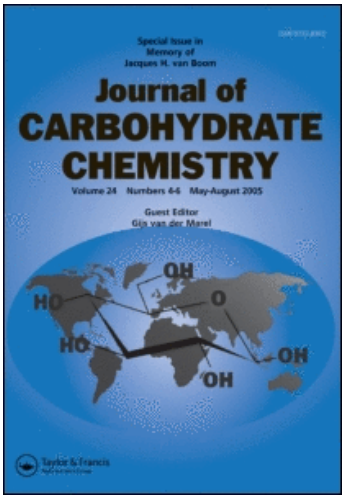


Journal of Carbohydrate Chemistry

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713617200

Enzymatic Galactosylation of Cello- and Chito-Oligomers

Harald Streicher[a]; Oliver Scheel[a]; Joachim Thiem[a]
[a] Department of Chemistry, University of Hamburg, Faculty of Science, Hamburg, Germany

To cite this Article Streicher, Harald , Scheel, Oliver and Thiem, Joachim(2008) 'Enzymatic Galactosylation of Cello- and Chito-Oligomers', Journal of Carbohydrate Chemistry, 27: 1, 41 — 49
To link to this Article: DOI: 10.1080/07328300802030779
URL: http://dx.doi.org/10.1080/07328300802030779

Journal of Carbohydrate Chemistry, 27:41–49, 2008

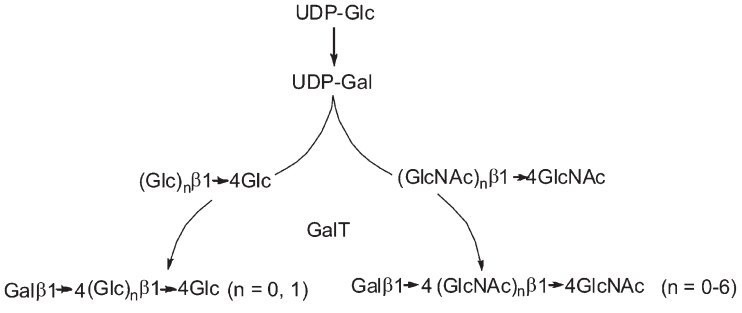
ISSN: 0732-8303 print 1532-2327 online
DOI: 10.1080/07328300802030779

Enzymatic Galactosylation of Cello- and Chito-Oligomers

Harald Streicher, Oliver Scheel, and Joachim Thiem

Faculty of Science, Department of Chemistry, University of Hamburg, Hamburg, Germany

Terminal galactosylations of cello- and chito-oligomers are studied employing galactosyltransferase with and without α-lactalbumin. The lactose synthase complex allows just for galactosylation of cellobiose in lower yield but not for higher cello-oligomers. In contrast, the affinity in galactosylation of chito-oligomers increases with higher members to reach the maximum at chitohexaose with only a 30% reduction in transfer rate. In addition to kinetic data, preparative studies with a number of acceptor substrates gave galactosylated oligosaccharides in high yields.



Keywords Enzyme catalysis, Galactosyltransferase, Cello-oligomers, Chito-oligomers

Received July 12, 2007; accepted January 1, 2008.
Address correspondence to Joachim Thiem, Faculty of Science, Department of Chemistry, University of Hamburg, Martin-Luther-King-Platz 6, D-20146, Hamburg, Germany. E-mail: joachim.thiem@chemie.uni-hamburg.de

INTRODUCTION

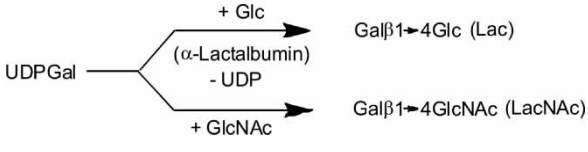
Among some others, galactosyltransferase (GalT, EC 2.4.1.22) is one of the glycosyltransferases that represents important exceptions from the one-enzyme-one-linkage hypothesis.[1] Dependent on the presence or absence of the so-called "specifier protein" α-lactalbumin, it shows two separate substrate specificities. GalT alone transfers galactose from UDP-Gal onto N-acetylglucosamine; however, GalT and α-lactalbumin together form the lactose synthase complex, which in turn transfers galactose from UDP-Gal onto glucose to give lactose in the mother milk of mammals (Formula 1).[2–6]

Earlier studies concerning the variability of the acceptor substrates focused on the use of unnatural substrates to achieve information on kinetics and perhaps the topology of the active center.[7–15] In following such an approach, the present study was to check the potential galactosylations of cello- and chito-oligomers. It can be considered that kinetic data and the results of preparative transformations should allow for further information regarding the bioreactions of GalT.

