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Some findings in transgalactosylations employing modified donor substrates

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ABSTRACT

The scope of transgalactosylation with β-galactosidase (bovine testis) was studied employing a series of modified donor substrates based on *p*-nitrophenyl β-D-galactopyranoside and as uniform acceptor allyl 2-*N*-acetamido-2-deoxy-α-D-galactopyranoside. Structurally diverse donor molecules were recognized by the enzyme and led to novel disaccharide components, yet an excessive structural distortion was not accepted.

1. Introduction

The Thomsen–Friedenreich antigen (T-antigen) Galβ1-3GalNAc represents the central component of the core A structure in O-glycoproteins. It is present in the preliminary stages of complex mucin oligosaccharides and occurs as such but also sialylated on cancer cell surfaces. Thus, oligosaccharides carrying this motif are of particular interest in cancer research and are considered as potential leads for the development of vaccines or anti-metastatically active cancer drugs.[1] Previously other approaches toward such disaccharides[2] as well as sialylated components[3] could be elaborated. With the intention to introduce markers into such systems, it was of special interest to arrive at components modified in the non-reducing ring. Therefore, this study was initiated to understand the limits given by the enzyme system β-galactosidase (bovine testis) in transgalactosylations[4–6] with slightly as well as considerably altered donor substrate structures.

2. Results and discussion

It was of interest to study the specificity of β-galactosidase (EC 3.2.1.23) from bovine testis[7] employing a number of donor substrates and allyl 2-acetamido-2-deoxy-α-D-galactopyranoside (**7**)[8,9] as reference acceptor substrates. Instead of previously reported laborious enzyme purifications[10,11] an approach described to give sufficiently clean β-galactosidase useful for preparative approaches was followed[4] and the material assayed as reported.[12]

Under standard conditions *p*-nitrophenyl β-D-galactopyranoside (**1**)[13] gave the β,1-3 glycosylated disaccharide Galβ1-3-GalNAcα1OAll (**8**) regio- and stereoselectively in 67% yield. Then modifications in the donor substrate were tested with the same acceptor **7**. First, the corresponding D-fucose donor with the 6-position deoxygenated **2**[14] was employed to give the β,1-3 fucosylated derivative **9** in 59% yield. Further, the complete 6 position was omitted and *p*-nitrophenyl α-L-arabinopyranoside **3**[15] was employed similarly to give the disaccharide **10** in 51% yield. In all cases the regioselectivity at the acceptor side was maintained, and so was the β-stereospecificity at the reducing end for the interglycosidic linkage. Merely the slightly decreasing yields give some indication for a less-optimal binding of the substrates within the active center of the enzyme. Apparently the missing primary hydroxyl group is involved in a stabilizing hydrogen bridging network but its absence is only of minor effect concerning the transgalactosylation.

Previously, employing the elongated donor substrate **4** an enzyme raw extract from barley containing β-amylase, an acetone precipitation from a raw extract of snails,[16] and also the β-galactosidase of *Bacillus circulans*[17] were shown to effect transgalactosylations. In the present case enzymatic transfer occurred again regio- and stereoselectively to give the β,1-3 disaccharide derivative **11** in amazing 40% yield. This remarkable T-antigen derivative shows two terminal double bonds which allow for interesting further modifications.

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Finally, the C-6 modified aldehyde-hydrate derivative **5** as well as the 6-deoxy-6-fluoro derivative **6**, both of which could be reacted enzymatically with other enzymes[16–18] were studied. However, by treatment under the above conditions with β-galactosidase (bovine testis) no transfer to the acceptor **7** but only slow hydrolysis could be observed. It may be considered that **5** with its additional hydroxyl group and enhanced hydrophilic character may provoke the formation of excessive hydrogen bonding or impair the ideal hydrogen bonding network. In contrast, in the 6-deoxy-6-fluoro donor **6** the smaller fluoro function should operate as acceptor but not as donor for hydrogen bridging bonds, and thus in the cited examples could be successfully employed. Yet, with this apparently more delicate enzyme system no transfer occurred (Scheme 1).

Long ago it was observed that β-galactosidase of *Aspergillus oryzae* could be used for the formation of glucopyranoside structures.[19] Thus, it was of interest to check, whether this β-galactosidase could work correspondingly. Indeed, under standard conditions and employment of *p*-nitrophenyl β-D-glucopyranoside (**14**)[20] the uniform acceptor **7** could be transferred to give the β,1-6-glucosylated disaccharide structure obtained as peracetate **15** in reasonable 28% yield. Apparently, the axial 4-hydroxy function in the galacto-structured donors **1**–**4** is required for correct orientation within the binding pocket. Thus, in this case surprisingly the 1-6-linked component was obtained selectively.

