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# Enzymatic Galactosylation of Non-Natural Glucosamide-Acceptors

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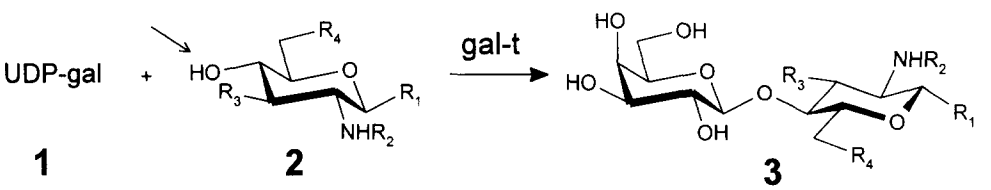
## ABSTRACT

**Commercial galactosyltransferase is used to galactosylate non-natural glucosamine derivatives. The enzyme tolerates various replacements of the natural N-acetyl group including charged and sulfonamide residues.**



As part of our program directed towards the synthesis of modified, more potent carbohydrate ligands for selectins[1)2)3)], we were prompted to explore the potential of glycosyltransferases. Glycosyltransferases form a class of enzymes which catalyze the regio- and stereospecific assemblage of glycosides in *vivo*[4)]. A sugar-residue is thereby transfered from a sugar-nucleotide-donor to an OH-group of an acceptor. On the whole this method has been tested in *vitro* for the synthesis of natural oligosaccharides[5)]. The limited knowledge of substrate recognition patterns of the enzymes has hampered the general applicability of this new and elegant method[6)7)].




**1** **2** **3**

Donor Acceptor

Scheme : Enzymatic galactosylation.

Most of the early, analytical investigations dealt with variations in the aglycon part of the acceptor (see entries 1-3 , table 1) and only one study probed the length of lipophilic N-acyl-residues on the acceptor (entry 5, table 1). A further study showed that an azido-group is tolerated as $R_4$ whereas an amino- or amido-group dramatically slowed down the turn-over rate (entry 6, table 1). More recent investigations with xylose[16)], 3-N-acetyl-glucosamine[17)] and conduritol[18)] acceptors revealed interesting new applications.

We wish to report here some of our unexpected and surprising findings concerning the galactosylations of glucosamine derivatives (entry 7, table 1, scheme).

Preparation of Acceptor Substrates:

Starting from the known, common precursor **2** ($R_2$=H)[19)20)] a number of N-acyl- or sulfonamides **2** have been prepared by standard acylations with the appropriate chlorides (see table 2, entries 1, 3, 8, 11-13, 18-25) or acid anhydrides (see table 2, entry 6)[21)22)]. Some hydroxyl-, thiol- or amino-functionalised acid-chlorides have been used in their corresponding allyloxy- or benzyloxy-protected forms (table 2, entries 4, 5, 10, 16) for the amidations. Those protecting groups have been removed in part by deallylation[20)] or hydrogenation. The formamide **2** ($R_2$=C(O)H)) is easily obtained (entry 2) by heating amine **2** ($R_2$=H) with methylformate in methanol and the thioacetamide **2** ($R_2$=C(S)$CH_3$) by heating ethyl-dithioacetate with the amine in dioxane[23)]. The β-nitropropionate (entry 15) is obtained by peptide coupling methodology[24)] and the oxo- and thioureas (entries 9, 14) were synthesized by stirring the amine with the corresponding isocyanates in dioxane. The guanylated compounds (entries 25, 26) were prepared by stirring the amines **2** ($R_2$=H or C(O)$(CH_2)_2NH_2$) with a versatile guanylating-reagent at slightly elevated temperature, followed by hydrogenation[25)]. Oxidation[26)] of the thiol with hydrogenperoxide in methanol finally yielded the sulfonate (table 2, entry 17).

| entry | R1 | R2 | R3 | R4 | Lit. |
|---|---|---|---|---|---|
| 1 | O-alkyl | acetyl | OH | OH | 8) 9) |
| 2 | O-peptides | acetyl | OH | OH | 10)11) |
| 3 | N-peptides | acetyl | OH | OH | 12) |
| 4 | OH | acetyl | O-ester, O-ether | OH | 13) |
| 5 | OH | acetyl, propionyl, butanoyl, hexanoyl, octanoyl | OH | OH | 14) |
| 6 | O-Bn | acetyl | OH | $N_3$, $NH_2$, HAc | 15) |
| 7 | $O(CH_2)_8COOMe$ | see text | OH | OH | |

Table 1.

