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Product Information

59505 β(1→4)Galactosyltransferase Kit

Cat.-No.	Name	Amount
48279	**β(1→4) Galactosyltransferase from bovine milk** ~ 1 U/mg[1] , E.C. 2.4.1.22	**5 x 1 mg**
40396	UDP-Galactose UDP-Gal; Uridine 5'-diphospho-α-D-galactose disodium salt *BioChemika*, ≥90% (HPLC)	**100 mg**
63536	**Manganese(II) chloride tetrahydrate** puriss. p.a., ACS, ≥99.0% (KT)	**500 mg**
93371	**Trizma®[2] hydrochloride** *BioChemika*, pH 7.4	**1 g**
61289	**α-Lactalbumin from bovine milk** *BioChemika*, calcium depleted, ≥90% (HPCE)	**25 mg**
79385	**Phosphatase alkaline from bovine intestinal mucosa** *BioChemika*, solution (clear), >10000 U/ml[3], E.C. 3.1.3.1	**200 µl**

1) 1 U corresponds to the amount of enzyme, which transfers 1 µmol of galactose from UDP-galactose to D-glucose per minute at pH 8.4 and 30°C in the presence of α-lactalbumin.
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]

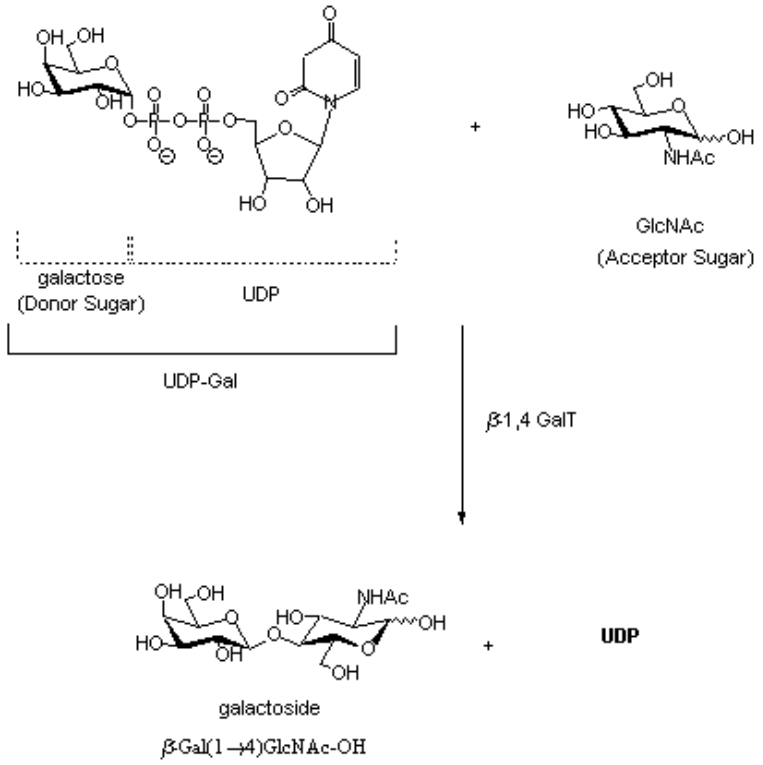
The presence of multiple functional groups and stereocenters in carbohydrates makes them challenging targets for the organic chemist. Decades of synthetic research have not yielded robust, automated protocols comparable to those developed for the preparation of peptides and oligonucleotides. Major issues 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}.