Chemoenzymatic reactions of glycals were reported earlier by Lehmann et al. who found hydrations with D-glucal and D-galactal.[21] Later, they could demonstrate transglycosylations of simple alcohols with β-galactosidase from *Escherichia coli* to give alkyl 2-deoxy-β-D-glycopyranosides.[22,23] Beau et al. could even elaborate that approach for the formation of β-linked 2′-deoxy-discaccharides with β-galactosidase of *Aspergillus oryzae*.[24] Here it was attempted to treat D-galactal (**16**)[25] with the acceptor **7** in the presence of β-galactosidase (bovine testis), and indeed the 2′-deoxy-T-antigen derivative **17** could be obtained readily. The yield of 9% should be considered positively in comparison to a multistep chemical approach for obtaining this complex structure.

Finally, in addition to the endocyclic glycal **16** it was of interest to check an exocyclic derivative. By facile regioselective monotosylation of **1**[13] the 6-*O*-tosylate **18** was obtained in 59% yield, and further pivaloylation led to compound **19** in 72% yield. Previously eliminations of primary tosylates with silver fluoride were reported,[26] and here advantageously tetra-*n*-butylammonium difluorotriphenylsilicate (TBAT)[27] could be employed in acetonitrile to give compound **20** in 75% yield. Cleavage of the pivaloates was with ammonia in methanol to lead to the desired exocyclic derivative **21** in 78% yield as a solid material, which by nmr spectra gives evidence of a mobile boat conformation. Unfortunately, incubation of **21** as potential donor substrate with β-galactosidase and the acceptor **7** did not show transfer but only minor hydrolysis. Obviously, the flat boat conformation leads to a complete distortion of all those hydroxyl groups required for an acceptable donor substrate in the reactive site of the enzyme (Scheme 2).

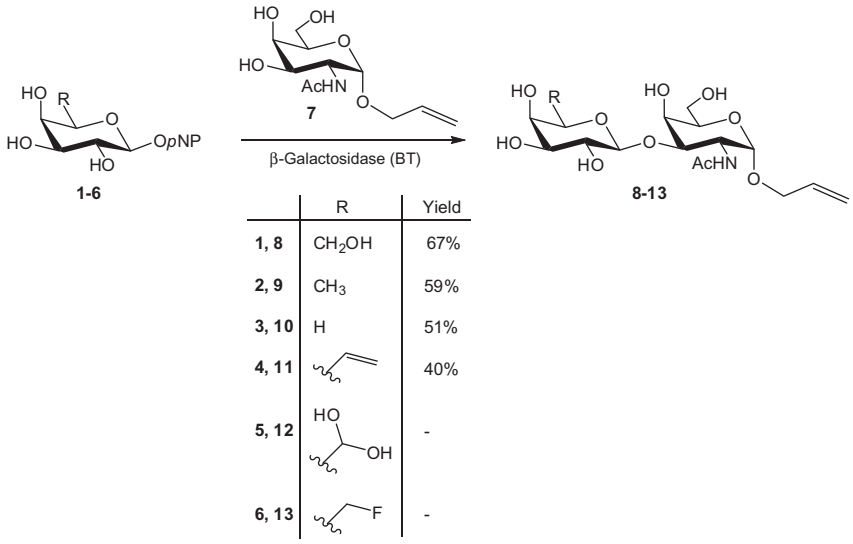
3. Conclusion

In this contribution transgalactosylations employing β-galactosidase (bovine testis) could be studied with a number of unusual donor substrates based on *p*-nitrophenyl β-D-galactopyranoside. Thus, regio- and stereoselective transfers could be achieved onto allyl 2-acetamido-2-deoxy-α-D-galactopyranoside to give modified Galβ1-3GalNAc (T-antigen) components. Structurally rather diverse donor substrates were recognized by the enzyme and led to novel disaccharide components, yet an excessive structural distortion was not accepted.

4. Experimental

4.1. General methods

Commercially available starting materials were used without further purification. Solvents were dried according to standard methods. TLC was performed on precoated aluminum plates (Silica Gel 60 F_{254}, Merck 5554), and charring was with 10% H_2SO_4 in ethanol for visualization. For column chromatography Silica Gel 60, 230–400 mesh, 40–63 μm (Merck) was used. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Avance-300 (75.48 MHz for ^{13}C), AMX-400 (400 MHz for ^{1}H, 100.61 MHz for ^{13}C) or on a Bruker DRX-500 (500 MHz for ^{1}H, 125.77 MHz for ^{13}C) at 300 K. Chemical shifts were calibrated to solvent residual peaks. Signals were assigned by ^{1}H,^{1}H-COSY, TOCSY-,Roesy-,^{1}H^{13}C-COSY, HSQC, and HMBC experiments. Optical rotations were measured using a Perkin–Elmer polarimeter 341 (589 nm) at 20 °C. Melting points were measured on a Reichert heating microscope or on a ST-apotec and are uncorrected. MALDI-TOF-MS was performed on a Bruker Biflex III with 2,5-dihydroxybenzoic acid as matrix in positive



	R	Yield
1, 8	CH_2OH	67%
2, 9	CH_3	59%
3, 10	H	51%
4, 11		40%
5, 12		-
6, 13		-

Scheme 1. Formation of T-antigen derivatives employing modified donor substrates in transgalactosylations with β-galactosidase (bovine testis).