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Formation of LacNAc mimetics employing novel donor substrates for enzymatic β1→4 galactosylation

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In examining C-6 modified 4-nitrophenyl β-D-galacto-pyranosides as donor structures the β-galactosidase (*Bacillus circulans*) revealed an unexpectedly broad substrate specificity which allowed successful syntheses of various disaccharide components.

The important role of carbohydrates in vital biological recognition processes has increasingly stimulated efforts in glycoconjugate research. Due to the complexity of conventional oligosaccharide synthesis, enzymatic methods have experienced growing interest,[1–5] which do not require protection and deprotection steps and therefore provide rapid access to natural oligosaccharides.

Unnatural and modified oligosaccharides are needed as potential drug candidates as well as for the investigation of glycoconjugate structure–function relationships in biochemical processes. Exploitation of enzymes for this purpose exhibiting the usual high regio- and stereospecificity is more difficult. In this case as certain flexibilities concerning their substrates are allowed, modification of the glycosyl residue may be possible before glycosylation. Then as an additional advantage the enzyme-catalysed reaction can be carried out as the last step.

N-Acetyllactosamine is a common structural element in biologically important glycoconjugates, for example as a typical terminal sequence of *N*-linked oligosaccharides, as the core unit of carbohydrates isolated from human milk[6] and as an essential component of ligands of various lectins.[7–9] An appropriate enzyme for its synthesis is the β-galactosidase from *Bacillus circulans*, which regioselectively catalyses the β1⟶4 linkage formation between a galactosyl donor and a 2-acetamido-glycosyl acceptor (Fig. 1). Generally it shows a high trans-glycosylation rate with extraordinary regioselectivity. Product yields range from 18 to 66%, depending on the leaving group of the donor and the utilized acceptor. As donor leaving groups, glucose, *para*-nitrophenol and *ortho*-nitrophenol are used commonly, others were also investigated.[10] The acceptor may have a glucosyl or a galactosyl α/β-configuration with its anomeric center either unprotected or protected. An acetamido group at C-2 is necessary to control the regioselectivity, in other cases, *e.g.* with a hydroxyl group at C-2, a mixture of β 1–3-, β 1–4- and β 1–6- linked regioisomeric disaccharides is obtained.[11]

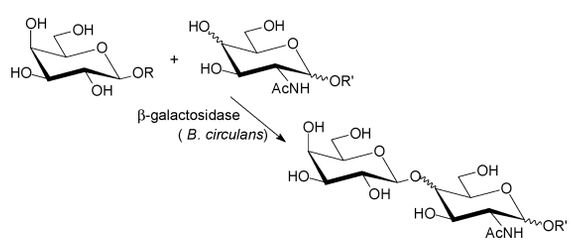
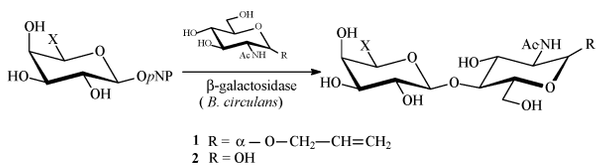


Fig. 1 Glycosylation catalysed by galactosidase from *B. circulans*.

Previously, two modified galactosides were reported as donor substrates that are the C-6-oxidized 4-nitrophenyl β-D-*galacto*-hexodialdo-pyranoside (**9**) and the 4-methylumbelliferyl 6-sulfo-β-D-galactopyranoside. The former could be successfully utilized for disaccharide synthesis, although the resulting disaccharide was not isolated, but further reacted *in situ*[12] and the latter could be converted into the corresponding sulfated disaccharide.[13]

Based on these findings, the primary alcohol function in galactosides does not seem to be crucial for recognition. To verify this assumption further C-6-modified donors were synthesized and tested as substrates for β-galactosidase (*B. circulans*) with allyl 2-acetamido-2-deoxy-α-D-glucopyranoside (**1**) as well as 2-acetamido-2-deoxy-D-glucopyranose (**2**) as acceptor molecules (Fig. 2).



1 R = α – O–CH_2–CH=CH_2
2 R = OH

Fig. 2 General reaction with derivatized galactopyranosides.

Both, the *p*NP β-D-fucopyranoside (**3**) and *p*NP α-L-arabinopyranoside (**4**) proved to be substrates for the β-galactosidase, yielding the expected disaccharides allyl β-D-fucopyranosyl-(1⟶4)-2-acetamido-2-deoxy-α-D-glucopyranoside (**5**) and α-L-arabinopyranosyl-(1⟶4)-2-acetamido-2-deoxy-α-D-glucopyranoside (**6**) in extraordinary high 76% and poorer 13% yield, respectively. The corresponding reaction with 2-acetamido-2-deoxy-α-D-glucopyranose (**2**) gave β-D-fucopyranosyl-(1⟶4)-2-acetamido-2-deoxy-D-glucopyranose (**7**) in 66% yield, but in case of the arabinopyranoside only traces of a newly formed compound **8** were detectable (Table 1).