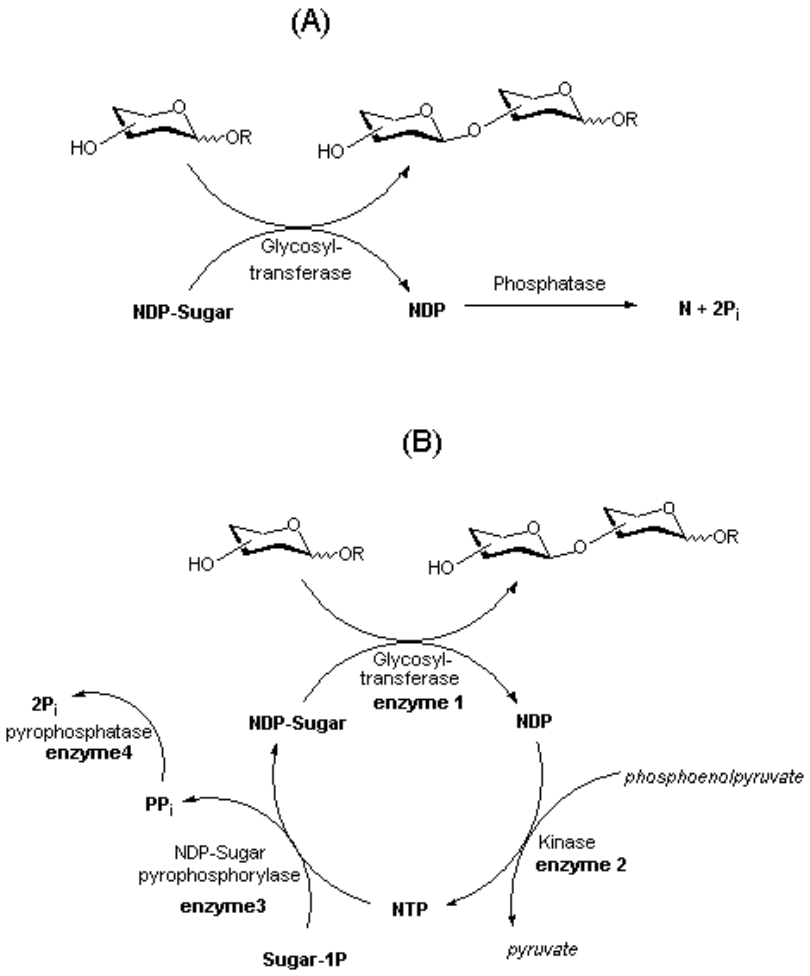
Glycosyltransferases are specific for the type of linkage (α or β), and the linkage position of the glycoside bond formed {*e.g.* α(1→3) or β(1→4)}. They had also been considered to be specific for a given glycosyl donor and acceptor, which led to the "one enzyme–one linkage" concept.[28,29] A number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

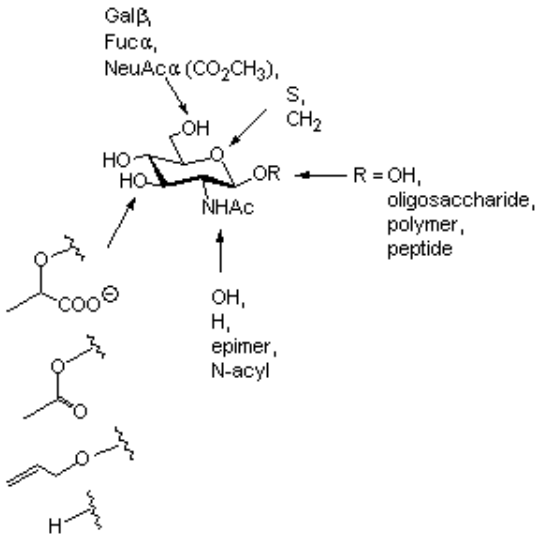
To learn more about our comprehensive portfolio of enzymes please have a look at the SIGMA-ALDRICH Enzyme Explorer – your research assistant on www.sigma-aldrich.com/enzymexplorer

1.2. β(1→4) Galactosyltransferase

β(1→4) Galactosyltransferase from bovine milk (β(1→4) GalT, EC 2.4.1.22) is one of the most extensively studied mammalian glycosyltransferases with regard to synthesis and substrate specificity.[2-6,45-51] β(1→4) GalT catalyses the transfer of galactose from UDP-galactose (UDP-Gal) to the OH at the 4-position of N-acetyl glucosamine (GlcNAc) and also β-linked GlcNAc subunits to yield β-lactosamine (β-LacNAc) and β-Gal(1→4)-β-GlcNAc structures.[52] When the enzyme forms a heterodimeric complex with α-lactalbumin, the specificity is altered and D-glucose becomes the preferred acceptor. Thus, addition of α-lactalbumin promotes the formation of lactose (β-Gal(1→4)-Glc-OH). Both α- and β-glycosides of glucose were utilized as acceptors in enzymatic galactosidation as well. The α-glucosides required the presence of α-lactalbumin.[5] Numerous other acceptor substrates for β(1→4)GalT catalyzed transfer of galactose have been described in the literature (see Table 1), *e.g.* 2-deoxyglucose, D-xylose, 5-thioglucose, N-acetylmuramic acid, and myoinositol. Moreover, 6-*O*-fucosylated and sialylated modifications served as acceptors[53] as well as 3-*O*-methyl-GlcNAc,[38,54] 3-deoxy-GlcNAc, 3-*O*-allyl-GlcNAcβOBu and 3-oxo-GlcNAc.[55] Several modifications of GlcNAc that were employed as acceptor substrates are illustrated in Scheme 3.[2]