Model compounds for these studies were cello-oligomers **3** obtained by acetolysis of cellulose and subsequent deacetylation.[16] Chito-oligomers **4** could be obtained following an older procedure that could be improved to obtain higher-molecular-weight derivatives.[17,18]

In the glucose series glucose itself (**3a**, n = 0) is the genuine natural and thus an excellent substrate. It was shown previously that cellobiose (**3b**, n = 1) could be accepted; however, kinetic data prove it to be a poor substrate.[7] This is also reflected in the preparative transformation, which results in quite low yields. Higher cello-oligomers were not hitherto studied, and that is in focus of this contribution.

In case of N-acetylglucosamine, there were kinetic data for chito-oligomers up to the tetramer (**4**, n = 3).[7] The affinities reported increase in going from GlcNAc to $(GlcNAc)_2$; however, for $(GlcNAc)_3$ and $(GlcNAc)_4$, they decreased again. A preparative galactosylation of chitobiose (**4**, n = 1) was reported previously to give Galß1-4GlcNAcß1-4GlcNAc (**6**, n = 1) in 18% yield.[19] This work was further to focus on galactosylation of higher chito-oligosaccharides employing GalT.



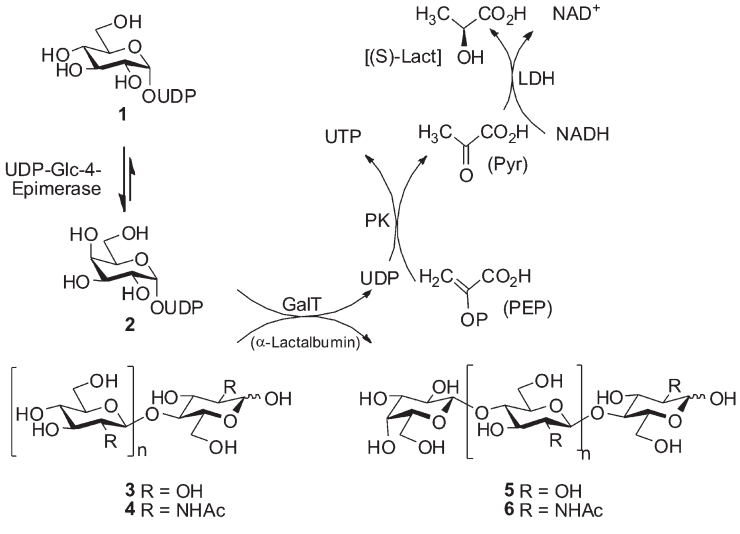
Formula 1: Galactosylation of glucose and N-acetylglucosamine.

RESULTS AND DISCUSSION

Kinetic studies of cello-oligomers made use of the transformations as depicted in Scheme 1. Employing UDP-Gal (**2**), galactosylation was catalyzed by galactosyltransferase (GalT) of the substrates **3** or **4**, respectively. The resulting UDP was phosphorylated with pyruvate kinase (PK) and phosphoenol pyruvate (PEP) to give UTP and pyruvate. Pyruvate was reduced with NADH and lactate dehydrogenase (LDH) to give (S)-lactate and NAD^+. This well-known approach allows for easy photometric assay of the decreasing extinction due to the decreasing concentration of NADH at 340 nm.[20]

For the genuine substrate glucose (**3a**, R = OH, n = 0) and for the unnatural substrate cellobiose (**3b**, R = OH, n = 1), the data from the literature could be confirmed. Thus, glucose showed a K_m of 5 mM (ref. 12, K_m = 5 mM) and cellobiose K_m = 826 mM (ref. 7, K_m = 833 mM). Tests with higher cello-oligomers (cellotriose **3c**, R = OH, n = 2; cellotetraose **3d**, R = OH, n = 3; cellopentaose **3e**, R = OH, n = 4) gave no products and thus evidence that none of these could function as substrates for GalT. Thus, the affinity from the mono- to the disaccharide decreases drastically, and higher gluco-oligomers of the ß,1-4-linked type are not recognized by the enzyme.

