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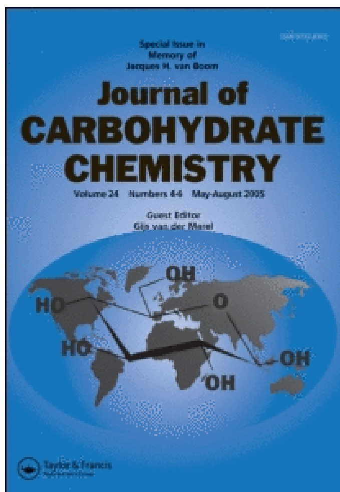


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J. CARBOHYDRATE CHEMISTRY, 17(8), 1239-1247 (1998)

# ENZYMATIC GLYCOSYLATION OF BRANCHED SYMMETRICAL NON-CARBOHYDRATE POLYOLS

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## ABSTRACT

Three glycosidases, α-mannosidase from *Jack beans*, β-galactosidase from *Aspergillus oryzae* and β-*N*-acetylhexosaminidase from *Aspergillus oryzae* were evaluated for their ability to catalyze the glycosylation of non-saccharidic polyols. A series of monoglycosylated derivatives of tris(2-aminoethyl)cyanuric acid, pentaerythritol and bis(hydroxymethyl)benzene were obtained in pure form and characterized by NMR-spectroscopy.

## INTRODUCTION

Oligoantennary glycomimetics, also refered to as "glycoclusters" or "clustered glycosides" and "glycodendrimers" have recently received much attention, as they often serve as especially potent inhibitors of carbohydrate-protein interactions due to their multivalency.[1] Most of the glycoclusters designed so far have been synthesized by chemical means[2] and only few examples are reported where transferases have been employed for the glycosylation of oligoantennary carbohydrate derivatives.[3] Although



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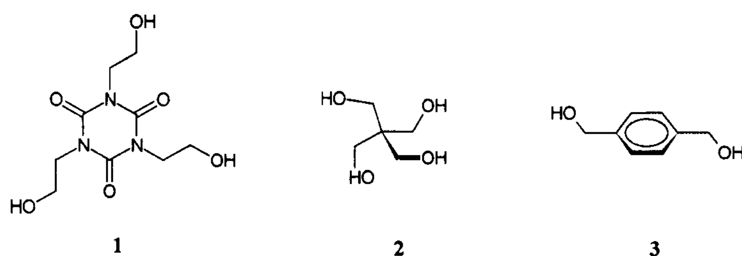


**Scheme 1.** Branched acceptor polyols

glycosyltransferase catalyzed glycosylation reactions proceed with excellent yields,[4] these enzymes cannot be used for the glycosylation of non-carbohydrate core structures, because of their stringent substrate specificity. The goal of the present study was the investigation of glycosidases for the glycosylation of symmetrical non-carbohydrate polyols such as compounds **1-3**. Glycosidases are advantageous for this purpose because of their easy availibility and handling, their low price and low specificity towards different acceptor molecules.[5] Furthermore, simple and unprotected glycosyl donors can be used as substrates for glycosidases and unprotected products produced may be directly used for biological testing.

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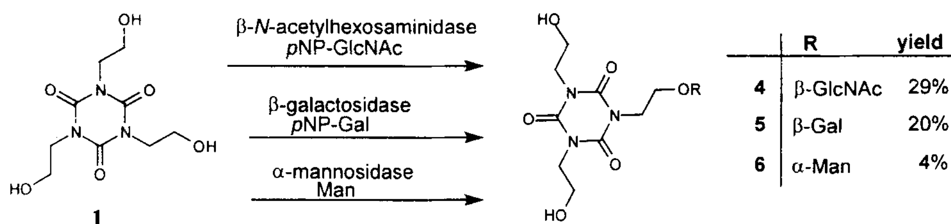
## RESULTS

Three readily available branched polyols, 1,3,5-tris(hydroxyethyl)cyanuric acid (**1**), pentaerythritol (**2**) and bis(hydroxymethyl)benzene (**3**) were selected as representatives of different types of oligovalent acceptors, e.g. heterocyclic, aliphatic and benzylic alcohols (Scheme 1).