β(1→4)GalT has been employed in solid-phase oligosaccharide synthesis on polymer supports like polyacrylamide or water-soluble poly(vinyl alcohol). The resulting galactosylated oligosaccharides are cleaved from the polymers photochemically or by using chymotrypsin.[56,57]

N-Acetylglucosaminyl amino acids and peptides were successfully galactosylated to afford glycopeptides with a disaccharide moiety.[58-60] Further extension of the carbohydrate chain was accomplished afterwards by employing α(2→6)Sialyltransferase.[58-60]



Scheme 3. Modifications of GlcNAc employed as acceptors in β(1→4)GalT catalyzed transfer of galactose.

The preparation of an asparagine-bound trisaccharide was accomplished by combined chemo-enzymatic synthesis.[58] Galactosidation of a *N*-acetylglucosaminyl oligopeptide followed by sialylation with α(2→3)Sialyltransferase and fucosylation with α(2→3)Fucosyltransferase yielded a glycopeptide containing a tetrasaccharide moiety.[61]

As different glycosides of *N*-acetylglucosamine and glucose can be used as acceptors in β(1→4)GalT catalyzed galactosidations, this enzymatic method was recently exploited in the modification of pharmacologically interesting glycosides.[15,16,62,63]

Several currently published syntheses of new drug-sugar conjugates derived from the broad range of naturally occurring glycosides accentuated the high potential of glycosylations in drug delivery, for example by increasing the solubility and bioavailability of large hydrophobic molecules under mild conditions. β(1→4)GalT catalyzed galactosidations of glycosides was successfully accomplished for elymoclavine-17-*O*-β-D-glucopyranoside,[15] stevioside and steviolbioside,[64] colchicoside,[65] coumarinic glycoside fraxin,[65] and different ginsenosides.[66,67]

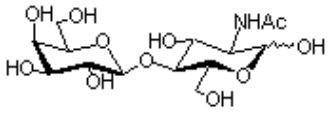
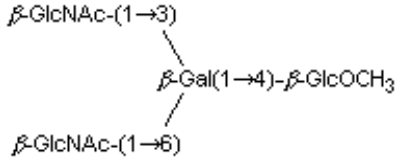
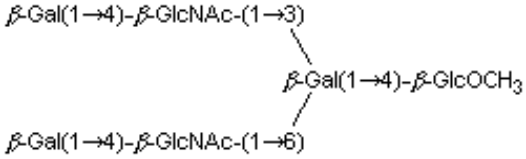
Transfer of galactose onto cyclodextrin was performed, because the recognition of the Gal-cyclodextrin conjugates by galectins was expected to enhance the drug delivery capabilities of the system.[68]