Correspondingly chito-oligomers (**4a**–**g**, R = NHAc, n = 0–6) were studied (Sch. 1) in 0.1 M aqueous solutions, but due to low solubility, the higher chito-oligomers had to be measured in 0.02 M aqueous solutions. Whereas in such cases the K_m values may be compared, the v_{max} values cannot.[20] Thus, both the pentamer (**4e**, n = 3) and the hexamer (**4f**, n = 5) were measured at two concentrations, and this allowed for correlation of the data for the heptamer (**4g**, n = 6).



Scheme 1: Galactosyltransferase (GalT)-catalyzed galactosylation of cello- and chito-oligomers. PK, pyruvate kinase; LDH, lactate dehydrogenase.

As depicted in Figure 1 (cf. also Table 1), the K_m values decrease steadily in going form GlcNAc (**4a**, n = 0: 3.6 mM) to chitohexaose (**4f**, n = 5) and chitoheptaose (**4 g**, n = 6), reaching the limiting value of $K_m = 0.2$ mM and thus the highest affinity. Figure 1 (cf. Table 1) shows the relative rate of the galactose transfer on to the chito-oligomers. Apparently, there is some decrease in going from GlcNAc (**4a**, n = 0) to chitotetraose (**4d**, n = 3) of about 70%, after which the rate remains constant. Overall, the affinity in going to higher chito-oligomers increased drastically, whereas there is a less substantial effect on the transfer rate.

For preparative use direct application of UDP-Gal (**2**) is too costly, and thus cheaper UDP-Glc (**1**) is employed and transformed in situ into **2** with UDP-glucose-4-epimerase. As observed in the kinetic studies, chitotriose (**4c**) can be used in 20 mM concentration; however, for the higher chito-oligomers this would result in substrate saturation, and thus chitotetraose (**4d**) and chitopentaose (**4e**) were employed in 5 mM concentrations. The literature data for formation of the trisaccharide galactosylchitobiose (**6b**) was 60%,[21] and here we observed a slight increase in the formation of the tetrasaccharide galactosylchitotriose (**6c**) to 63% yield after 3 d of incubation and workup. Alternative syntheses of **6b** from chitobiose (**4b**) as well as of **6c** from chitotriose (**4c**) and p-nitrophenyl ß-D-galactopyranoside by transgalactosylation employing ß-galactosidase (*B. circulans*) in 10% [22] and 27%,[23] respectively, were

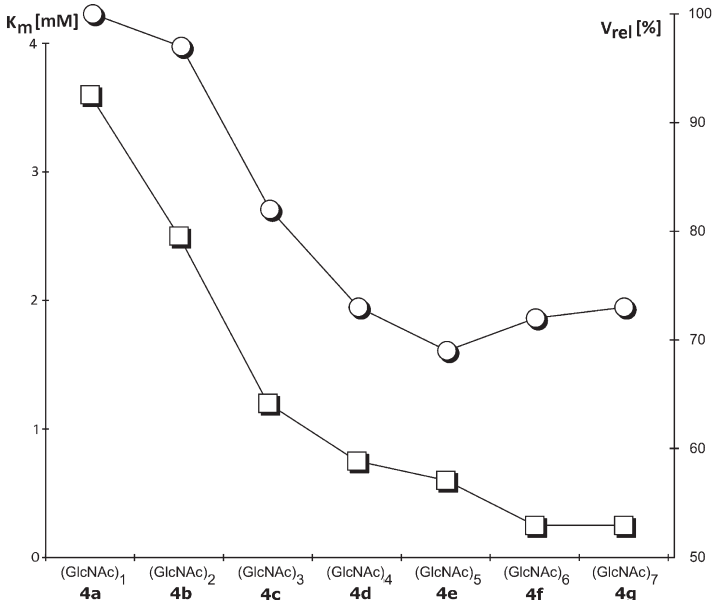


Figure 1: Michaelis constants (K_m, □) and relative rates (v_{rel},O) of chitooligomer acceptor substrates in GalT-catalyzed galactosylation.