Galactosylation Experiments:

All the starting acceptors listed in table 2 have been subsequently galactosylated with bovine UDPGal:N-acetylglucosaminide β1,4galactosyltransferase (E.C.2.4.1.22)[27)] on a preparative scale (see scheme). Besides the expected good turn-over of glucosamine-derivatives bearing small N-acylgroups we found - surprisingly - that the transferase accepted all the other glucosamine-derivatives favourably. Highly bulky substituents were also tolerated, with excellent results, provided that their low solubility is enhanced by the addition of DMSO. DMSO can be added to at least 15% (vol/vol) without any adverse effects on the enzyme. The enzyme also tolerates both negatively and positively charged N-acylgroups (entries 5, 16, 17, 26) close to the 'reaction-pocket'. This is contradictory to the

common notion of a small lipophilic N-acyl-binding pocket on the enzyme for substrate recognition[14)17)28)]. A NHC=O moiety is also not exclusive for enzyme recognition. Replacement by a NHC=S moiety (entries 7-9) or even by a sulfonamide (entries 23, 24) leads to excellent yields of **3**.

Nonetheless some limitations were encountered (compare low yields of entry 10). Both the substrate and the product suffered partial oxidative decomposition during incubations.

The structural features of all new compounds **3** - in comparison to the known one (entry 1) - were proven by $^{1}H$-, $^{13}C$ NMR- and MS-spectra. Some selected data are included in table 2. The connectivity of the disaccharides has been supported further by ensuing stereospecific glycosylations e. g. at the 3-OH-group of the glucosamine moieties (see succeeding paper).

In addition to the reports of Hindsgaul et al.[29)] and Kodama et al.[30)] these results demonstrate that such transferases may be successfully employed for efficient and predictable synthesis of non-natural oligosaccharides.

It proved to be favourable to add calf intestine alkaline phosphatase (CIAP, E.C.3.1.3.1) to the incubation mixture in order to destroy the uridine-diphosphate (UDP) by-produkt, which was found to inhibit the β1,4galactosyltransferase[12)31)].

*Representative experimental procedure: 14.9 mg (32.9 μmol) of glucosamide* **2** *($R_2$ = C(O)Ph), 23.2 mg (37.3 μmol) UDP-galactose (Sigma), 2 mg bovine serum albumine (Boehringer) and 12.3 mg (52.5 μmol) $MnCl_2 \cdot 6H_2O$ were put together into 1.8 ml sodium-cacodylate buffer solution (0.1 m, pH=7.52) containing 150 μl DMSO and sonicated to give a milky but homogeneous mixture. After addition of 625 mU (250μl) galactosyltransferase (Sigma G-5507, 6U/mg prot., stock solution containing 25U/10ml cacodylate buffer as above) and 30 U (2 μl) alkaline phosphatase (Boehringer No. 108146, 7500U/498μl) the mixture was briefly vortexed and stirred at 37°C. When TLC-control (mixtures of $CH_2Cl_2$-MeOH), after variable incubation periods, showed the consumption of the acceptors* **2** *the precipitates were centrifuged off and the supernatant purified on a short silica-gel column (eluent: mixtures of $CH_2Cl_2$-MeOH). The resulting product* **3** *($R_2$ = C(O)Ph) was obtained as a white powder after lyophilisation from dioxane-water (15.8 mg, 81%).*

