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Acceptor Analogues as Potential Inhibitors of Bovine β-1,4-Galactosyl Transferase

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Abstract: A series of 4- and 6-substituted derivatives of benzyl *N*-acetyl-β-D-glucosaminide were prepared and assessed as inhibitors of bovine β-1,4-galactosyl transferase. Of the azido, amino, and acetamido compounds tested, only the 4-amino compound (**2**) produced significant inhibition of this enzyme.

UDP-D-Galactose : D-glucose β-1,4-D-galactosyl transferase [EC 2.4.1.22] (hereafter referred to as β-1,4-Gal T) catalyzes the transfer of D-galactose from UDP-D-galactose to form β-1,4-linkages to glucose and to the non-reducing terminal *N*-acetyl-D-glucosamine of oligosaccharides in the presence and absence respectively of α-lactalbumin[1]. The donor[2] and acceptor[3] specificities of the bovine enzyme have been extensively studied with both natural and synthetic substrates. Despite the volume of work, few inhibitors of this enzyme[4], or any other glycosyl transferase, have been reported (reviewed in ref 5).

Studies in these laboratories have demonstrated[6] that a 2-amino-2-deoxy-D-mannose-containing glycolipid may act as an acceptor analogue inhibitor of a dolichol-phosphate-mannose dependent mannose-α-1,2-mannose mannosyl transferase from *Trypanosoma brucei*. We interpreted these observations[7] in terms of a model for acceptor binding to glycosyl transferases put forward by Hindsgaul[8]. This model proposes that for some, but not all, glycosyl transferases there is a critical hydrogen bond between the hydroxyl group to be glycosylated on the acceptor and a basic residue at the active site of the enzyme (Figure 1A).

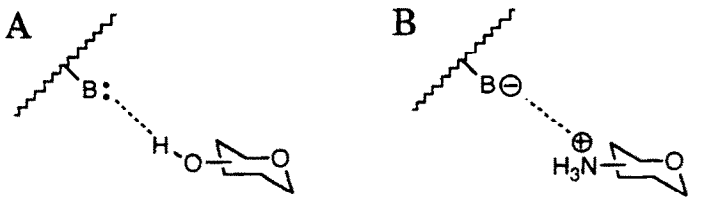
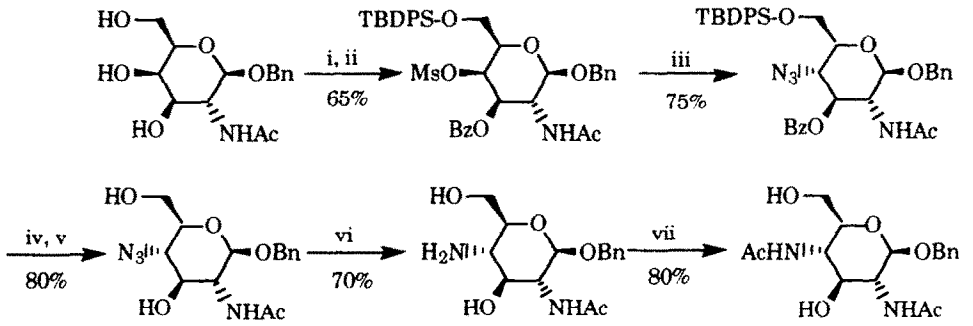


Figure 1 *Representations of: **A** - a glycosyl transferase / acceptor substrate interaction; **B** - a prospective glycosyl transferase / acceptor analogue inhibitor interaction.*

For amino-substituted acceptor analogues, which would be protonated and hence cationic under normal assay conditions (pH 7.5), the enzyme-acceptor hydrogen bond may be replaced by a charge-charge interaction in the enzyme-inhibitor complex (Figure 1B). This requires the active-site base to be anionic, which, given the occurrence of active-site carboxylic acids / carboxylate anions in glycosidases[9] may not be unreasonable. Comparison may be drawn between amino-

substituted acceptors as glycosyl transferase inhibitors and amino-substituted aglycone analogues as glycosidase inhibitors[10]. The current study was undertaken in an attempt to exploit the putative acceptor / active-site base interaction of bovine β-1,4-Gal T in the development of inhibitors of this enzyme.

Hydrophobic glycosides[3e] have K_m values in the same range (150-200μM) as the natural bianntennary glycan substrates for β-1,4-Gal T. Current studies have therefore focussed on benzyl *N*-acetyl-β-D-glucosaminide (hereafter referred to as benzyl β-GlcNAc) which has a K_m value of 120μM for the bovine enzyme. Moreover, we have demonstrated[7] that benzyl β-GlcNAc is taken up, and subsequently glycosylated, by Chinese hamster ovary cells. Synthetic targets for this study were therefore benzyl β-GlcNAc derivatives in which the OH group at the 4-position of the sugar ring was replaced by N_3 (negative control; potential hydrogen-bond acceptor), NH_2 (protonated under the assay conditions; potential charge-charge interaction), and NHAc (neutral; potential hydrogen-bond donor and acceptor). The requisite compounds, (**1**) to (**3**), were prepared from benzyl *N*-acetyl-β-D-galactosaminide as outlined in Scheme 1[11].



(**1**) (**2**) (**3**)

i. TBDPS-Cl / DMF / imidazole, ii a) BzCl / CH_2Cl_2 / pyridine, -50°, b) MsCl, 0° to 20°, iii NaN_3 / DMF, 110°, iv MeONa, v Bu_4NF / THF, vi PPh_3 / MeOH / pyridine / NH_4OH, vii Ac_2O / $NaHCO_3$ aq

Scheme 1

Previous studies[3c,8,12] have shown that the removal of hydrogen-bond donating ability from the 4-position of an acceptor abolishes its binding to β-1,4-Gal T. Similar modification[3c,4c] of the 3- or 6- positions of the acceptor has a much less dramatic effect. 6-Substituted derivatives (N_3, NH_2, and NHAc) of benzyl β-GlcNAc were therefore synthesized[11], using standard procedures, for use as 'negative' controls in inhibition experiments.