A number of glycosidases were evaluated for their biological activities towards the polyols **1-3**. From those β-galactosidase from *Aspergillus oryzae*,[6] β-*N*-acetyl-hexosaminidase from *A. oryzae*[7] and α-mannosidase from *Jack beans*[8] were chosen as catalysts for enzymatic glycosylation because these three enzymes showed the ability to catalyze the transfer of respective hexopyranosyl residues onto the symmetrical acceptor alcohols.




**Scheme 2.** Enzymatic glycosylation of cyanuric acid derivative **1**

The enzymatic galactosylations and *N*-acetyl-D-glucosaminylations were carried out as transglycosylation reactions with the respective *p*-nitrophenyl glycosides as donors. Enzymatic mannosylations were performed in the reverse hydrolysis mode with an excess of D-mannose as substrate.[9]

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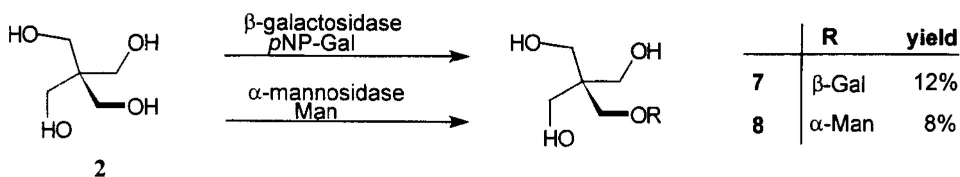
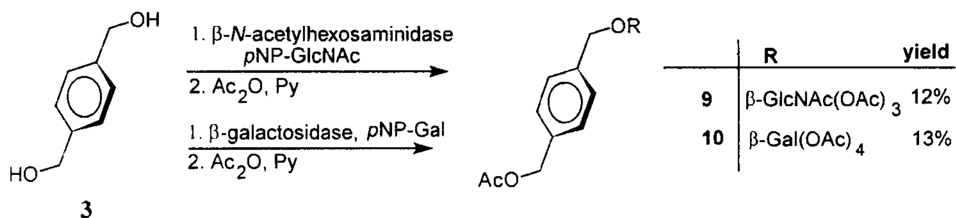
Cyanuric acid (**1**) served as an acceptor for all three glycosidases used and was converted into the monoglycosides **4**, **5** and **6**, obtained in 29, 20 and 4% isolated yield, respectively (Scheme 2). Di- or tri-*O*-glycosylated products were not isolated.

Pentaerythritol (**2**) and the benzylic diol **3** were rather poor substrates in the enzymatic glycosylations. No product could be detected when the hexosaminidase was incubated with the tetraalcohol **2**. However, the galactosidase- and mannosidase-catalyzed glycosylation of **2** lead to the monoglycosides **7** and **8** (Scheme 3) in 12 and 8% isolated yield, respectively. Though the aromatic diol bis(hydroxymethyl)benzene (**3**) was not glycosylated by the α-mannosidase from *Jack beans*, a mono-*N*-acetyl-glucosaminylated product **9** was formed with β-*N*-acetyl hexosaminidase, and β-galactosidase furnished the analogous monogalactoside. After gel permeation chromatography on BioGel P-2 and subsequent acetylation of the product fractions, followed by purification of the acetylated products on silica gel, the monoglycosides **9** and **10** were isolated in pure form in 12 and 13% yield, respectively (Scheme 4).

All enzymatic reactions carried out were complicated by the formation of large amounts of byproducts. This made the purification and isolation of the glycosylation products especially difficult. Nevertheless, all reported yields refer to pure products, which were unequivocally characterised by NMR spectroscopy.




