Synthesis of the First Tricomponent Bisubstrate Analogue That Exhibits Potent Inhibition against GlcNAc:*β*-1,4-Galactosyltransferase†

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Enzymatic glycosyl transfer[1] is one of the most extensively studied subjects in the field of glycoscience, from both the chemical/mechanistic as well as the biological/biosynthetic point of view. Among the various glycosyl transfer reactions, those utilizing sugar nucleotides, the so-called Leloir donors, as suppliers of sugar residues and oligosaccharide chains as acceptors are important because many biologically active carbohydrate chains are constructed mainly by these substitution reactions.[2] The characteristic sequences of carbohydrate chains are regulated by the specificities of glycosyltransferases as represented by the one linkage−one enzyme concept.[3] Although in the last decade more than 20 glycosyltransferases participating in the biosynthesis of various glycoconjugates have been cloned, none of them could be structurally elucidated by X-ray crystallography.[4] Therefore, details of enzymic glycosyl transfer, e.g., which functional groups are responsible for the substitution reactions and how the glycosyl transfers proceed using the same Leloir donor with inversion or with retention of the configuration at the anomeric center, remain to be elucidated. Because it has been difficult to purify and crystallize glycosyltransferases, the biochemical approach using mimetics of the two substrates of glycosyltransferases is still effective for elucidating details of these reactions. In this respect the inhibitory activity of the mimetics toward glycosyltransferases is a remarkably useful indicator of recognition. Some of the important galactosyltransferases are UDP-Gal:GlcNAc-R *β*-1,4-galactosyltransferase, UDP-Gal:Gal*β*-1,4-GlcNAc-R α-1,3-galactosyltransferase, and UDP-Gal:Fucα-1,2-Gal-R α-1,3-galactosyltransferases.[5] In this paper, the first bisubstrate analogue to be composed of three components, i.e., an electrophilic glycosyl residue (Gal), a nucleotide leaving group (UDP), and a nucleophilic glycosyl acceptor (GlcNAc*β*-OMe) for the *β*-1,4-galactosyltransferase, was synthesized and was found to remarkably inhibit this glycosyltransferase.

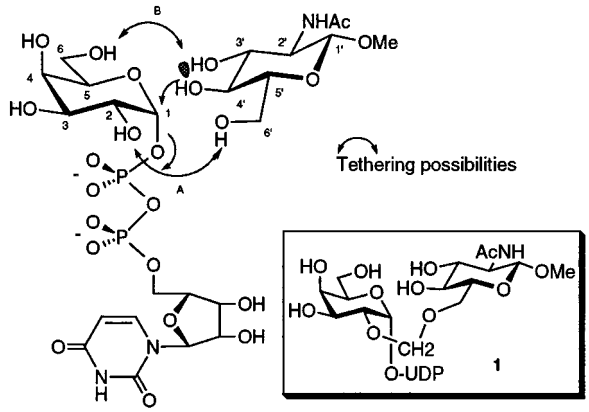

Figure 1. Deduced S_N2 transition state of glycosyl transfer with GlcNAc:*β*-1′4-galactosyltransferase and tethering possibilities between two substrates, i.e., UDP-Gal and GlcNAc *β*-OMe, and the synthetic target **1**.

The first bisubstrate analogue of glycosyltransferase was designed and synthesized by Hindsgaul and coworkers[6] for GDP-Fuc:Gal-R α-1,2-fucosyltransferase. Their bisubstrate analogue was composed of a leaving group (GDP) and an acceptor moiety (Gal*β*-OPh), and this dicomponent bisubstrate analogue was found to be a potent inhibitor with a K_i value of 2.3−16 μM. Synthetic studies based on a similar idea for the bisubstrate analogue are now in progress.[7] A tricomponent bisubstrate analogue for fucosyltransferase was reported,[8] but no information was given concerning its activity. For human α-1,3-fucosyltransferase V, an alternative possibility of a tricomponent inhibitor design was shown by synergistic inhibition[9] of an aza sugar and GDP.

The novel tricomponent bisubstrate analogue **1** for *β*-1,4-galactosyltransferase was designed based on a simple model of an S_N2-like transition state of two substrates as shown in Figure 1. This model has two strategic characteristics for the design of a glycosyl transferase inhibitor, i.e., the use of natural UDP as the leaving group instead of its chemically unreactive mimics such as phosphonate[10] and malondiamido[8] structures and the linking of the acceptor (GlcNAc*β*-OMe) and the donor (Gal moiety) via a methylene tether. The former characteristic is based on the observation that suitable structural modification of the Gal moiety can retard only the glycosylation step (dynamic binding[11]) without affecting the substrate affinity (passive binding) toward the enzyme. The introduction of a tether is expected to

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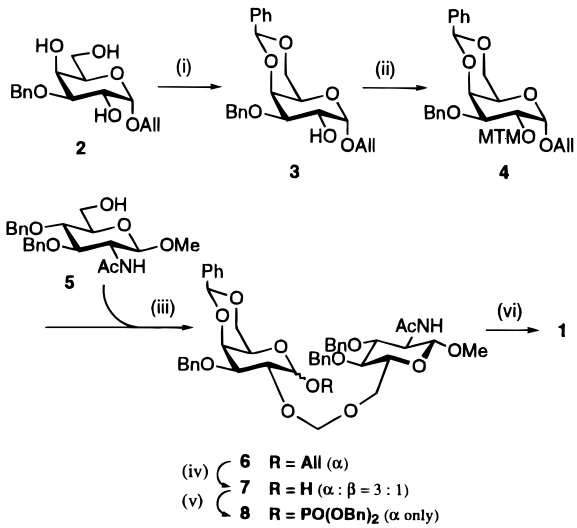
Scheme 1. Synthesis of 6′,2-Tethered Bisubstrate Analogue 1[a]