The synthetic compounds were screened as inhibitors of, and substrates for, bovine Gal T[13]. At 100-500μM concentrations, only the 4-amino compound (**2**) showed detectable inhibition with benzyl β-GlcNAc as substrate ([S]=120μM; K_m=120μM). Further analyses showed compound (**2**) to be a competitive inhibitor of the bovine enzyme with *N*-acetyl-D-glucosamine as substrate (Figure 2A). The inhibition constant, Ki=850μM, was determined from separate experiments using both benzyl β-GlcNAc and GlcNAc as substrates.

The synthetic compounds were also evaluated as alternate substrates for β-1,4-Gal T at 100μM concentration; that is, in the range of K_m for benzyl β-GlcNAc (Table 1). The results indicate that of the 4-substituted compounds prepared, only the 4-amino compound (**2**) was able

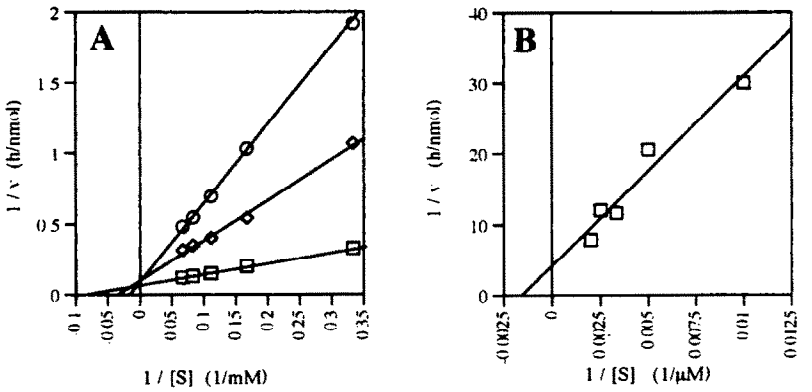


Figure 2: ***A.*** *Benzyl 4-amino-4-deoxy β-GlcNAc (**2**) ([I] = 0, 2.52, 5.04mM) as an inhibitor of bovine β-1,4-Gal T with GlcNAc as substrate*
B. *Benzyl 4-amino-4-deoxy β-GlcNAc (**2**) as a substrate for bovine β-1,4-Gal T*

to stimulate significant production of neutral radioactive material from UDP-[^{3}H]galactose. The 4-acetamido compound (**3**) acts as neither a substrate for nor inhibitor of β-1,4-Gal T, indicating that the enzyme is unable to use efficiently the hydrogen-bond donating ability of an acetamido group; steric constraints cannot be discounted however. Further studies of β-1,4-Gal T with (**2**) as the acceptor gave K_m = 670μM and $V_{max} \approx 1\%$ of that of the benzyl β-GlcNAc (Figure 2B). The K_m value is in reasonable agreement with the K_i value (850μM) determined from earlier experiments.

Table 1: Analogues of Benzyl N-acetyl-β-D-Glucosaminide as Substrates for Bovine β-1,4-Galactosyl Transferase

Results, reported as percentages of the initial rate of the reaction with benzyl *N*-acetyl-β-D-glucosaminide (4-OH) as 100%, were obtained from experiments performed in triplicate. All compounds were assayed at 100μM† concentration[13]

Compound	% Rate	Compound	% Rate
4-OH	100		
4-N_3 (**1**)	n.d.	6-N_3	27
4-NH_2 (**2**)	2	6-NH_2	4
4-NHAc (**3**)	n.d.	6-NHAc	2

n.d. - not detectable, † - similar results were obtained at 1mM concentration

Modification (OH→N_3, NH_2, NHAc) of the 6-position of benzyl β-GlcNAc renders the molecule a moderate or weak substrate for β-1,4-Gal T. In contrast, substitution of the acceptor at the 6-position is known to be tolerated by β-1,4-Gal T[3c], and even a bulky 6-α-L-fucosyl residue is accommodated by the enzyme. However, a *Saccharomyces cerevisiae* mannose-α-1,2-mannose mannsoyl transferase uses 6-azido and 6-amino acceptor analogues only very poorly[14].

At least two mechanisms could explain the apparent ability of the 4-amino compound (**2**) to act as a substrate for β-1,4-Gal T. It might act as a true acceptor for the transferase, forming a hydrolytically labile[15] β-glycosylamine intermediate that hydrolyzes *in situ* with the liberation of [^{3}H]galactose, or it might bind to the transferase and stimulate the hydrolysis of UDP-galactose without acting as an acceptor for galactosyl transfer[16].

Conclusions

Inhibition of bovine β-1,4-Gal T by benzyl 4-amino-4-deoxy-β-GlcNAc (**2**) is modest, with K_i=850μM being notably higher than K_m=120μM for the corresponding alcohol. The data obtained confirm that there is a highly specific interaction between the 4-position of the acceptor and the

enzyme. It appears that it is not possible to exploit the full potential of the interaction between the acceptor and the acceptor binding site of β-1,4-Gal T by replacement of the 4-hydroxy group of the acceptor with an amino group. Related observations have been made regarding carbohydrate-lectin interactions[17]. In contrast, Lowary and Hindsgaul have reported[18] the strong inhibition of human serum α-1,3-GalNAc transferase by a 3-amino disaccharide acceptor analogue, suggesting that there may be an anionic residue adjacent to the acceptor binding site of this enzyme.

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