**Scheme 3.** Enzymatic glycosylation of pentaerythritol (**2**)




**Scheme 4.** Enzymatic glycosylation of *p*-bis(hydroxymethyl)benzene (**3**).

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## DISCUSSION

In all enzymatic experiments a complex mixture of byproducts was obtained as observed by TLC during the reactions. This mixture was mainly comprised of asymmetrically substituted oligosaccharide-functionalized core molecules, which was obvious from the NMR spectra. These byproducts arise from further glycosylation at the glycosidic moiety of monoglycosylated primary products. Even though the reactions were not monitored by HPLC, it was clear that the monoglycosylated products obtained were the only derivatives formed in the enzymatic reactions which could be isolated in preparative useful amounts.

The course of the enzymatic reaction can be explained by the specificity of the applied hydrolases, which prefer to glycosylate hydroxy groups of the first attached glycosyl moiety rather than the next OH group of the non-carbohydrate core. This might be facilitated by the aglycon moiety as it is well known that enzymatic glycosylation reactions are often significantly influenced by the nature not only of the glycon but also the aglycon part of a saccharide.[8]

Steric hindrance may also be an important contributing factor for the monoglycosylation of the core. The monoglycosylated substrate might not fit into the active site of the enzyme in a manner that allows glycosylation of the remaining hydroxy groups of the core molecule. Furthermore, non-physiological primary hydroxy groups of the core polyol have to compete with OH groups of the first transferred glycosyl moiety, for which the applied enzymes are naturally specialized. Consequently, relatively large amounts of *p*NP di- and trisaccharides were also detected in the enzymatic transglycosylation reaction mixtures. This is not surprising as the enzymatic synthesis of *p*NP oligosaccharides is well documented in the literature.[11] A study which was published during our investigations is in agreement with our findings.[12] It is probably the only thus far reported example of the utilization of glycosidases for the multiple glycosylation of non-carbohydrate polyols. In the latter communication β-hexosidase from bovine liver was used for the attachment of two L-arabinosyl residues to an aromatic diol. The L-arabinosyl residue, however, bears no primary hydroxy groups which would compete with the benzylic OH groups of the aromatic core polyol for glycosyl transfer.

Even though products with more then one glycosylated core-OH group could not be isolated in our enzymatic syntheses, the results obtained are promising for selective glycosylation of the symmetrical polyols, which is difficult to perform by chemical means. It might be favourably combined with subsequent chemical glycosylations to afford mixed glycoclusters. Orthoester formation, which often occurs in chemical glycosylations, especially in α-mannosylations[13] can be avoided using enzymes. Thus, the use of glycosidases for selective monoglycosylation of oligovalent symmetrical alcohols, as has also been employed for enzymatic discrimination of heterotopic hydroxy groups,[14] can be considered as an advantageous supplementation to chemical glycosylations of polyols.

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## EXPERIMENTAL

**General methods.** The reactions were monitored on silica gel 60-coated TLC plates (Merck) with propanol-water (7:3 (v/v), 1% ammonia) or propanol-nitromethane-

water (10:9:2 (v/v/v)). Yields were determined gravimetrically. NMR spectra were recorded in $D_2O$ or $CDCl_3$ on a Bruker AC-250P (62.89 MHz for $^{13}C$) or a Bruker AMX 400 (100.67 MHz for $^{13}C$). Chemical shifts are given in ppm relative to TMS (0.00 ppm) as internal standard or $D_2O$ at 4.65 ppm. Flash chromatography was carried out with Merck silica gel (0.040-0.063 mm). Gel filtration was performed with BioGel P-2 (Bio-Rad) using a 100 x 2.5 cm column. α-Mannosidase from *Jack beans* (EC 3.2.1.24) and β-galactosidase from *A. oryzae* (EC 3.2.1.23) were purchased from Sigma. β-*N*-Acetylhexosaminidase from *A. oryzae* was prepared according to Hunková et. al.[15] and the corresponding *p*-nitrophenyl glycosides were synthesized according to published procedures.[16] FAB-MS was carried out on a VG Analytical 70-250S.