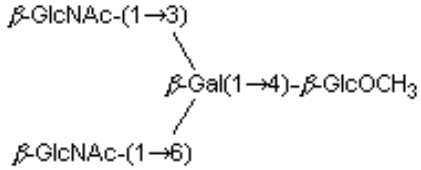
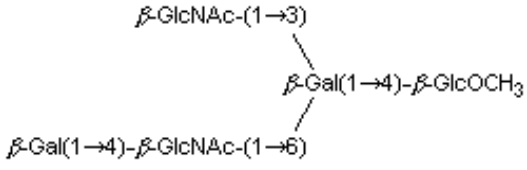
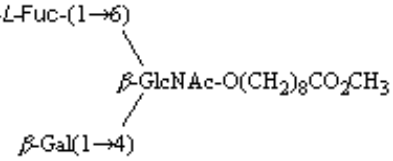
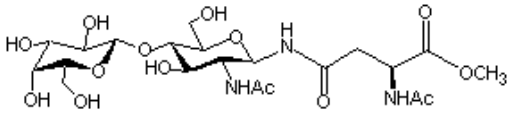
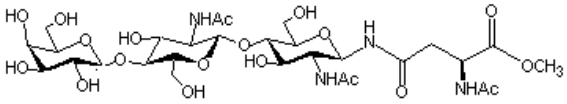
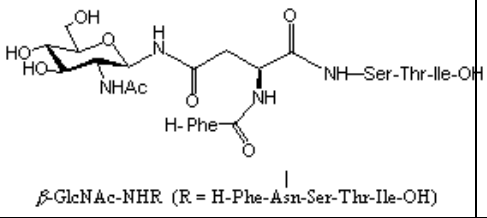
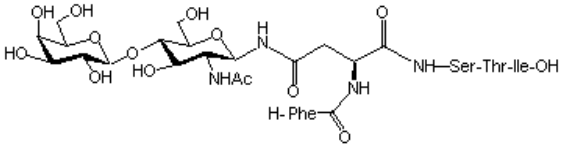
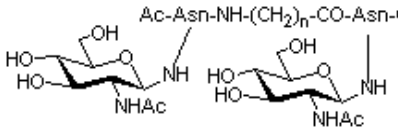
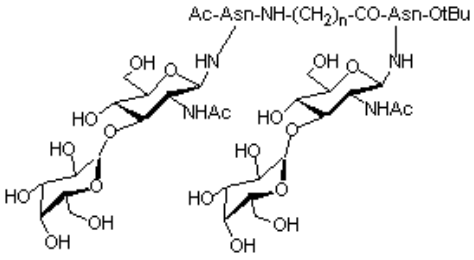
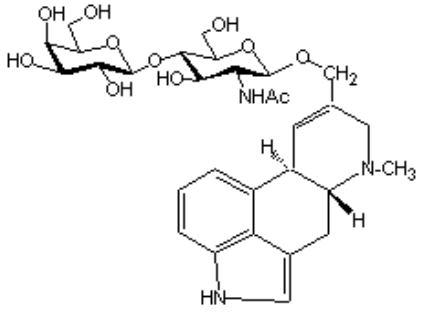
Employing C-glycoside analogues of the naturally occurring glycopeptide linkages (*N*-acetylglucosamine β-linked to either asparagine or serine) the corresponding C-lactosides were isolated in excellent yields.[69]

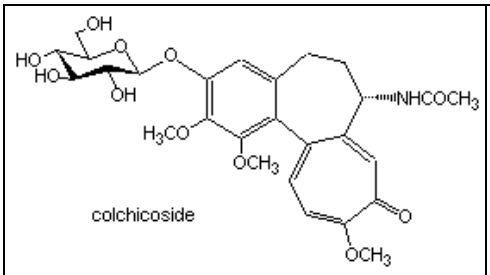
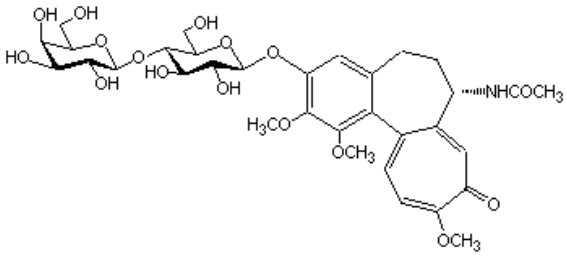
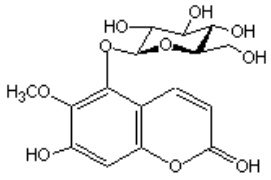
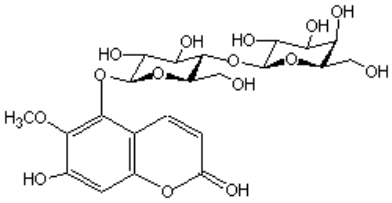
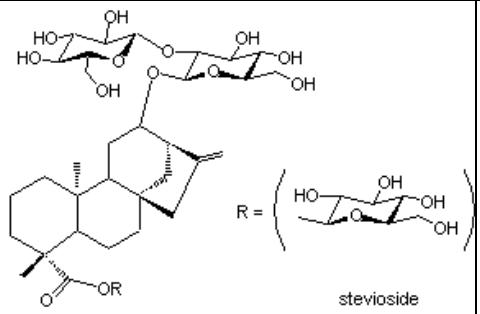
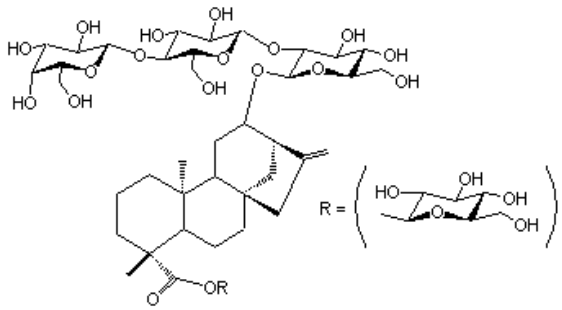
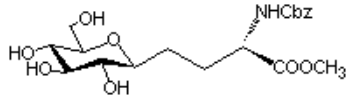
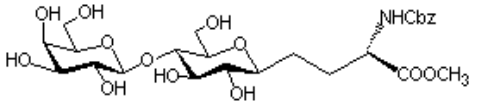
Neither D-mannose, D-allose, D-galactose, nor D-ribose is a substrate.[4,5] Monosaccharides displaying a negative charge, such as glucuronic acid and α-glucose-1-phosphate are also not accepted as substrates. Azasugars and glucals are considered to be very weak acceptors.[54]

