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Synthesis of Novel Disaccharides Based on Glycosyltransferases: β1,4Galactosyltransferase[1]

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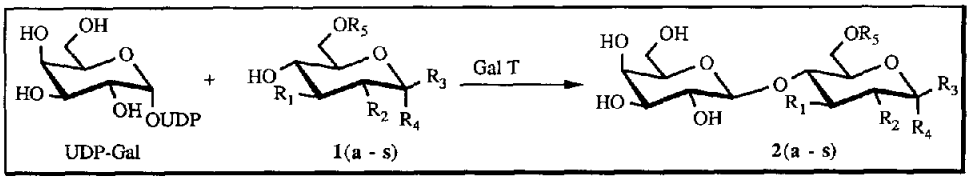
Abstract: β1,4Galactosyltransferase and β-galactosidase have been investigated with regard to their acceptor specificity and used in the synthesis of galactosides using 5-thioglucose, deoxyazaglucose, glucal, modified N-acetylglucosamine and glucose derivatives as acceptors.

Sophisticated chemical procedures for protection/deprotection, activation, and coupling are now available for the stereocontrolled synthesis of oligosaccharides.[2] Synthesis of oligosaccharides containing unnatural sugar derivatives, however, still represents a significant problem; different protection and deprotection strategies are often required for such a synthesis. Enzymatic oligosaccharide synthesis based on glycosyltransferases[3] or glycosidases[4] is potentially a useful alternative to the chemical synthesis. Glycosyltransferases are highly specific with regard to their glycosidic linkages, and no protection/deprotection steps are required. Procedures for the regeneration of sugar nucleotides are also available for large-scale processes.[5,6] Synthesis of oligosaccharides containing unnatural monosaccharide units based on glycosyltransferases, however, has not been well explored. As part of our interests in the development of enzymatic procedures for the synthesis of uncommon disaccharides and oligosaccharide building blocks containing removable protecting groups, we here report our initial study on the use of β1,4galactosyltransferase (GalT, EC 2.4.1.22, from bovine milk) and β1,4galactosidase (EC 3.2.1.23, from *E. coli*) in the synthesis of several galactosides.

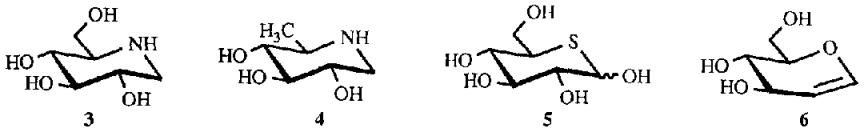
It is known that GalT from bovine milk accepts N-acetylglucosamine (GlcNAc) and its glycosides (β is better than α-glycoside) as substrates.[7] Glucose and its α- and β-glucosides are also acceptable; however, lactalbumin is required for α-glucosides. Some 6-O-glycosyl derivatives (e.g. Fucα1,6GlcNAcOR, NeuAc(OMe)α2,6GlcNAcβOR) and 3-O-methyl GlcNAc were reported to be weak substrates for the enzyme.[8] Several UDP derivatives of galactose analogs (e.g. glucose, 4-deoxy-glucose, arabinose, N-acetylgalactosamine, and glucosamine) were also acceptable as donors with a rate ~ <5% that of GlcNAc.[9]

Our initial study on the acceptor specificity of GalT indicates that the enzyme is highly specific for GlcNAc and glucose, and their related glycosides. A substantial decrease of activity was observed for GlcNAc derivatives with modification at position 3. While some weak activities were observed for 3-substituted β-glycosides, no activity was observed for the corresponding α-glycoside (e.g. **1 g**). Glucose and derivatives are acceptable in the presence of lactalbumin. The same trend was observed with 3-substituted glucose derivatives. The 3-oxo-(**1 n**) and 3-epimeric (**1 o**) derivatives of GlcNAc are acceptable at 2% and



