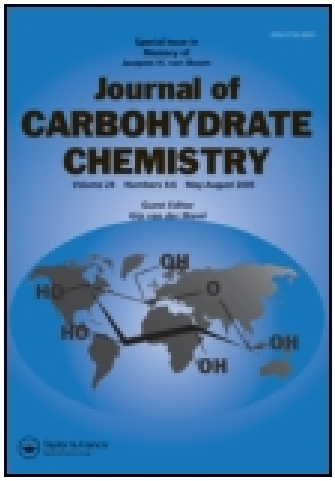


Journal of Carbohydrate Chemistry
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/lcar20

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Published online: 20 Aug 2006.

To cite this article: Lars Kröger & Joachim Thiem (2005) Facile One-Step Syntheses of Modified O-Glycoprotein Galβ1-3GalNAc Structures by Transglycosylation Employing Three β-Galactosidases from Bovine Testes, Xanthomonas manihotis, and Bacillus circulans , Journal of Carbohydrate Chemistry, 24:7, 717-722

To link to this article: http://dx.doi.org/10.1080/07328300500208131

*Journal of Carbohydrate Chemistry, 24:717–722, 2005*

*ISSN: 0732-8303 print 1532-2327 online*
*DOI: 10.1080/07328300500208131*



# Facile One-Step Syntheses of Modified *O*-Glycoprotein Galβ1-3GalNAc Structures by Transglycosylation Employing Three β-Galactosidases from Bovine Testes, *Xanthomonas manihotis*, and *Bacillus circulans*

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Natural *O*-glycoproteins such as the Thomsen-Friedenreich antigen or gangliosides contain the motif Gal$\beta$1-3GalNAc as an important disaccharide with significant biologic activity. The arrangement of spatial functionalities in this structure are of particular interest with regard to the development of potential leads en route to pharmaceuticals. Therefore, it was desired to obtain access to a range of modified derivatives of the aforementioned motif paying particular attention to introducing specific deoxy functions instead of hydroxyl groups.

**Keywords** Chemoenzymatic glycosylations, $\beta$-Galactosidases, T Antigen

Received January 24, 2005; accepted March 7, 2005.
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This paper is dedicated to Prof. Rosa M. de Lederkremer, Buenos Aires, on the occasion of her 70th anniversary in recognition of her substantial contributions to carbohydrate chemistry.

Compared to the well-elaborated classical glycosylation procedures, the in vitro application of enzymes excels in absolute stereospecificity, mild reaction conditions, and the absence of protection-deprotection steps.[1] Glycosidases are enzymes that normally catalyze the cleavage of glycoside bonds, as opposed to synthesizing glycosyltransferases. Instead of transferring an enzyme-bound glycosyl residue to water, the β-galactosidases can transfer it to a different acceptor. By utilizing novel deoxy acceptor structures in enzymatic glycosylation reactions with different affinities for the employed enzymes, we anticipated obtaining additional information about hydrogen bonding of the substrate in the active site of the enzymes. With this knowledge a map of hydrogen donor and acceptor interactions may be envisaged, thus allowing prediction of binding affinities and enabling a more systematic application of biocatalysts. In this project, we decided to employ three different β-galactosidases from bovine testes,[2] *Xanthomonas manihotis*,[3] and *Bacillus circulans* (recomb., *bga*C-gene).[4]

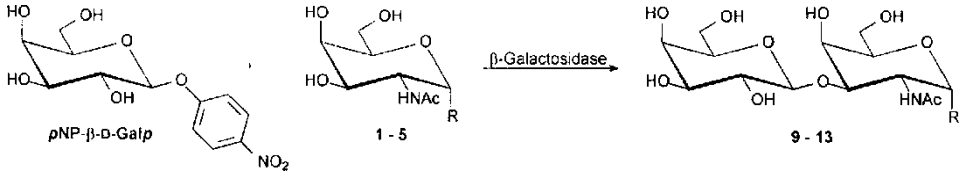
A suitable donor for β-galactosidase-catalyzed glycosylations is *p*-nitrophenyl β-D-galactopyranoside (*p*NP-β-D-Gal*p*), releasing *p*-nitrophenol (*p*NP-OH). Its incubation with allyl 2-acetamido-2-deoxy-β-D-galactopyranoside **1**[5] in the presence of β-galactosidase from bovine testes gave allyl 2-acetamido-2-deoxy-3-*O*-(β-D-galactopyranosyl)-α-D-galactopyranoside **9** in 67% yield as the single product (Table 1); no formation of regioisomers was observed.[a] This synthesis of **9** is superior to all previously published classical organic methods with regard to conditions employed, efficiency, and total yield.[6]

The regioselective gaiactosylation of methyl glycoside **2**[7] employing the β-galactosidase from bovine testes was quite successful as well, whereas the yield was slightly lower in case of the less water-soluble acceptors benzyl and 2-(trimethylsilyl)-ethyl glycosides **3**[8] and **4**,[9] respectively. The crude reaction mixture resulting from the treatment of benzyl 2-acetamido-2-deoxy-β-D-galactopyranoside **3** in the presence of *p*NP-β-D-Gal*p* had a very low solubility in aqueous buffers, preventing the use of size exclusion chromatography during workup. Hence, after the reaction went to completion, *p*-nitrophenol was extracted, and the crude mixture was acetylated and purified.