With regard to the nucleotide sugar donors, several modified substrates were utilized, but the rate of enzyme-catalyzed transfer turned out to be rather slow.[4,5]

Table 1. Acceptors and products of β(1→4)GalT catalyzed transfer of galactose.

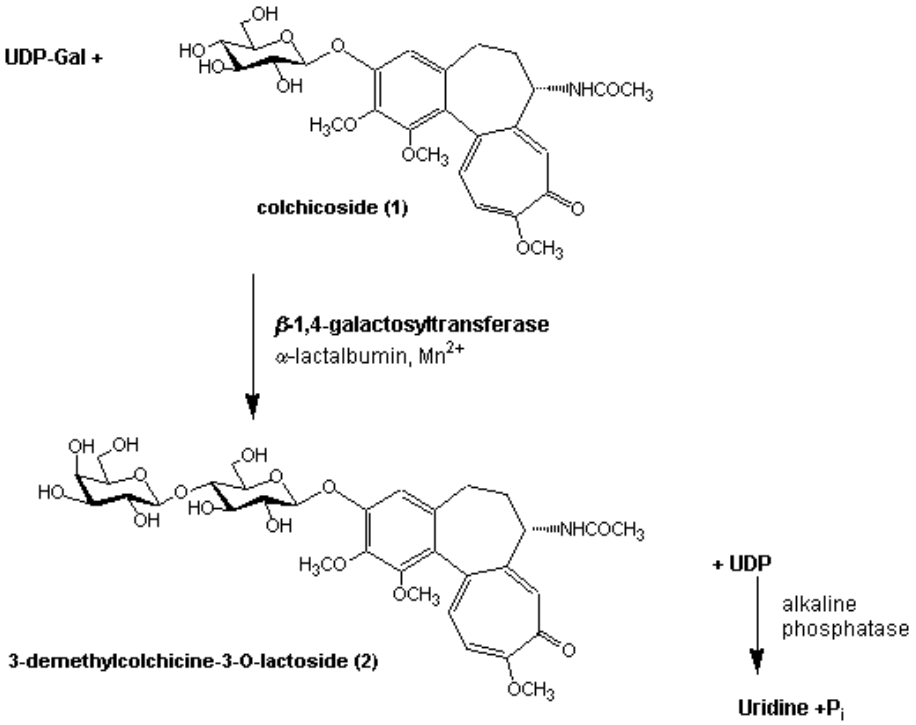
Acceptor Substrate	→	Product	Ref.
GlcNAc-OH	→	 β-Gal(1→4)GlcNAc-OH	[46,47]
Glc-OH	→	β-Gal(1→4)-Glc-OH	[46,47]
β-GlcNAc-hexanolamine	→	β-Gal(1→4)- β-GlcNAc- hexanolamine	[47]
β-GlcNAc-hexanolamine-agarose	→	β-Gal(1→4)- β-GlcNAc- hexanolamine-agarose	[47]
β-GlcNAc(1→4)-Gal-OH	→	β-Gal(1→4)-β-GlcNAc(1→4)-Gal-OH	[46]
β-GlcNAc(1→4)-GlcNAc-OH	→	β-Gal(1→4)-β-GlcNAc(1→4)-GlcNAc-OH	[47,58]
β-GlcNAc(1→6)-Gal-OH	→	β-Gal(1→4)-β-GlcNAc(1→6)-Gal-OH	[38,54]
β-GlcNAc(1→3)-Gal-OH	→	β-Gal(1→4)-β-GlcNAc(1→3)-Gal-OH	[38,54]
β-Glc-$OCH_2C_6H_4(NO_2)$-CONH-polymer	→	β-Gal(1→4)-β-Glc-$OCH_2C_6H_4(NO_2)$-CONH-polymer	[56,57]
β-Glc(1→4)-β-Glc-$OCH_2C_6H_4(NO_2)$-CONH-polymer	→	β-Gal(1→4)-β-Glc(1→4)-β-Glc-$OCH_2C_6H_4(NO_2)$-CONH-polymer	[56,57]
β-Glc(1→4)-β-Glc-OCH_2-NH-*L*-Phe-CO-polymer	→	β-Gal(1→4)-β-Glc(1→4)-β-Glc-OCH_2-NH-*L*-Phe-CO-polymer	[56,57]
 β-GlcNAc-(1→3) β-Gal(1→4)-β-GlcOCH$_3$ β-GlcNAc-(1→6)	→	 β-Gal(1→4)-β-GlcNAc-(1→3) β-Gal(1→4)-β-GlcOCH$_3$ β-Gal(1→4)-β-GlcNAc-(1→6)	[54,60]