| | $R_2$ (**2** or **3**) | % (mg) | glcN: H-1** | gal: H-1** | glcN: C-1, 2, 4** | gal: C-1, 3, 4** | C-others** (aglycon, NHR) |
|---|---|---|---|---|---|---|---|
| 1 |  | 95 (35.0) | 4.46 | 4.49 | 101.91, 56.05, 80.10 | 104.08, 74.57, 69.55 | 70.61, 23.04 |
| 2 |  | 88 (28.4) | 4.38 | 4.45 | 101.90, 55.10, 79.96 | 104.30, 74.05, 69.72 | 70.67, 164.02 |
| 3 |  | 90 (30.8) | 4.37 | 4.44 | 102.90, 56.61, 80.72 | 104.90, 75.21, 70.41 | 71.57, 18.85 |
| 4 |  | 84 (34.6) | 4.42 | 4.55 | 102.83, 56.83, 80.51 | 104.88, 74.50, 70.54 | 72.60, 63.05 |
| 5 |  | 75 (65.8) | 4.43 | 4.53 | 101.76, 55.94, 79.29 | 103.76, 75.92, 69.43 | 71.42, 44.38 |
| 6 |  | 80 (27.2) | 4.08 | 4.19 | 101.81, 57.06, 81.90 | 105.23, 75.79, 70.17 | 70.42, 120.85 |
| 7 |  | 100 (29.3) | 4.41 | 4.51 | 102.58, 61.80, 80.67 | 105.04, 74.77, 70.34 | 70.62, 33.85 |
| 8 |  | 85 (18.6) | n.d. | n.d. | 102.24, 59.49, 80.21 | 104.59, 74.36, 70.07 | 71.14, 14.65 |
| 9 |  | 98* (40.0) | 4.49 | 4.49 | 102.83, 60.47, 82.20 | 104.53, 74.94, 69.78 | 70.61, 14.28 |
| 10 |  | 29* (6.5) | 4.45 | 4.50 | 102.74, 57.16, 81.12 | 105.03, 74.75, 70.06 | 70.90, 44.08 |
| 11 |  | 100 (17.5) | 4.32 | 4.36 | 103.14, 58.54, 80.90 | 105.04, 74.27, 70.33 | 70.75, 117.25 |
| 12 |  | 90* (28.0) | 4.36 | 4.49 | 103.15, 58.33, 80.93 | 104.99, 74.74, 70.28 | 70.78, 15.05 |
| 13 |  | 90 (26.0) | 4.48 | 4.41 | 102.69, 58.02, 80.56 | 104.69, 74.43, 70.11 | 71.06, 16.20 |
| 14 |  | 72* (21.7) | 4.38 | 4.41 | 103.38, 57.79, 79.49 | 104.39, 75.59, 70.29 | 71.19, 15.62 |

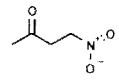
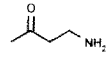
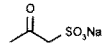
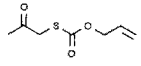
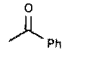
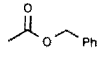
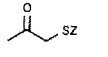
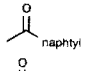
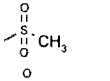
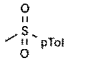
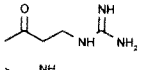
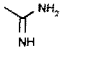
| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 15 |  | 42 (13.8) | 4.41 | 4.46 | 102.29, 56.51, 80.50 | 104.56, 74.33, 69.90 | 72.29, 34.75 |
| 16 |  | 82 (17.0) | 4.42 | 4.49 | 101.82, 55.94, 79.61 | 103.95, 76.08, 69.56 | 71.55, 36.64 |
| 17 |  | 77 (38.7) | 4.41 | 4.53 | 102.26, 56.92, 79.65 | 104.16, 73.82, 69.86 | 72.12, 62.35 |
| 18 |  | 72 (34.1) | 4.31 | 4.39 | 102.13, 56.86, 80.15 | 104.35, 76.45, 69.36 | 71.13, 119.80 |
| 19 |  | 81* (15.8) | 4.42 | 4.61 | 102.72, 57.20, 81.03 | 104.89, 74.29, 70.24 | 71.13, 132.77 |
| 20 |  | 81* (17.0) | 4.35 | 4.36 | 103.43, 58.50, 81.19 | 105.03, 74.80, 69.78 | 70.62, 67.54 |
| 21 |  | 76 (24.4) | n.d. | n.d. | 102.09, 56.78, 80.13 | 104.33, 74.09, 69.81 | 70.42, 35.58 |
| 22 |  | 78* (15.6) | 4.42 | 4.45 | 101.98, 56.77, 80.65 | 104.21, 74.00, 69.57 | 70.58, 124.44 |
| 23 |  | 87 (29.8) | 4.47 | 4.47 | 102.26, 60.37, 79.52 | 103.96, 74.16, 69.65 | 71.69, 42.89 |
| 24 |  | 82* (16.3) | 4.21 | 4.48 | 102.97, 60.98, 80.59 | 104.94, 74.89, 70.38 | 70.73, 21.52 |
| 25 |  | 64 (29.7) | 4.48 | 4.52 | 102.59, 56.58, 81.24 | 105.18, 74.77, 70.25 | 70.64, 38.81 |
| 26 |  | 73 (29.1) | 4.39 | 4.46 | 103.78, 60.60, 81.13 | 105.62, 75.10, 71.11 | 73.47, 180.55 |

Table 2: * addition of DMSO; ** relative to methanol, J ~ 7.2 Hz (dublett each), n.d. not determined.

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