Table 1: Kinetic data of $(GlcNAc)_n$ acceptor substrates in galactosylation with GalT.

Chito-oligomer substrates	K_m (mM)	v_{max} (μmol min^{-1})	v_{rel} (%)
GlcNAc **4a**	3.6	7.5	100
$(GlcNAc)_2$ **4b**	2.5	6.5	87
$(GlcNAc)_3$ **4c**	1.2	6.3	84
$(GlcNAc)_4$ **4d**	0.7	5.3	71
$(GlcNAc)_5$ **4e**	0.6	5.0	67
$(GlcNAc)_6$ **4f**	0.2	5.2	69
$(GlcNAc)_7$ **4g**	0.2	5.3	71

reported. For galactosylations of chitotetraose (**4d**) and chitopentaose (**4e**), a considerably improved transfer and yield was obtained after 2 d of incubation and workup to give the pentasaccharide galactosylchitotetraose (**6d**) in 81% yield and the hexasaccharide galactosylchitopentaose (**6e**) in 98% yield. As obvious from these data, the slight decrease in reaction rate is of little influence regarding a preparative approach. In particular, generally a high transfer could be observed for oligomers larger than the tetrasaccharide.

SUMMARY

These findings underline the anticipated biological function of galactosyltransferase. In mammals and the presence of α-lactalbumin, the lactose synthase complex is formed and suited to accept and galactosylate glucose as the only substrate to give lactose (**5b**). A minor deviation allows for the acceptance of the unnatural substrate cellobiose (**3b**), which is still galactosylated in 23% yield to give the trisaccharide galactosylcellobiose (**5b**)[19]; however, higher cello-oligomers (**3c**, **3d**, **3e**) did not show any transfer.

In contrast, the GalT-catalyzed galactosylation of a terminal N-acetylglucosamine unit did not require any copeptide and improved drastically with the increasing oligosaccharide chain as indicated by the improving affinity, and this again reflects in the preparative yields. It would be interesting to look into the acceptor properties of pure malto-oligomers and terminally N-acetylglucosaminylated oligosaccharides resembling chito-oligomers, and studies in this direction are in progress.

EXPERIMENTAL

General Remarks

Commercially available starting materials were used without further purification. Solvents were dried according to standard methods. Purifications of

the products were carried out by column chromatography using Biogel P2 or P4. The enzymatic reactions were incubated in a Thermomixer Comfort (Merck) at 600 rpm. The nuclear magnetic resonance spectra were recorded on Bruker AM-300 or AMX-400. All chemical shifts are quoted in ppm downfield from TMS or referred to the characteristic signal of HDO in D_2O (4.63 ppm). Mass spectra were recorded on Bruker MALDI-Tof Biflex III. GalT from bovine milk (EC 2.4.1.22), UDP-galactose-4-epimerase (EC 5.1.3.2), PK (EC 2.7.1.40), LDH (EC 1.1.1.27), UDP-Glc, and UDP-Gal were from Sigma.

Kinetic Data

The maximum reaction rate (v_{max}) and the Michaelis constant (K_m) were determined for the acceptor substrates cello-oligomers **3a–3e** (R = OH, n = 0–4) and chito-oligomers **4a–4g** (R = NHAc, n = 0–6). In a total volume of 1 mL the Tris-HCl buffer (100 mM, pH 7.5) containing $MgCl_2$ (10 mM), $MnCl_2$ (10 mM), and KCl (37 mM) and NADH (30 μM), PEP (100 μM), UDP-Gal (20 μM), and α-lactalbumin (0.1 mg/mL) were incubated with PK (10U), LDH (10U), GalT (0.1U), and the Glc_n acceptor substrates (**3a–3e**) in 0.1 aqueous solution. In case of the GlcNAc acceptor substrates (**4a–4g**), α-lactalbumin was omitted. The decreasing extinction was followed for 5 to 10 min at 340 nm and the linear part of the graph analyzed employing the Lineweaver-Burk and also the Hanes approach.