On removal of the anomeric hydroxyl group such as in 2-acetamido-1,5-anhydro-2-deoxy-D-galactitol **5**,[9] no effect on the recognition by the enzyme from bovine testes was observed compared to the methyl or allyl glycosides. Apparently, the absence of the 2-acetamido group in 2-deoxy glycoside **6**

[a]Donor *p*NP-β-D-Gal*p* and 10 equivalents of the acceptor were dissolved in McIlvain buffer (50 mM, pH 4.3) and warmed to 37°C. β-Galactosidase from bovine testes (4.7 U/mmol) was added, and the mixture was incubated for 50 hr at 37°C. The reaction was stopped by heating to 90°C for 5 min, followed by extraction of liberated *p*NP-OH. Purification was achieved by size exclusion chromatography on Biogel P-2 columns and filtration through mixed-bed ion exchange resin.

**Table 1:** Enzymatic galactosylation of GalNAc derivatives.



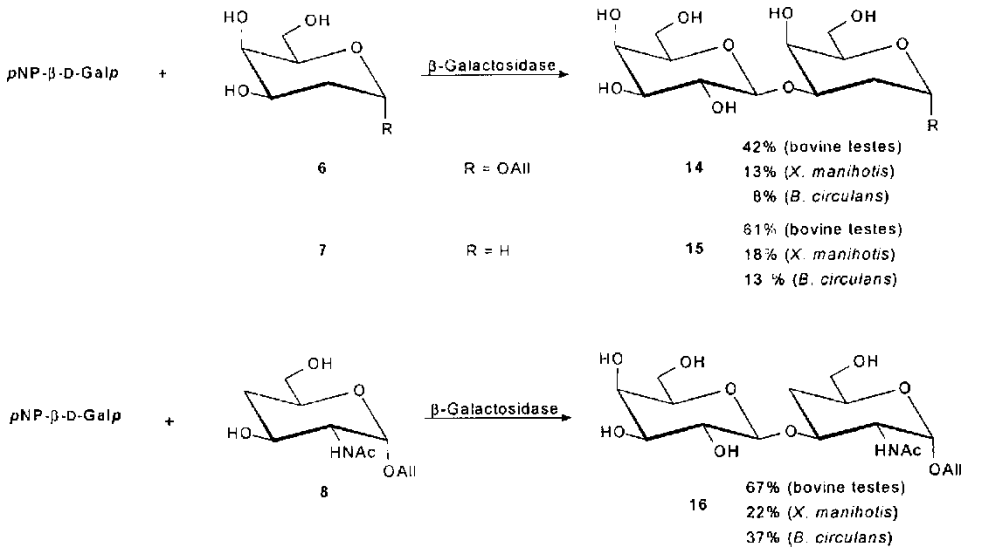

| Acceptor | R | Product | $\delta_{13C}$C-3 (ppm)[a] | $^3J_{1',2'}$ (Hz) | Enzyme | Yield (%) |
|---|---|---|---|---|---|---|
| **1** | OAll | **9** | 77.64 | 7.6 | Bovine testes | 67 |
| | | | | | *X. manihotis* | 21 |
| | | | | | *B. circulans* | 34 |
| **2** | OMe | **10** | 77.70 | 7.9 | Bovine testes | 66 |
| | | | | | *X. manihotis* | 24 |
| | | | | | *B. circulans* | 33 |
| **3** | OBn | **11**[b] | 73.78[b] | 7.6[b] | Bovine testes | 41 |
| | | | | | *X. manihotis* | 16 |
| | | | | | *B. circulans* | 38 |
| **4** | OSE | **12** | 77.41 | 7.6 | Bovine testes | 47 |
| | | | | | *X. manihotis* | 19 |
| | | | | | *B. circulans* | 35 |
| **5** | H | **13** | 80.22 | 7.6 | Bovine testes | 70 |
| | | | | | *X. manihotis* | 19 |
| | | | | | *B. circulans* | 31 |

[a]Assigned by HMBC-NMR experiments.
[b]Isolated as the corresponding peracetylated product.

prevents an optimal binding into the active site of the enzyme, giving lower yields of the disaccharide product **14** (Sch. 1). In contrast, 1,5-anhydro-2-deoxy-D-*lyxo*-hexitol **7**[10] was glycosylated by β-galactosidase from bovine testes in high yields. Besides, the chemoenzymatic glycosylation of allyl 2-aectamido-2,4-dideoxy-α-D-*xylo*-hexopyranoside **8** was accomplished regioselectively at position 3 in particularly high yields.