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**Enzymatic β-galactosylation and β-*N*-acetylglucosaminylation; general procedure.** The acceptor alcohol (40 mg) and the donor *p*NP glycoside (40 mg) were suspended in 0.1 M buffer solution (2 mL) and 20 U of respective glycosidase were added. For the glycosylation with β-galactosidase (*A. oryzae*) a 0.1 M phosphate buffer ($KH_2PO_4/K_2HPO_4$; pH 5.0; 6 mg $MgSO_4$/mL) was used and in the case of β-*N*-acetyl-hexosaminidase (*A. oryzae*) a 0.1 M McIlvaine-buffer (citric acid/$Na_2HPO_4$; pH 5.0) was employed. Further 20 mg portions of *p*NP-donor were added after 60 and 120 min. The reaction was stopped before total consumption of donor by addition of methanol (1 mL) and heating to 80 °C. Denatured enzyme was removed by filtration and the products were separated on a Bio-Rad P-2 column (100 x 2.5 cm) using a 35 mM $NH_4HCO_3$-buffer. Product fractions were collected and freeze-dried. Peracetylation of **9** and **10** was performed with 2:1-mixture of pyridine and acetic acid anhydride after GPC. The acetylated compounds were purified on silica gel.

**Enzymatic α-Mannosylation; general procedure.** D-Mannose (160 mg) and acceptor alcohol (20 mg) were dissolved in 0.1 M McIlvaine-buffer (0.5 mL; pH 5.0) and α-Mannosidase (5 U) from *Jack beans* was added. The reaction mixture was incubated for four days at 37 °C. The reaction was then stopped by heating to 100 °C for 5 min. Subsequently, the mixture was diluted tenfold with water and submitted to gel chromatography. Product fractions were collected and freeze-dried.

Yields of the reactions and NMR data of the products are listed below.

**1-(2 – Acetamido – 2-deoxy-β-D-glucopyranosylethyl) -3,5-bis(hydroxyethyl)-cyanuric acid (4).** 29% isolated yield (20 mg, 0.04 mmol); $^1H$ NMR ($D_2O$): δ 4.43 (d,

1H, H-1), 4.00 (m, 2H, $CH_2O$), 3.93 (t, 4H, 2 $CH_2O$), 3.91-3.87 (m, 1H, $C\underline{H}_aH_bCH_2$), 3.76 (dd, 1H, H-6), 3.72-3.67 (m, 1H, $CH_a\underline{H}_bCH_2$), 3.64 (t, 4H, 2 $CH_2N$), 3.57 (dd, 1H, H-6'), 3.49 (dd, 1H, H-2), 3.33 (dd, 1H, H-3), 3.23-3.16 (m, 2H, H-4, H-5) ppm; $J_{1,2}$ = 8.3, $J_{2,3}$ = 10.2, $J_{3,4}$ = 8.1, $J_{5,6}$ = 2.0, $J_{5,6'}$ = 5.6, $J_{6,6'}$ = 11.7 Hz; $^{13}C$ NMR (100.62 MHz, $D_2O$): δ 173.98 (NAc), 151.39, 151.11 (2x) (3 C=O), 102.86 (C-1), 78.26, 76.23 (C-4, C-5), 72.25 (C-3), 66.73 ($CH_2O$), 63.01 (C-6), 60.00 (2x) (2 $CH_2O$), 57.44 (C-2), 46.00 (2x), 43.44 (3 $CH_2N$), 23.32 (NAc) ppm; FAB-MS 487.2 $[M+Na]^+$ (487.16 calculated for $C_{17}H_{28}N_4O_{11}Na$).