	→		[54,60]
α-*L*-Fuc-(1→6)-β-GlcNAc-O$(CH_2)_8CO_2CH_3$	→		[53]
β-GlcNAc-R ; R = Ac-Asn-OMe	→		[58]
β-GlcNAc(1→4)-β-GlcNAc-R ; R = Ac-Asn-OMe	→		[58]
	→		[59]
β-GlcNAc-R ; R = Gly-Gly-Asn-Gly-Gly	→	β-Gal(1→4)-β-GlcNAc-R ; R = Gly-Gly-Asn-Gly-Gly	[59]
	→		[61]
elymoclavine 17-O-(2-acetamido-2-deoxy-β-D-glucopyranoside)	→		[15]

 colchicoside	→		[65]
 fraxin	→		[65]
 stevioside	→		[64]
	→		[69]

1.3. Practical Notes

Procedure 1:



Scheme 4. Enzymatic galactosidation of colchicoside (**1**)

The galactosylation of the alkaloide colchicoside **1** to yield 3-demethylcolchicine-3-*O*-lactoside **2** is highly instructive for carbohydrate chain elongations of glycosides bearing a hydrophobic aglycone and may serve as a representative experimental procedure. Both substrate and product are easily detected by UV at λ = 254 nm and the enzymatic conversion can be conveniently monitored by TLC and HPLC.

A solution of 50 mM Trizma® hydrochloride buffer pH 7.4 (**93371**) containing colchicoside (**1**, 22 mg/ml, 40 mM), UDP-galactose (**40396**, 29 mg/ml, 50 mM), $MnCl_2 \cdot 4H_2O$ (**63536**, 400 μg/ml ,2 mM), α-lactalbumin (**61289**, 1 mg/ml) and alkaline phosphatase (**79385**, 1 μl/ml, 10 U/ml) was incubated at 30°C with 0.5 U/ml (reaction 1) or 1 U/ml (reaction 2) β(1→4)GalT (**48279**).

The pH was adjusted by adding 0.25 M NaOH and both reactions were controlled by TLC (Figure 1) and HPLC (Figure 1).

