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Preliminary communication

Methyl-3-*O*-(2-acetamido-2-deoxy-6-thio-β-D-glucopyranosyl)-β-D-galactopyranoside: a slow reacting acceptor-analogue which inhibits glycosylation by UDP-D-galactose–*N*-acetyl-D-glucosamine-(1 → 4)-β-D-galactosyltransferase

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Inhibitors of glycosyltransferase, especially those specific for their glycosyl acceptors, would be of potential use for studies on biological functions of cell-surface carbohydrates. For fucosyltransferase[1] (EC 2.4.1.69) and glucosaminyltransferase V (EC 2.4.1.155, ref. 2), inhibitors in the form of transition-state and glycosyl-acceptor analogues, respectively, have been found. However, in the case of UDP-D-galactose–*N*-acetyl-D-glucosamine-(1 → 4)-β-D-galactosyltransferase, (EC 2.4.1.90), only UDP-D-glucose was reported[3] to competitively inhibit galactosyl transfer. Accordingly, our approach to investigate the recognition mechanism of galactosyltransferase for the acceptor was begun with the elucidation of the role of the hydroxyl group of the acceptor, i.e., the 2-acetamido-2-deoxy-β-D-glucopyranosyl (β-GlcNAc) moiety by modifying the 3-, 4-, and 6-positions with either a fluoro or a sulfhydryl group. Investigation of the kinetics of these chemically modified compounds may elucidate the acceptor recognition mechanism of the enzyme. For both the fluoro and sulfhydryl analogues, the nature of hydrogen-bond formation is expected to be biased to a hydrogen-accepting and -donating role, respectively. In this communication, we would like to report the effect of the substitution at the 6-position of the β-GlcNAc moiety on the kinetics and competitive inhibition of bovine (1→4)-β-D-galactosyltransferase by the 6′-sulfhydryl disaccharide analogue **12**.

Our preliminary investigation showed that the 6-deoxy analogue (**2**) of methyl 2-acetamido-2-deoxy-β-D-glucopyranoside (**1**) had a K_m (app.) value of 0.5 mM,

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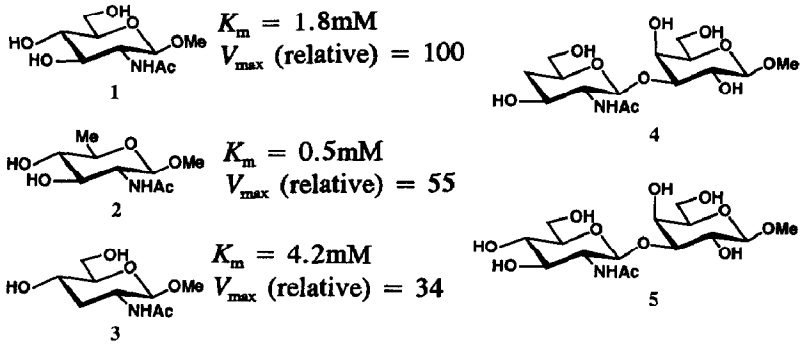
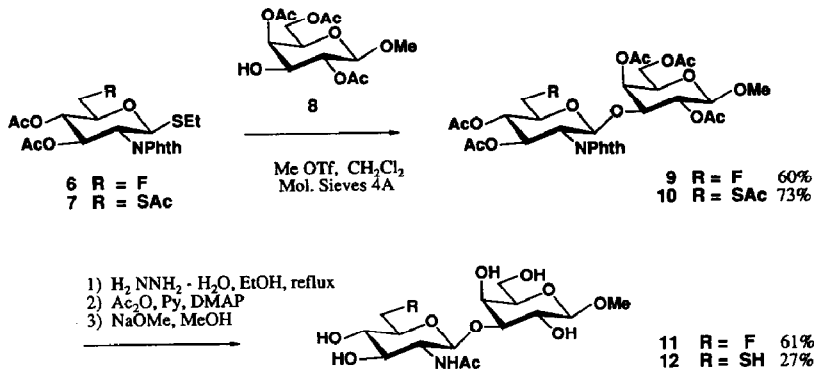