General Preparation (GP)

The acceptor substrate and UDP-Glc (**1**) were dissolved in degassed cacodylate buffer (100 mM, pH 7.5) containing $MnCl_2$ (5 mM). UDP-glucose-4-epimerase and galactosyltransferase were added and the mixture incubated at 30°C. Every 24 h the pH was adjusted to 7.5; the progress was checked by the UDP assay. After termination proteins were removed by centrifugation and the residue chromatographed on Dowex 2 × 8 (Cl^-) ion exchange resin. Elution with doubly distilled water (250 mL) left charged material on the column and gave the products. After concentration, purification was by gel chromatography (Biogel P2 and P4) and final freeze drying.

*β-D-Galactopyranosyl-(1-4)-β-D-glucopyranosyl-(1-4)-D-glucose (**5b**)*

Following the GP cellobiose (**3b**, 69 mg, 200 μmol, 20 mM) was mixed with UDP-Glc (**1**, 131 mg, 200 μmol, 20 mM), α-lactalbumin (5 mg), UDP-Glc-4-epimerase (2U, twice), and Gal T (1U, twice) in buffer (10 mL) and incubated for 5 d; the second enzyme charge was added on day 3. Workup and purification on Biogel P2 gave **5b** (23 mg, 23%) as colorless amorphous material. ^1H NMR

(D_2O): $\delta = 5.23$ (d, $J_{1,2} = 3.9$ Hz, 1H, H-1α), 4.67 (d, $J_{1,2} = 7.9$ Hz, 1H, H-1ß), 4.55 (d, $J_{1',2'} = 7.9$ Hz, 1H, H-1′, 4.46 (d, $J_{1'',2''} = 7.9$ Hz, 1H, H-1″).

A corresponding preparative size approach for galactosylation of cellotriose (**3c**, 100 mg, 198 μmol, 20 mM) did not give any compound **5c**.

*ß-D-Galactopyranosyl-(1-4)-2-acetamido-2-deoxy-ß-D-glucopyranosyl-(1-4)-2-acetamido-2-deoxy-ß-D-glucopyranosyl-(1-4)-2-acetamido-2-deoxy-D-glucose (**6c**)*

Following the GP tri-*N*-acetylchitotriose (**4c**, 60 mg, 96 μmol, 20 mM) was mixed with UDP-Glc (**1**, 63 mg, 96 μmol, 20 mM), and UDP-Glc-4-epimerase (4U) and GalT (2U) and were incubated in buffer (4.8 mL) for 3 d. Workup and purification on Biogel P2 gave tetrasaccharide **6c** (48 mg, 63%) as colorless amorphous material; $[\alpha]_D^{20}$: -108° (*c* 0.08, H_2O) [Lit.:[8] -114° (*c* 0.1, H_2O)]; ^1H NMR (D_2O): $\delta = 5.21$ (d, $J_{1,2} = 2.6$ Hz, 1H, H-1α), 4.71 (d, $J_{1,2} = 8.3$ Hz, 1H, H-1ß), 4.62 (each d, $J_{1',2'} = J_{1'',2''} = 7.8$ Hz, 2H, H-1′ and H-1″), 4.84 (d, $J_{1''',2'''} = 7.8$ Hz, 1H, H-1‴;), 3.95 (dd, $J_{3''',4'''} = 3.3$ Hz, $J_{4''',5'''} = 1.1$ Hz, 1H, H-4‴), 3.60 (dd, $J_{1''',2'''} = 7.8$ Hz, $J_{2''',3'''} = 9.9$ Hz, 1H, H-2‴), 2.08 (each s, 6H, CH_3), 2.06 (s, 3H, CH_3). $C_{30}H_{51}N_3O_{21}$: 789.30; FAB-MS *m/z* 790 $[M + H]^+$, 812 $[M + Na]^+$.