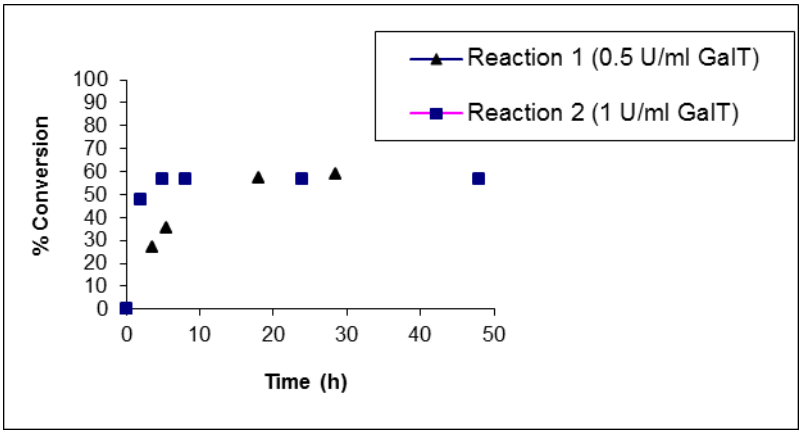


Figure 1. Conversion (%) of colchicoside (**1**) controlled by HPLC.[71]

The final conversion rate (~58%) was reached after 5 h using 1 U/ml β(1→4)GalT (reaction 2). After incubation with 0.5 U/ml β(1→4)GalT (reaction 1) the final conversion rate was reached after 18 h. The product (**2**) was isolated by flash chromatography and characterized as the 3-O-lactosyl derivative of colchicine.[65,72]

Procedure 2:

For the galactosylation of *N*-acetyl-D-glucosamine to yield *N*-acetyl-lactosamine, the following small scale procedure may serve as a representative experimental protocol:
379 mg Trizma® hydrochloride buffer pH 7.4 (**93371**) and 198 mg $MnCl_2 \cdot 4H_2O$ (**63536**) are dissolved in 50 ml in MilliQ water to prepare the reaction buffer pH 7.4.
To 1.0 ml of the reaction buffer is added 2.2 mg GlcNAc (No **01140**, 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*), 7.3 mg UDP-galactose (**40396,** at least 1.2 equiv.), 1 mg bovine serum albumine (No **05470**), 1 µl alkaline phosphatase (**79385**,10 U/ml) and 50 mU of β(1→4)GalT (**48279**). The reaction mixture is incubated at 37°C and the conversion is monitored regularly by TLC.[73] The non UV-active sugars are visualized using either anisaldehyde (No **10440**) spray reagent[74] or 2´,7´-dichlorofluorescein (No **35848**) dipping solution.[75]
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 β(1→4)GalT.

Procedure 3:

For the galactosylation of D-glucose in the presence of α-lactalbumin yielding lactose (lactose-synthase reaction), the following small scale procedure may serve as a representative protocol:
To 1.0 ml of reaction buffer as described in **Procedure 2** is added 1.8 mg D-Glc-OH (No **49139**, preferably 10 mM or higher, *see comments above*), 7.3 mg UDP-galactose (**40396**, at least 1.2 equiv.), 0.2 mg α-lactalbumin (**61289**), 1 µl alkaline phosphatase (**79385**,10 U/ml) and 50 mU of β(1→4)GalT (**48279**). The reaction mixture is incubated at 37°C and the conversion is monitored regularly by TLC.
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 β(1→4)GalT.

Procedure 4:

Reinhold Öhrlein and his coworkers proposed the following protocol for enzymatic galactosydation of a glucosamide acceptor:[52]

32.9 μmol acceptor substrate, 37.3 μmol (23.2 mg) UDP-galactose, 2 mg bovine serum albumine (No **05470**), 52.5 μmol (12.3 mg) $MnCl_2 \cdot 6H_2O$ were put together into 1.8 ml sodium cacodylate buffer solution (pH 7.52) containing 150 μl DMSO and sonicated to give a milky but homogeneous mixture. After addition of 625 mU β(1→4)GalT (250μl from a 2.5 U/ml stock solution in cacodylate buffer as above) and 30 U alkaline phosphatase the mixture was briefly vortexed and stirred at 37°C. Consumption of the acceptor substrate was controlled by TLC. The precipitates were filtered off and the product was isolated from the supernatant in 81% yield.

⇒ The effect of organic cosolvents:[65]

Galactosylation of glycosides bearing a hydrophobic aglycone may suffer from poor solubility of the acceptor substrate. Recent systematic investigations of the stability of β(1→4)GalT in different aqueous reaction mixtures and the effect of organic cosolvents are very instructive for choosing an appropriate solvent mixture (Table 2).[65] Solvents like dimethyl sulfoxide, acetone, dioxane and ethanol seemed to be beneficial, stabilizing this enzyme, while other solvents such as *N,N*-dimethylformamide, acetonitrile and tetrahydrofuran enhanced the inactivation process.