**1-(β-D-Galactopyranosylethyl)-3,5-bis(hydroxyethyl)cyanuric acid (5).** 20% isolated yield (13 mg, 0.03 mmol); $^1H$ NMR ($D_2O$): δ 4.28-4.20 (m, 2H, H-1, $C\underline{H}_aH_b$), 4.16-4.08 (m, 2H, $CH_a\underline{H}_b\underline{H}_cH_d$), 4.04 (t, 4H, 2 $CH_2$), 3.90-3.82 (m, 2H, H-4, $CH_c\underline{H}_d$), 3.78 (t, 4H, 2 $CH_2$), 3.72-3.69 (m, 2H, H-6, H-6'), 3.52-3.46 (m, 3H, H-2, H-3, H-5) ppm; $^{13}C$ NMR (100.58 MHz, $d_4$-MeOH): δ 151.39 (2x), 151.32 (3 C=O), 105.31 (C-1), 76.96 (C-5), 75.13 (C-3), 72.73 (C-2), 70.45 (C-4), 67.38 ($CH_2O$), 62.66 (C-6), 60.06 (2 $CH_2O$), 45.97 (2x), 43.61 (3 $CH_2N$) ppm, FAB-MS 446.3 $[M+Na]^+$ (446.14 calculated for $C_{15}H_{25}N_3O_{11}Na$).

**1-(α-D-Mannopyranosylethyl)-3,5-bis(hydroxyethyl)cyanuric acid (6).** 4% isolated yield (1.3 mg, 0.003 mmol); $^1H$ NMR ($D_2O$): δ 4.87 (dd≈s, 1H, H-1), 4.04 (t, 4H, 2 $CH_2O$), 3.97-3.61 (m, 10H, 6 ring-H, $OCH_2$, $CH_2N$), 3.77 (t, 4H, 2 $CH_2N$) ppm; $^{13}C$ NMR (62.89 MHz, $D_2O$): δ 153.05 (3x) (3 C=O), 96.79 (C-1), 75.29, 73.12, 72.50, 69.31 (C-2, C-3, C-4, C-5), 68.38 (C-6), 63.59, 61.11 (2x) (3 $CH_2O$), 47.98 (2x), 47.18 (3 $CH_2N$) ppm.

***O*-(1-β-D-galactopyranosyl)pentaerythritol (7).** 12% isolated yield (10 mg, 0.03 mmol); $^1H$ NMR ($D_2O$): δ 4.21 (d, 1H, H-1), 3.77-3.75 (m, 3H, H-4, 2 H-6), 3.65-3.55 (m, 2H, $CH_2O$), 3.53 (ddd≈m, 1H, H-5), 3.49 (dd, 1H, H-3), 3.45 (s, 6H, 3 $CH_2OH$), 3.37 (dd, 1H, H-2) ppm; $J_{1,2}$ = 7.7, $J_{2,3}$ = 10.2, $J_{3,4}$ = 4.4 Hz; $^{13}C$ NMR (100.62 MHz, $D_2O$): δ 103.86 (C-1), 75.48, 73.00, 72.24 (C-2, C-3, C-5), 69.29 (C-6), 69.02 (C-4), 61.38 ($CH_2O$-gal), 61.16 (3x) (3 $CH_2OH$), 45.75 ($\underline{C}(CH_2)_4$) ppm; FAB-MS 299.1 $[M+H]^+$ (299.13 calculated for $C_{11}H_{22}O_9H$).

***O*-(1-α-D-mannopyranosyl)pentaerythritol (8).** 8% isolated yield (4 mg, 0.01 mmol); $^1H$ NMR ($d_4$-MeOH): δ 4.74 (dd≈s, 1H, H-1), 3.90 (dd≈s, 1H, H-2), 3.80-3.61 (m, 6H, 5 ring-H, man-$OC\underline{H}_aH_b$), 3.60 (s, 6H, 3 $CH_2OH$), 3.37 (d, 1H, J= 9.4 Hz, man-

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$OCH_aH_b$) ppm; $^{13}C$ NMR (62.89 MHz, $d_4$-MeOH): δ 99.48 (C-1), 72.00, 70.28, 69.58, 66.03 (C-2, C-3, C-4, C-5), 64.80 (C-6), 60.38 (3 $CH_2OH$), 57.26 ($CH_2O$-man), 44.51 ($C(CH_2)_4$) ppm; FAB-MS 321.3 $[M+Na]^+$ (321.12 calculated for $C_{11}H_{22}O_9Na$).