*ß-D-Galactopyranosyl-(1-4)-2-acetamido-2-deoxy-ß-D-glucopyranosyl-(1-4)-2-acetamido-2-deoxy-ß-D-glucopyranosyl-(1-4)-2-acetamido-2-deoxy-ß-D-glucopyranosyl-(1-4)-2-acetamido-2-deoxy-glucose (**6d**)*

Following the GP tetra-*N*-acetylchitotetraose (**4d**, 100 mg, 120 μmol, 5 mM) was mixed with UDP-Glc (**1**, 79 mg, 120 μmol, 5 mM), UDP-Glc-4-epimerase (4U), and GalT (2U) and incubated in buffer (24 mL) for 2 d. Workup and purification on Biogel P4 gave the pentasaccharide **6d** (97 mg, 81%) as a white amorphous solid; $C_{38}H_{64}N_4O_{26}$: 992.95; FAB-MS *m/z* 994 $[M + H]^+$, 1016 $[M + Na]^+$, 1032 $[M + K]^+$.

*ß-D-Galactopyranosyl-(1-4)-2-acetamido-2-deoxy-ß-D-glucopyranosyl-(1-4)-2-acetamido-2-deoxy-ß-D-glucopyranosyl-(1-4)-2-acetamido-2-deoxy-ß-D-glucopyranosyl-(1-4)-2-acetamido-2-deoxy-ß-D-glucopyranosyl-(1-4)-2-acetamido-2-deoxy-ß-D-glucose (**6e**)*

Following the GP penta-*N*-acetylchitopentaose (**4d**, 100 mg, 97 μmol, 5 mM) was mixed with UDP-Glc (**1**, 64 mg, 97 μmol, 5 mM), UDP-Glc-4-eimerase (4U), and GalT (2U) and incubated in buffer (19.4 mL) for 2 d. Workup and purification on Biogel P2 gave the hexasaccharide **6e** (114 mg, 98%) as a colorless amorphous material; $C_{46}H_{77}N_5O_{31}$: 1195.45; FAB-MS *m/z* 1218 $[M + Na]^+$.

ACKNOWLEDGMENTS

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

REFERENCES

[1] Beyer, T.A.; Sadler, J.-E.; Rearick, J.I.; Paulson, J.C.; Hill, R.L. Glycosyltransferases and their use in assessing oligosaccharide structure and structure-function relationships. Adv. Enzymol. **1981**, *52*, 23–175.

[2] Brew, K.; Vanaman, T.C.; Hill, R.L. Role of α-lactalbumin and the A protein in lactose synthetase. Unique mechanism for the control of a biological reaction. Proc. Natl. Acad. Sci. USA **1968**, *59*, 491–497.

[3] Klee, W.A.; Klee, C.B. The role of alpha-lactalbumin in lactose synthetase. Biochem. Biophys. Res. Commun. **1970**, *39*, 833–841.

[4] Barker, R.; Olsen, K.W.; Shaper, J.H.; Hill, R.L. Agarose derivatives of uridine diphosphate and N-acetylglucosamine for the purification of a galactosyltransferase. J. Biol. Chem. **1972**, *247*, 7135–7147.

[5] Hill, R.L.; Brew, K. Lactose synthetase. Adv. Enzymol. **1975**, *43*, 411–490.

[6] Yadav, S.; Brew, K. Identification of a region of UDP-galactose:N-acetylglucosamine beta 4-galactosyltransferase involved in UDP-galactose binding by differential labeling. J. Biol. Chem. **1990**, *265*, 14163–14169.

[7] Schanbacher, F.L.; Ebner, K.E. Galactosyltransferase acceptor specificity of the lactose synthetase A protein. J. Biol. Chem. **1970**, *245*, 5057–5061.

[8] Khatra, B.S.; Herries, D.G.; Brew, K. Kinetic properties of human milk galactosyl transferase. Eur. J. Biochem. **1974**, *44*, 537–560.