Assay conditions: acceptor (0.5–3 mM); $MnCl_2$ (40 mM); (1→4)-β-D-galactosyltransferase (0.83×10^{-5} units for the acceptor **1**); and UDP-[U-^{14}C]-D-galactose (3 μM). Inhibition assay: **4** (2 mM); acceptor **5** (0.5 mM). The other three components were the same as above.

and the 3-deoxy analogue (**3**) of **1** had a K_m (app.) value of 4.2 mM. However, the 4′-deoxy analogue (**4**) of methyl 3-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-β-D-galactopyranoside (**5**) did not inhibit the reaction of bovine (1 → 4)-β-D-galactosyltransferase[4]. These results suggested that the 4-hydroxyl group of GlcNAc moiety is necessary for binding with the galactosyltransferase. A similar result was also reported[5] using the corresponding *O*-methyl analogues of GlcNAc. However, the K_m value of the 6-deoxy analogue **2** suggested that chemical modification at the 6-position of GlcNAc may not cause a severe decrease in the affinity to the galactosyltransferase. Furthermore, a preliminary investigation also showed that the disaccharide **5** had higher affinity for the galactosyltransferase than had **1**. Therefore, we planned the syntheses of the 6′-fluoro (**11**) and 6′-sulfhydryl (**12**) analogues of **5** as candidates for a galactosyltransferase inhibitor.

Two glycosyl donors **6** and **7** were prepared from ethyl 2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside[6]. Selective fluorination of the primary hydroxyl group of this thioglycoside with diethylaminosulfur trifluoride in CH_2Cl_2 (ref. 7) at −40°–room temperature, followed by acetylation (Ac_2O, pyridine, room temperature), gave **6** in 33% overall yield. Selective tosylation of the primary hydroxyl group of the same thioglycoside (TsCl, pyridine, room temperature) and subsequent acetylation afforded, in 83% yield, its 6-tosylate, which was transformed in 82% yield to the corresponding 6-acetylthio derivative **7** by reaction with potassium thioacetate in *N*,*N*-dimethylformamide at 80°. Glycosylation of methyl 2,4,6-tri-*O*-acetyl-β-D-galactopyranoside (**8**) with **6** or **7** in the presence of methyl triflate in CH_2Cl_2 at room temperature afforded the (1→3)-β-linked disaccharides **9** and **10**, respectively. *N*-Dephthaloylation of **9** and **10** followed by *N*,*O*-acetylation and *O*-deacetylation afforded corresponding disaccharides **11** and **12**, respectively, both of which were shown to be analytically pure by high-performance liquid chromatography and by their 500 MHz ^{1}H-NMR spectra [D_2O, sodium 3-(trimethylsilyl)propionate as the external standard], **16**: δ 4.66 (bd, 2 H, $J_{6'a,F} = J_{6'b,F}$ 47.4



Hz, H-6′a,6′b), 4.70 (d, 1 H, $J_{1',2'}$ 8.5 Hz, H-1′); **17**: δ 4.64 (d, 1 H, $J_{1',2'}$, 8.4 Hz, H-1′), 3.24 (bd, 1 H, $J_{6'a,6'b}$ 14.3 Hz, H-6′a), 2.81 (dd,1 H, $J_{5,6'b}$ 9.1 Hz, H-6′b).

Galactosylation of the disaccharide **5** and its modified analogues **11** and **12** by bovine (1→4)-β-D-galactosyltransferase was assayed by the method of Babad and Hassid[8]. Incubations were performed at 37° for 15–60 min in 20 mM Tris–HCl buffer (pH 7.5) (30 μL), which contained the following assay components: synthetic disaccharide acceptor (2–0.5 mM), manganese(II) chloride (40 mM), bovine serum albumin (1×10^{-2} μg), bovine galactosyltransferase (1.2×10^{-6} units), and UDP-[U-^{14}C]-D-galactose (3 μM, 9.92 GBq/mmol). The reaction was traced up to the 15% consumption of UDP-D-galactose. The kinetic parameters K_m and V_{max} were obtained by using the computer program based on the method of Wilkinson[9]. The disaccharide **5** proved to be a good acceptor of the galactosyl residue with a K_m (app.) value of 1.08 ± 0.16 mM. The 6′-fluoro analogue **11** showed a K_m (app.) value of 1.03 ± 0.44 mM, and the V_{max} value was the same as that for **5**. On the other hand, the 6′-sulfhydryl analogue **12** was an extremely poor galactosyl acceptor. Studies of the galactosylation of **12**, in which the enzyme was increased over 25-fold, indicated that the V_{max} value was very much lower than that of **5**, while the K_m value was not strongly affected (see Table I).