**(2-Acetamido-2-deoxy -3,4,6-tri-*O*-acetyl - β-D-glucopyranosylmethyl) -4- (*O*-acetylhydroxymethyl)benzene (9).** 12% isolated yield (14 mg, 0.03 mmol); $^1H$ NMR ($CDCl_3$): δ 7.31 (d, 2H, 2 aryl-H), 7.28 (d, 2H, 2 aryl-H), 5.44 (d, 1H, NH), 5.21 (dd≈t, 1H, H-3), 5.11-5.06 (m, 3H, H-4, $CH_2OAc$), 4.89 (d, 1H, $OCH_aH_b$), 4.64 (d, 1H, H-1), 4.60 (d, 1H, $OCH_aH_b$), 4.27 (dd, 1H, H-6), 4.17 (dd, 1H, H-6'), 3.97 (ddd, H-2), 3.67 (ddd, 1H, H-5), 2.10 (s, 6H, 2 OAc), 2.02, 2.01 (each s, each 3H, 2 OAc), 1.92 (s, 3H, NAc) ppm; $J_{1,2}$ = 8.2, $J_{NH,H-2}$ = 8.6, $J_{2,3}$ = 9.7, $J_{3,4}$ = 9.7, $J_{4,5}$ = 10.2, $J_{5,6}$ = 4.5, $J_{5,6'}$ = 2.5, $J_{6,6'}$ = 12.2 Hz; $^{13}C$ NMR (62.89 MHz, $CDCl_3$): δ 170.99, 170.88, 170.74, 170.14, 169.38 (5 $C(O)CH_3$), 136.98 (aryl-C), 135.85 (aryl-C), 128.39 (2x) (2 aryl-CH), 128.17 (2x) (2 aryl-CH), 99.52 (C-1), 77.35 (C-5), 71.89 (C-4), 70.23 ($CH_2O$), 68.52 (C-3), 65.90 ($CH_2O$), 62.08 (C-6), 54.50 (C-2), 23.28 (NAc), 20.97, 20.74, 20.65, 20.59 (4 OAc) ppm.

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**(2,3,4,6- Tetra – *O* -acetyl-β-D-galactopyranosylmethyl)-4-(*O*-acetyl-hydroxy-methyl)benzene (10).** 13% isolated yield (18 mg, 0.03 mmol); $^1H$ NMR ($CDCl_3$): δ 7.27 (m, 4H, 4 aryl-H), 5.41-5.35 (m, 2H, H-2, H-4), 5.32-5.20 (m, 2H, $CH_2OAc$), 5.00 (dd, 1H, H-3), 4.89 (d, 1H, $OCH_aH_b$), 4.65 (d, 1H, $OCH_aH_b$), 4.53 (d, 1H, H-1), 4.28 (dd, 1H, H-6), 4.15 (dd, 1H, H-6'), 3.87 (ddd, 1H, H-5), 2.16, 2.07, 2.02, 1.99, 1.98 (each s, each 3H, 5 OAc) ppm; $J_{1,2}$ = 8.1, $J_{2,3}$ = 9.7, $J_{3,4}$ = 3.0, $J_{5,6}$ = 6.1, $J_{5,6'}$ = 2.5, $J_{6,6'}$ = 11.2 Hz; $^{13}C$ NMR (62.89 MHz, $CDCl_3$): δ 170.99, 170.88, 170.74, 170.14, 169.38 (5 $OC(O)CH_3$), 136.96 (aryl-C), 135.81 (aryl-C), 127.96 (2 aryl-CH), 127.32 (2 aryl-CH), 99.50 (C-1), 70.46 (C-5), 70.40 (C-3), 70.31 ($CH_2OAc$), 68.42 (C-2), 66.62 (C-4), 66.50 ($CH_2O$), 60.82 (C-6), 20.96, 20.31, 20.24, 20.12, 20.10 (5 OAc) ppm.

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