Cmpd	R_1	R_2	R_3	R_4	R_5	Rel. Rate(%)
1a	OH	AcNH	(H,	OH)	H	100
1b	OH	AcNH	H	CH_3O-	H	75
1c	OH	AcNH	H	CH_2=$CHCH_2O$-	H	25
1d	$CH_3CHOCO_2^-$	AcNH	(H	OH)	H	0.6
1e	OH	AcNH	(H,	OH)	CH_3CO-	4
1f	AcO-	AcNH	(H,	OH)	H	0.4*
1g	CH_2=$CHCH_2O$-	AcNH	H	OMe	H	0
1h	$CH_3(CH_2)_2O$-	AcNH	(H,	OH)	H	0.5
1i	H	AcNH	CH_2=$CHCH_2O$-	H	H	1.0
1j	CH_2=$CHCH_2O$-	AcNH	$CH_3(CH_2)_3O$-	H	H	0.3
1k	MeOCO-	AcNH	CH_2=$CHCH_2O$-	H	H	-*
1l	Ally-OCO-	AcNH	CH_2=$CHCH_2O$-	H	H	-*
1m	$MeOCH_2O$-	AcNH	CH_2=$CHCH_2O$-	H	H	2.0
1n	O	AcNH	CH_3O-	H	H	2.0
1o	epi-OH	AcNH	(H,	OH)	H	0.04
1p	OH	AcNH	-SPh	H	H	-
1q	O	AcNH	H	OMe	H	0.1
1r	OH	Phthalimido	H	SPh	H	0
1s	epi-OH	AcNH	H	OMe	H	0
1t	OH	OH	(OH,	H)	H	100
1u	CH_2=$CHCH_2O$-	OH	(OH,	H)	H	0
1v	MeO-	OH	(OH,	H)	H	10
1w	OH	OH	SPh	H	H	0.1
1x	OH	OH	SPh	H	CH_2Ph	0.04
3						3
4						0.1
5						70
6						0.4

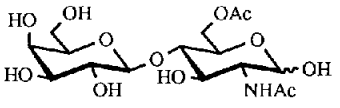
Conditions: UDP-Gal (0.2 mM), acceptor (25 mM), lactalbumin (0.1 mg/mL for Glc derivatives, **1t** - **1x** and **3** - **6**), $MnCl_2$ (5 mM), phosphoenol pyruvate (1 mM), NADH (0.3 mM), pyruvate kinase (10 U) and L-lactate dehydrogenase (10 U) in 1 mL of cacodylate (0.05 M), pH 7.0 was added 40 mU of Gal T. (Fitzgerald, D. K.; Colvin, B.; Maval, R.; Ebner, K. E. *Anal. Biochem.* **1970**, *36*, 43). The initial rate was determined based on the decrease of absorbance at 340 nm (ε=6.22 $mM^{-1}cm^{-1}$). The detection limit of this method is about 0.1%. The rates for Glc derivatives (the bottom portion) are compared to that of Glc, and the rates for GlcNAc derivatives (the top portion) are compared to GlcNAc. *3->6 O-Acyl migration occurs. No 3-O-acyl product was formed.



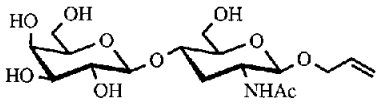
0.04% rate, respectively. 6-O-Acetyl-GlcNAc (4%) and deoxynojirimycin (**3**, 3%) are also reasonable substrates. 5-Thioglucose (**5**) is a good substrate with a relative rate 70% that of glucose. Azasugar and glucal **6**, however, are weak substrates.