On treatment of the acceptor glycosides **1–5** with β-galactosidases from *X. manihotis*[b](Xm) or *B. circulans*[c] (Bc) the formation of a second disaccharide product identified as being the corresponding β1-6-regioisomer was observed. In the first case (Xm), the ratio was approximately 10 : 1, but in the second case

[b]Donor *p*NP-β-D-Gal*p*, 3 equivalents acceptor, β-galactosidase from *Xanthomonas manihotis* (230 U/mmol), and some BSA were dissolved in sodium acetate buffer (100 mM, pH 5.5. 10% MeCN) and incubated for 50 hr at 37°C. Purification see note a.
[c]Donor *p*NP-β-D-Gal*p*, 3 equivalents acceptor, and β-galactosidase from *Bacillus circulans* (*bga*C-gene, 5.9 U/mmol) were treated in potassium phosphate buffer (100 mM, pH 6.0. 20% DMF) for 4 1/2 hr at 37°C. Purification see note a.




**Scheme 1:** Enzymatic galactosylation of deoxy derivatives. $\delta_{13C}$ C-3 (ppm) **14**: 73.94, **15**: 77.51; **16**: 75.59; $^{3}J_{1',2'}$ (Hz) **14**: 7.6, **15**: 7.9, **16**: 7.9.

(Bc) the unwanted regioisomer was formed in considerable amounts giving a ratio of 3 : 2. To facilitate purification of the desired products, in situ hydrolysis, introduced by Larsson et al.,[11]d of the unwanted disaccharide was applied. The crude reaction mixtures were diluted and treated with β-galactosidase from *Escherichia coli*, which has a high specificity for hydrolyzing β1-6-glycosidic linkages, thus leaving the desired disaccharide linkage intact. In this way, a nonspecific transglycosylating enzyme can be used for chemoenzymatic syntheses by employing it in conjunction with a highly specific hydrolyzing enzyme. However, due to a lower activity and regiospecificity, the yields are reduced compared to the results with β-galactosidase from bovine testes.

A decrease in yields was observed for all acceptor substrates lacking a 2-acetamido function. This applies to the enzyme from *X. manihotis* and was even more pronounced for that from *B. circulans*. Again, the accompanying β1-6-regioisomers were hydrolyzed by β-galactosidase from *E. coli*. In contrast, the 4-deoxy derivative **8** was galactosylated only at position 3 and no formation of any regioisomers was observed.

The respective 6-deoxy derivative allyl 2-acetamido-2-deoxy-α-D-fucopyranoside[12] was also studied but apparently was not recognized by any of the

[d]After denaturation of the respective transglycosylating β-galactosidase, the reaction mixture was diluted 10-fold with sodium phosphate buffer (50 mM, pH 7.0. 1 mM $MgCl_2$). The β-galactosidasc from *Escherichia coli* was added and the mixture heated to 37°C for 4 hr. Workup see note a.

employed enzymes; the hydroxyl group at position 6 seems to be essential for binding to all β-galactosidases tested.

The β-galactosidases from bovine testes, *X. manihotis*, and *B. circulans* all show a useful tolerance toward modifications of the acceptor structure, giving access to derivatives of the Galβ1-3-GalNAc motif in moderate to convincing yields. Further modifications will be tested to ascertain the binding requirements for the different enzymes, enabling more accurate predictions of reactions.



## ACKNOWLEDGMENTS

Support of this work by the Deutsche Forschungsgemeinschaft (SFB 470) and the Fonds der Chemischen Industrie is gratefully acknowledged.

## REFERENCES

[1] (a) Křen, V.; Thiem, J. Glycosylation employing bio-systems: from enzymes to whole cells. Chem. Soc. Rev. **1997**, *26* (6), 463–473; (b) Fernández-Mayoralas, A. Synthesis and modification of carbohydrates using glycosidases and lipases. Top. Curr. Chem. **1997**, *186*, 1–20; (c) Koeller, K.M.; Wong, C.-H. Synthesis of complex carbohydrates and glycoconjugates: enzyme-based and programmable one-pot strategies. Chem. Rev. **2000**, *100* (12), 4465–4494.

