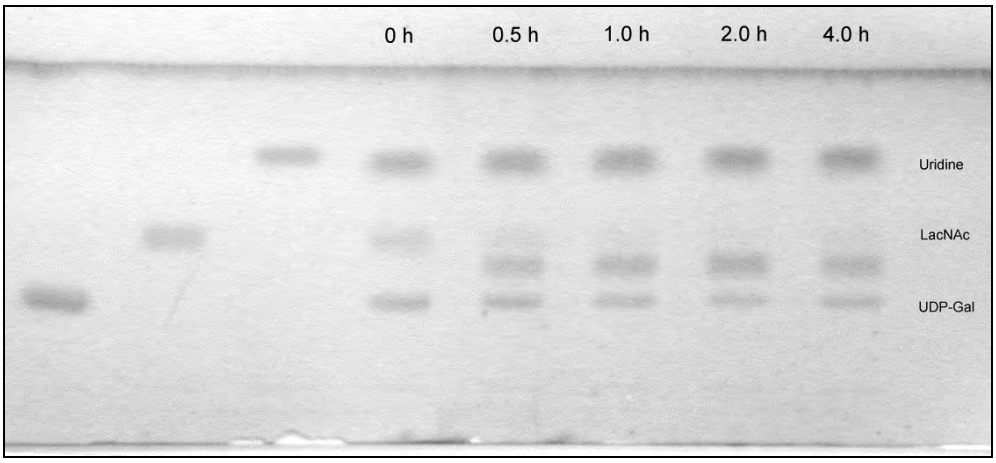


Figure 1. Monitoring of enzymatic galactosidation of LacNAc by TLC.

Procedure 2:
Reinhold Öhrlein and his coworkers proposed the following protocol for enzymatic galactosydation of several different non-natural N-acyl-disaccharides:[59]
14.6 μmol N-acyl-disaccharide acceptor substrate, 20.9 μmol (12.8 mg) UDP-galactose and 1.7 mg bovine serum albumine, are added to a mixture of 1150 μl bidistilled water, 200 μl DMSO and 400 μl sodium cacodylate buffer solution (50 mM, pH 6.52) containing 93.4 μmol (18.5 mg) $MnCl_2 \cdot 6H_2O$. The mixture is briefly vortexed and then incubated at 37°C with 375 mU α(1→3)GalT and 45 U alkaline phosphatase for 48 h.
Finally, the products are isolated chromatographically from the turbid mixtures. Yields range from 40-70 %.

1.4. References

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[65] TLC, on precoated Silica Gel plates with fluorescent indicator 254 nm (No **99573**, **09916**).
Eluent: n-butanol/AcOH/H_2O (2:1:1).
[66] Determination of carbohydrates with anisaldehyde spraying reagent: A mixture of 5 ml H_2SO_4 and 1 ml glacial acetic acid in 100 ml ethanol is cooled down and 3 ml anisaldehyde (No **10440**) is added. The spray reagent can be stored at 4°C for several weeks. The developed TLC plate is dried, sprayed with anisaldehyde reagent and

heated up slowly on a hot-plate or applying a flameless heat gun until the coloured spots are well visible (please note, that the background will turn magenta).

[67] Determination of carbohydrates with 2´,7´-dichlorofluorescein dipping solution: Solution A: Saturated solution of lead tetraacetate (No **15370**, ~2%) in glacial acetic acid. Solution B: 2´,7´-dichlorofluorescein (No **35848**, 0.2-1%)) in ethanol. Prior to usage 5 ml solution A are mixed with 5 ml solution B in and filled up to 200 ml with toluene. The TLC plates are dipped 10 sec in the freshly prepared solution and dried carefully afterwards. Spots are visible under UV at λ = 366 nm.

1.5. List of Abbrevations

Ac	acetyl
All	allyl
***t*Bu**	tert.-butyl
CMP	cytidine monophosphate
Fuc	fucose
Gal	galactose
GalNAc	N-acetyl galactosamine
GalT	galactosyltransferase
GDP	guanosine diphosphate
Glc	glucose
GlcNAc	N-acetyl glucosamine
GlcUA	glucuronic acid
Man	mannose
N	nucleoside
NDP	nucleoside diphosphates
NeuAc	N-acetyl neuraminic acid
NTP	nucleoside triphosphates
Oct	octyl
P_i	phosphate
UDP	uridine diphosphate

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