[a] Reagents and conditions: (i) $PhCH(OMe)_2$, *p*-TsOH, DMF, 60 °C (65%); (ii) $MeSCH_2Cl$, NaH, NaI, DMF (70%); (iii) MeOTf, MS4A, CH_2Cl_2 (51%); (iv) (1) *t*-BuOK, DMSO, 60 °C, (2) $HgCl_2$, acetone−H_2O (79%); (v) *n*-BuLi, $(BnO)_2POCl$, THF, −78 °C (27%); (vi) (1) Pd/C, H_2, *n*-Bu_3N, MeOH, (2) UMP-Imd, DMF.

provide a positive effect for inhibitory activity in two respects, i.e., by providing a higher affinity due to more rigid orientation of two substrates and by the above-described retardation as shown in the case of UDP-2-*O*-Me-Gal.[12] A rationalized tethering strategy is deduced from the behavior of many substrate mimics toward this glycosyltransferase. Two tethering possibilities, i.e., through the 2-position of Gal and the 6-position of GlcNAc or through the 6-position of Gal and the 3-position of GlcNAc, are shown in Figure 1. The former analogue **1** was selected as the first target, because the 2-position of Gal in UDP-Gal has a remarkable enzymatic tolerance assessed from the well-established transfer chemistry of unnatural donors[13] and our recent finding that UDP-2-OMe-Gal is well recognized by the β-1,4-GalTase[12] and also because the enzyme has a considerable affinity for GlcNAc analogues in which the hydroxyl group at the 6-position is substituted with a sulfhydryl group or fluorine atom.[14]

The methylene-tethered bisubstrate tricomponent analogue **1** was synthesized as shown in Scheme 1. An *O*,*O*-methylene tether was designed to form via *O*-[(methylthio)methyl] ether, and allyl 3-*O*-benzyl-4,6-*O*-benzylidene-2-*O*-[(methylthio)methyl]-α-D-galactopyranoside (**4**) was prepared from allyl α-D-galactopyranoside by selective 3-*O*-benzylation[15] and successive 4,6-*O*-benzylidenation. Coupling of **4** and methyl 2-acetamido-3,4-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside (**5**)[16] with methyl triflate in dichloromethane gave a tethered disaccharide **6** in a moderate yield. The *O*-deallylation at the anomeric position of the Gal moiety via alkaline isomerization to the corresponding enol ether and successive phosphorylation with dibenzyl phosphorochloridate afforded exclusively the α-dibenzyl phosphate **8**, the low yield of which was attributed to its lability in a silica gel column. Catalytic hydrogenolysis of the *O*-benzyl and *O*-benzylidene groups followed by condensation with UMP-imidazolide led to the target compound **1**, which was obtained as the bis(triethylammonium) salt[17] after purification on ion exchange and gel permeation columns.

The diphosphate structure of **1** was confirmed by two doublet signals in its ^{31}P NMR spectrum[18] and by $^1H-^{31}P$ HMBC cross peaks between Gal H-1 and P-1 and between Rib H-5 and P-2. The tethered linkage was also confirmed by $^1H-^{13}C$ cross peaks between the tether methylene group and the Gal C-2 methine group as well as the GlcNAc C-6 methylene group.[19] 1D- and 2D-ROESY spectra of **1** showed the NOE correlation of H-1 of the Gal moiety with methylene proton(s) in the tether and with a H-6 proton (δ 4.101) of the GlcNAc moiety, which is deduced to be a pro S proton from its coupling constant[20] with H-5. This indicates that the Gal and GlcNAc moieties in the most stable conformation of **1** in D_2O are not situated as close as is depicted in Figure 1. The potent inhibitory activity observed for **1** proved that the tether is well fitted for the conformational change to a quasi-transition state.

The tricomponent bisubstrate analogue **1** showed a remarkably potent inhibitory activity toward UDP-Gal:GlcNAc-R β-1,4-galactosyltransferases from bovine milk (K_i = 1.35 μM for acceptor:GlcNAc and K_i = 3.3 μM for donor:UDP-Gal). This is the strongest such activity reported. An inhibition assay was carried out using the radioactive donor, ^{14}C-UDP-Gal, and GlcNAcβ-OMe as previously reported.[14,21] The inhibition modes analyzed by Lineweaver−Burk plots were distinctly competitive for the acceptor and presumably competitive for the donor. Further investigations are being conducted to clarify the meaning of these results.

The bisubstrate analogue **1** is the first tricomponent example that shows a very strong inhibition. Thus, it should be possible to find substrate analogues similar to this for the other glycosyltransferases and these may then provide useful information for the elucidation of the active site architecture as well as the mechanism of the glycosyltransferase reaction using a Leloir donor.

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Supporting Information Available: NMR spectra for compound **1** (^1H, ^{13}C, and ^{31}P NMR spectra, $^1H-^{31}P$ and $^1H-^{13}C$ HMBC spectra, and 1D and 2D ROESY spectra) and Lineweaver−Burk plots and their replots for estimation of K_i values of **1** (11 pages).

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