Reaction of the C-6 oxidized donor 4-nitrophenyl β-D-*galacto*-hexodialdo-pyranoside (**9**) could be confirmed and the disaccharide allyl β-D-*galacto*-hexodialdopyranosyl-(1⟶4)-2-acetamido-2-deoxy-α-D-glucopyranoside (**10**) isolated in 66% yield. Treatment of this donor **9** with **2** gave the disaccharide **11** in 19% yield.

The precursor of the aldehyde **9** is the 6,7-olefin structure **12**,[14] which surprisingly readily could be glycosylated to give the disaccharides allyl 6,7-dideoxy-β-D-*galacto*-hept-6-eno-pyranosyl-(1⟶4)-2-acetamido-2-deoxy-α-D-glucopyranoside (**13**) or 6,7-dideoxy-β-D-*galacto*-hept-6-enopyranosyl-(1⟶4)-2-acetamido-2-deoxy-D-glucopyranose (**14**) in 31% and 20%, respectively.

Reductive amination of aldehyde **9** with the amino acid taurine led to a novel carbohydrate–amino acid conjugate,[15] which however was not accepted by the enzyme.

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Table 1 Galactoside derivatives employed and LacNAc mimetics obtained

No	X	No	Yield (%)	No	Yield (%)
3	CH_3	**5**	76	**7**	66
4	H	**6**	13	**8**	traces
9	CHO	**10**	66	**11**	19
12	$CH{=}CH_2$	**13**	31	**14**	20
15	CH_2NH_2	**16**	~10[a]	—	—

[a] After acetylation.

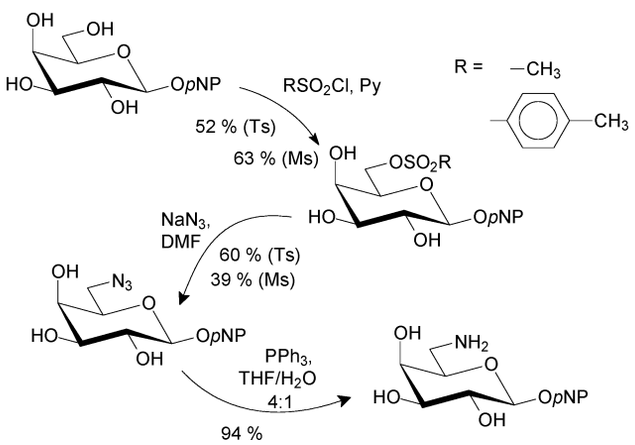

Fig. 3 Synthesis of an amine functionalized galactosyl donor.

To check the steric requirements, 4-nitrophenyl 6-*O*-allyl-β-D-galactopyranoside, which represents a kind of chain-elongated analogue of the previously recognized 6,7-olefin **12**, was prepared. However, this donor also was not recognized. Amine **15** was synthesized *via* 4-nitrophenyl 6-*O*-sulfonyl-β-D-galactopyranoside and 4-nitrophenyl 6-azido-6-deoxy-β-D-galactopyranoside (Fig. 3). Neither the 6-*O*-mesylate nor the 6-azido-6-deoxy compounds were accepted by the enzyme. However, to our surprise and pleasure the reaction of amine **15** with acceptor **1** could be achieved and resulted in the formation of allyl 6-amino-6-deoxy-β-D-galactopyranosyl-(1⟶4)-2-acetamido-2-deoxy-α-D-glucopyranoside (**16**).

To summarize the results, the synthesis of various LacNAc mimetics could be achieved employing the β-galactosidase from *Bacillus circulans*. Apparently, a certain flexibility in the enzyme's substrate specificity can be assigned, which proves that the primary alcohol function of the donor is not crucial for recognition by this β-galactosidase. Complete lack of a C-6 group diminishes the reaction yield and substituents at C-6 should not be too bulky. Again, charged functionalities do not present a hindrance for the enzyme catalyzed formation of disaccharides.†

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Notes and references

† Enzyme catalysed transgalactosylations to disaccharides **5**, **6**, **10**, **13** and **16** were performed according to the procedure described by Farkas and Thiem;[16] in the case of compound **10** the reaction was performed in phosphate buffer without acetonitrile as cosolvent.

Enzyme catalysed transgalactosylations with 2-acetamido-2-deoxy-D-glucopyranose (**2**) as acceptor were performed as follows: 150 μmol glycopyranosyl donor and 300 mg (9 equivalents) of acceptor **2** were dissolved in 4 ml of 50 mM acetate buffer pH 5.0 and incubated with 4 U β-galactosidase (*B. circulans*) for 3 h at 55 °C. The reaction was stopped by heating the solution to 100 °C. The crude reaction mixture was lyophilized. The product was separated from remaining educts and by-products chromatographically on a biogel P2 column with water as eluent.

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