Despite the weak activity with unnatural substrates, GalT was used as a catalyst in the synthesis of several galactosides. In a representative procedure for the synthesis of **7**, 5-thioglucose (100 mg, 500 μmol), GalT (5 U, $28.45/U), UDP-glucose (350 mg, 500 μmol), α-lactalbumin (0.1 mg/mL) and UDP-glucose epimerase (10 U)[10] were dissolved in 10 mL of 50 mM sodium cacodylate (pH 7.0) containing 5 mM of $MnCl_2$. The reaction mixture was incubated at 37°C or 2 days. The product was isolated via a Dowex 1 formate column followed by gel filtration (Bio Gel P-2) to give 90 mg of the disaccharide in 50% yield. A similar procedure was applied to the syntheses of **2i, 2j, 8** and **10** with a longer reaction time (4 days) and each was obtained with the yield in the range of 20-40%. It is worth noting that no byproducts were observed; the low yields were due to the incomplete reaction. In reactions with 3-O-acyl-GlcNAc derivatives (**1f, 1k, 1l**), migration of the 3-0-acyl group to 6-OH was observed,[11] resulting in the recovery of the disaccharide containing a 6-0-acyl group in the acceptor moiety (e.g. **2e** in ~40% overall yield) and no 3-O-acyldisaccharide was obtained.

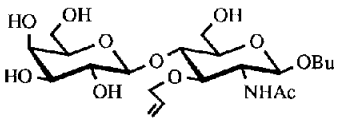
These syntheses demonstrate that GalT can be used as catalyst for the preparation of galactosides with weak acceptor substrates. Given that the enzyme is relatively stable,[5] GalT (and perhaps other glycosyltransferases) seems amenable for the synthesis of a number of unusual oligosaccharides. Since β-galactosidase can also be used in the synthesis of β-galactosides,[4] the enzyme was investigated for the synthesis of GalT products. 5-Thioglucose (**5**) was reacted with *p*-nitrophenyl β-galactoside in the presence of β-galactosidase. The product obtained (29%), however, possesses a β1,6 linkage.[12] When compound **3** or **4** was used as a substrate, no product was obtained.



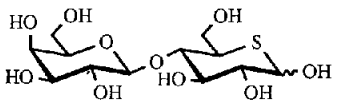
2e



2i



2j



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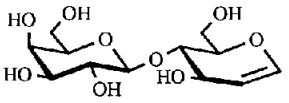

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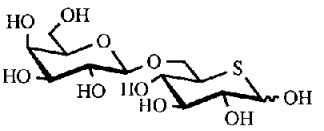

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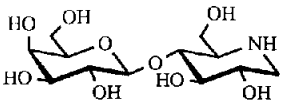

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Since **3**, **4**, **5** and **6** are potent inhibitors of glucosidases (e.g. β-glucosidase),[13] their glycosides may be potent inhibitors of other sequence-specific glycosidases. Compounds **2i**, **2n**, and **2o** are expected to be inhibitors of α1,3-fucosyltransferase due to the lack of appropriately oriented 3-OH group.[14] Compound **2j** is useful for the synthesis of Le^x (Galβ1,4(Fucα1,3)GlcNAc).[15]

In summary, we have demonstrated that GalT can be used as an effective catalyst for the assembly of specific glycosidic bonds containing unusual sugar derivatives without complicated protection and deprotection steps. A number of novel oligosaccharide structures may become accessible via the glycosyltransferase reaction. Work is in progress to explore the unusual catalytic properties of other glycosyltransferases.

References and Notes

§ Also at University of California, San Diego.

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10. This enzyme catalyzes the epimerization of UDP-Glc to UDP-Gal. UDP-Glc is less expensive than UDP-Gal. See Wong et al. in ref. 5.
11. We have confirmed by ^{1}H-NMR that the migration occurs before galactosidation. The CH_2 protons at C-6 showed a downfield shift (~0.5 ppm) upon acylation.
12. The glycosidic linkages were determined based on ^{1}H and ^{13}C-NMR data and NOE analysis. A significant downfield shift (0.1-0.3 ppm) for the protons attached to the carbon involved in glycosidic linkage was observed. This is further confirmed via acetylation of the disaccharide product. A downfield shift (1.3-1.8 ppm) was observed for the protons with the geminal OH group acetylated. All other compounds prepared in this study were further confirmed by ^{1}H-, ^{13}C-NMR, and HRMS.
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