Data obtained from these experiments are also indicating, that portionwise addition of β(1→4)GalT may be advantageous to drive the reaction to complete conversion.

Table 2. Degrees of conversion of colchicoside into its corresponding lactoside in the presence of various amounts of organic cosolvents

Cosolvent	5% (v:v)	10% (v:v)	15% (v:v)
blank	71	71	71
dimethylsulfoxide	72	74	75
methanol	77	80	84
ethanol	80	85	86
acetone	76	76	67
dioxane	72	67	25
acetonitrile	69	66	55
N,N-dimethylformamide	64	55	39
tetrahydrofurane	55	1	0

1.4. References

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[70] TLC, on precoated Silica Gel plates with fluorescent indicator 254 nm (No **99573**, **09916**).
Eluent: AcOEt/MeOH/H_2O (8:4:1).
[71] RP-HPLC, Column: 100 RP-18 (5 μm); Eluent: H_2O (**95304**)/acetonitrile (**00687**)/trifluoroacetic acid (**91703**) {92:8:0.05}; Flow rate: 1 ml/min; Detection: UV at λ = 254 nm.
[72] (S)-N-{3-[4-*O*-(*β*-D-galactopyranosyl)-*β*-D-glucopyranosyl]-5,6,7,9-tetrahydro-1,2,10-trimethoxy-9-oxo-benzo[a]heptalen-7-yl}-acetamide (3-demethylcolchicine-3-*O*-lactoside, (**2**); mp 220°C; $[\alpha]_D$ = - 104.6 (c = 0.5, in water); ^{1}H-NMR (Me_2SO-d_6 80°C): δ 8.35 (d, 1H, $J_{NH,7}$ 7.5 Hz, -NHCO), 7.18 (s, 1H, H-8), 7.12 (d, 1 H, $J_{11,12}$ 9.5 Hz, H-12), 7.01 (d, 1 H, H-11), 6.88 (s, H1, H-4), 5.01 (d, 1H, $J_{1',2'}$ 7.5 Hz, H-1'), 4.39 (dt, 1H, $J_{6ax,7}$ 7 Hz, $J_{6eq,7}$ = $J_{NH,7}$ 7.5 Hz, H-7), 4.33 (d, 1H, $J_{1,2}$, H-1"), 3.90, 3.88, 360 (1, 3H each, CH_3O), 1.88 () s, 3H, CH_3CO); ^{13}C-NMR (Me_2SO-d_6): δ 178.4 (C-9), 169.2 (NHCO), 163.9 (C-10), 151.2, 151,0 and 150.7 (C-7a, C-3, and C-1), 141.5 (C-2), 135.5 (C-12a), 135.0 (C-12), 134.3 (C-4a), 130.5 (C-8), 127.0 (C-1a), 112.6 (C-11), 111.3 (C-4), 104.0 (C-1"), 100.3 (C-1'), 80.3 (C-4'), 75.7 (C-5"), 75.4 and 75.2 (C-5' and C-3'), 73.3 (C-2'), 73.2 (C-3"), 70.8 (C-2"), 68.3 (C-4"), 61.3 (CH_3O), 61.2 (CH_3O), 60.6 (C-6"), 60.3 (C-6'), 56.4 (CH_3O), 51.6 (C-7), 35.8 (C-5), 29.4 (C-6), 22.6 (CH_3).
[73] TLC, on precoated Silica Gel plates with fluorescent indicator 254 nm (No **99573**, **09916**).
Eluent: n-butanol/AcOH/H_2O (3:1:1).
[74] 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).
[75] 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
Asn	asparagine
***t*Bu**	tert.-butyl
Cbz	carbobenzoxy
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
Gly	glycine
LacNAc	lactosamine
Man	mannose
Me	methyl
N	nucleoside
NDP	nucleoside diphosphates
NeuAc	N-acetyl neuraminic acid
NTP	nucleoside triphosphates
P_i	phosphate
Phe	phenylalanine
UDP	uridine diphosphate

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Precautions and Disclaimer:
This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.