TABLE I

Kinetic parameters for galactosylation of modified disaccharides **11** and **12** by bovine (1→4)-β-D-galactosyltransferase [a]

Compound	6′-Substituent	K_m (mM)	V_{max} (relative)
5	OH	1.08 ± 0.16	1.0
11	F	1.03 ± 0.44	1.0
12	SH	– [b]	– [b]

[a] The amount of the galactosyltransferase was 1.2×10^{-6} units for the acceptor **5**. [b] The corresponding parameters estimated from β-D-galactosyltransfer reaction using over 25-fold enzyme were as follows: $K_m < 9$ mM, V_{max} (relative) < 0.06.

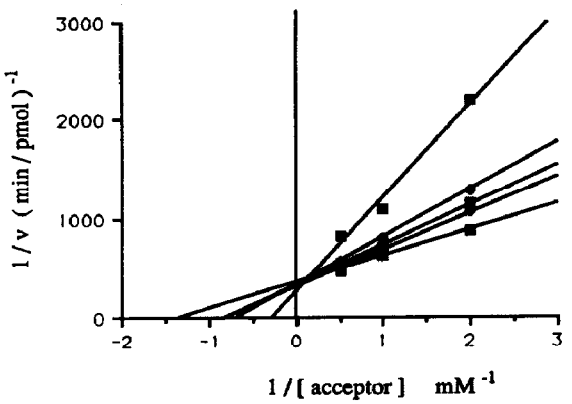


Fig. 1. Double-reciprocal plot of (1→4)-β-D-galactosytransferase reaction for **5** as an acceptor. Incubations were performed as described in the text. The concentration of UDP-[U-^{14}C]-D-galactose was fixed at 3 μM, while the concentration of **5** was changed together with that of **12**: 0, 300, 600, 900, and 2000 μM. Each data point is the average of at least three values.

Furthermore, **12** was found to be an inhibitor of the (1 → 4)-β-D-galactosyltransferase. The K_i value obtained for the 6′-sulfhydryl analogue **12** as determined by an inhibition assay toward the galactosylation of the disaccharide **5** using 1.6×10^{-6} units of the (1→4)-β-D-galactosyltransferase was 1.00 ± 0.09 mM, and the inhibition was shown to be competitive (Figs. 1 and 2)[10]. Thus the systematic chemical modification by the substitution of a hydroxyl group with either a hydrogen, a fluoro, or a sulfhydryl group was demonstrated to be effective for investigating the function of the hydroxy group and also for design of synthetic inhibitors. Further studies on the mechanism of the inhibition shown by the 6′-sulfhydryl group, which make use of compounds having similar substitution at the 3- and 4-positions, are in progress.

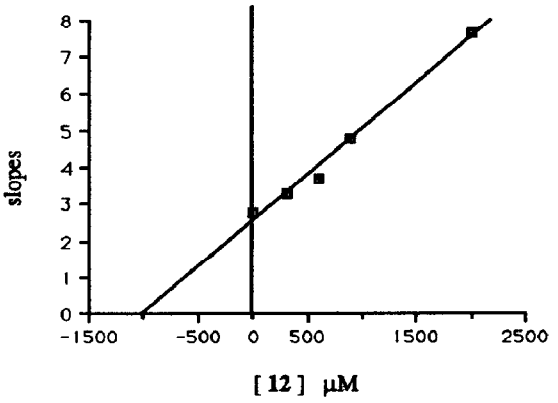


Fig. 2. Secondary plot of slopes obtained from the data of Fig. 1 vs. [**12**]: K_i of 1.00 ± 0.09 mM.

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