[2] (a) Alessandrini, A.; Schmidt, E.; Zilliken, F.; György, P. Enzymatic synthesis of β-D-galactosides of N-acetylglucosamine by various mammalian tissues. J. Biol. Chem. **1956**, *220* (1), 71–78; (b) Gambert, U.; Gonzales Lio, R.; Farkas, E.; Thiem, J.; Verez Bencomo, V.; Lipták, A. Galactosylation with β-galactosidase from bovine testes employing modified acceptor substrates. Bioorg. Med. Chem. **1997**, *5* (7), 1285–1291.

[3] (a) Wong-Madden, S.T.; Landry, D. Purification and characterization of novel glycosidases from the bacterial genus Xanthomonas. Glycobiology **1995**, *5* (1), 19–28; (b) Fujimoto, H. Regioselective synthesis of β-D-Gal-(1 → 3)-D-GlcNAc using β-galactosidase from *Xanthomonas manihotis*. J. Carbohydr. Chem. **1997**, *16* (6), 967–970.

[4] (a) Ito, Y.; Sasaki, T. Cloning and characterization of the gene encoding a novel β-galactosidase from *Bacillus circulans*. Biosci. Biotechnol. Biochem. **1997**, *61* (8), 1270–1276; (b) Fujimoto, H.; Miyasato, M.; Ito, Y.; Sasaki, T.; Ajisaka, K. Purification and properties of recombinant β-galactosidase from *Bacillus circulans*. Glycoconj. J. **1998**, *15* (2), 155–160.

[5] Nashed, M.A.; El-Sokkary, R.I.; Rateb, L. "Standardized intermediates" for oligosaccharide synthesis. A convenient preparation of partially benzylated derivatives of allyl 2-acetamido-2-deoxy-α-D-galactopyranoside having chain extension at position 4. Carbohydr. Res. **1984**, *131* (1), 47–52.

[6] (a) Lee, R.T.; Lee, Y.C. Synthesis of allyl 3-deoxy- and 4-deoxy-β-D-galactopyranoside and simultaneous preparations of Gal(1 → 2)- and Gal(1 → 3)-linked disaccharide glycosides. Carbohydr. Res. **1994**, *251* (1), 69–79; (b) Roy, R.; Baek, M.-G.; Rittenhouse-Olsen, K. Synthesis of *NN*-bis(acrylamido)acetic acid-based T-antigen glycodendrimers and their mouse monoclonal IgG antibody binding properties. J. Am. Chem. Soc. **2001**, *123* (9), 1809–1816; (c) Baek, M.-G.; Roy, R.

Synthesis and protein binding properties of T-antigen containing glycoPAMAM dendrimers. Bioorg. Med. Chem. **2002**, *10* (1), 11–17.

[7] Horton, D.; Rodemeyer, G.; Saeki, H. A synthesis of 2-acetamido-2,6-dideoxy-D-galactose (*N*-acetyl-D-fucosamine). Carbohydr. Res. **1977**, *59* (2), 607–611.

[8] Flowers, H.M.; Shapiro, D. Synthesis of 2-acetamido-2-deoxy-3-*O*-(β-D-galactopyranosyl)-α-D-galactose. J. Org. Chem. **1965**, *30* (6), 2041–2043.

[9] Kröger, L. Darstellung und chemoenzymatische Umsetzung modifizierter Substrate zur Synthese von Ligandbausteinen für Myelin assoziiertes Glycoprotein. In *Ph. D. Thesis*; University of Hamburg, **2003**.

[10] Hayashi, M.; Yamada, K.; Arikita, O. Practical and catalytic synthesis of 1,5-anhydrohex-1-en-3-uloses. Tetrahedron **1999**, *55* (28), 8331–8340.

[11] (a) Hedbys, L.; Johansson, E.; Mosbach, K.; Larsson, P.-O.; Gunnarsson, A.; Svensson, S. Synthesis of 2-acetamido-2-deoxy-3-*O*-β-D-galactopyranosyl-D-galactose by the sequential use of β-D-galactosidases from bovine testes and *Escherichia Coli*. Carbohydr. Res. **1989**, *186* (2), 217–223; (b) Hedbys, L.; Johansson, E.; Mosbach, K.; Larsson, P.-O.; Gunnarsson, A.; Svensson, S.; Lonn, H. Synthesis of Galβ 1-3GlcNAc and Gal β1-3GlcNAcβ-SEt by an enzymatic method comprising the sequential use of β-galactosidases from bovine testes and *Escherichia coli*. Glycoconj. J. **1989**, *6* (2), 161–168.

[12] Paulsen, H.; Rutz, V.; Brockhausen, I. Synthese von modifizierten Derivaten der 2-Acetamido-2-desoxy-D-galactose zur Untersuchung der Substratspezifität der Core-1-β3-Gal-Transferase und der Core- 3-β3-GlcNAc-Transferase der Biosynthese von *O*-Glycoproteinen. Liebigs. Ann. Chem. **1992**, 735–745.