[9] Rao, A.K.; Mendicino, J. Influence of glycopeptide structure on the regulation of galactosyltransferase activity. Biochemistry **1978**, *17*, 5632–5638.

[10] Blanken, W.M.; van Vliet, A.; den Eijnden, D.H. Branch specificity of bovine colostrum and calf thymus UDP-Gal: N-acetylglucosaminide beta-1,4-galactosyltransferase. Biol. Chem. **1984**, *259*, 131–15135.

[11] Pâquet, M.R.; Narasimhan, S.; Schachter, H.; Moscarello, M.A. Branch specificity of purified rat liver Golgi UDP-galactose:N-acetylglucosamine ß-1,4-galactosyltransferase. Preferential transfer of galactose on the GlcNAcß 1,2-Manα 1,3-branch of a complex biantennary Asn-linked oligosaccharide. J. Biol. Chem. **1984**, *259*, 4716–4721.

[12] Berliner, L.J.; Davis, M.E.; Ebner, K.E.; Beyer, T.A.; Bell, J.E. The lactose synthase acceptor site: a structural map derived from acceptor studies. Mol. Cell. Biochem. **1984**, *62*, 37–42.

[13] Palcic, M.M.; Srivastava, O.P.; Hindsgaul, O. Transfer of D-galactosyl groups to 6-O-substituted 2-acetamido-2-deoxy-D-glucose residues by use of bovine D-galactosyltransferase. Carbohydr. Res. **1987**, *159*, 315–324.

[14] Wong, C.H.; Ichikawa, Y.; Krach, T.; Gautheron-Le Narvor, C.; Dumas, D.P.; Look, G.C. Probing the acceptor specificity of ß-1,4-galactosyltransferase for the development of enzymatic synthesis of novel oligosaccharides. J. Am. Chem. Soc. **1991**, *113*, 8137–8145.

[15] Ernst, B.; Oehrlein, R. Substrate and donor specificity of glycosyl transferases. Glycoconj. J. **1999**, *16*, 161–170.

[16] Wolfrom, M.L.; Thompson, A. Acetolysis. Methods Carbohydr. Chem. **1963**, *3*, 143–150.

[17] Bredehorst, R.; Pomato, N.; Scheel, O.; Thiem, J. High-yield manufacture of water-soluble dimeric to decameric chitin oligomers. US Pat. 5.705.634 (01.06.1998); Chem. Abstr. **1996**, *125*, 279–065t.

[18] Scheel, O.; Thiem, J. Cleavage of chitin by means of aqueous hydrochloric acid and isolation of chito-oligosaccharides in Handbook of Chitin and Chitosan. Muzzarelli, R.A.A., Peter, M.G., Eds.; *Atec Edizione, Grottammare, I*; 1998; 165–170.

[19] Thiem, J.; Wiemann, T. Synthesis of galactose-terminated oligosaccharides by use of galactosyltransferase. Synthesis **1992**, 141–145.

[20] Gosselin, S.; Alhussaini, M.; Streiff, M.B.; Takabayashi, K.; Palcic, M.M. A continuos spectrophotometric assay for glycosyltransferases. Anal. Biochem. **1994**, *220*, 92–97.

[21] Nunez, H.A.; Barker, R. Enzymic synthesis and carbon-13 nuclear magnetic resonance conformational studies of disaccharides containing ß-D-galactopyranosyl and ß-D-[1-13C]galactopyranosyl residues. Biochemistry **1980**, *19*, 489–495.

[22] Gambert, U.; Conradt, H.S.; Nimtz, M.; Thiem, J. Galactosidase-assisted synthesis en route to type I and type II structures of chitooligomers. J. Carbohydr. Chem. **2000**, *19*, 621–629.

[23] Kren, V.; Dvorakova, J.; Gambert, U.; Sedmera, P.; Havlicek, V.; Thiem, J.; Bezouska, K. ß-Glucosylation of chitooligomers by galactosyltransferase. Carbohydr. Res. **1998**, *305*, 517–523.