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Stereoselective Chemical Synthesis of Sugar Nucleotides via Direct Displacement of Acylated Glycosyl Bromides

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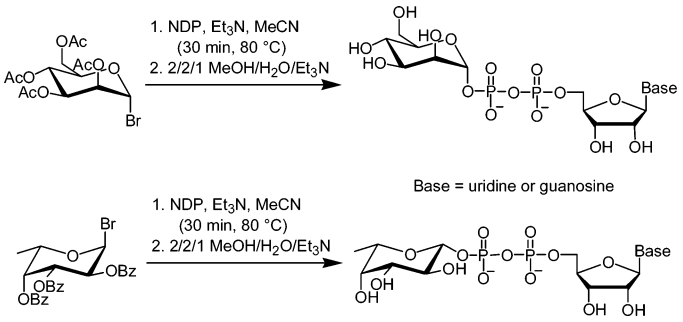
ABSTRACT



The use of Leloir glycosyltransferases to prepare biologically relevant oligosaccharides and glycoconjugates requires access to sugar nucleoside diphosphates, which are notoriously difficult to efficiently synthesize and purify. We report a novel stereoselective route to UDP- and GDP-α-D-mannose as well as UDP- and GDP-β-L-fucose via direct displacement of acylated glycosyl bromides with nucleoside 5′-diphosphates.

Oligosaccharides and glycoconjugates play a critical role in many biochemical recognition processes.[1,2] Through establishing structure−function relationships, glycobiology aims to further our understanding of the roles of complex carbohydrates in both eukaryotes and prokaryotes. In Nature, Leloir glycosyltransferases[3] catalyze the transfer of a monosaccharide unit from a nucleoside diphosphate donor to an acceptor substrate bearing a free hydroxyl group such as an amino acid, a carbohydrate, or a natural product aglycon. The use of glycosyltransferases to synthesize biologically relevant oligosaccharides has become an attractive alternative to total synthesis in recent years. Glycosyltransferase-catalyzed syntheses generally offer the advantages of both regio- and stereospecificity in addition to eliminating the need for laborious protection/deprotection procedures.[4,5]

In mammalian systems, Leloir glycosyltransferases primarily utilize the following seven sugar nucleotide donors: UDP-α-D-Glc,[6] UDP-α-D-Gal, UDP-α-D-GlcNAc, UDP-α-D-GalNAc, GDP-α-D-Man, GDP-β-L-Fuc, and CMP-β-D-NeuAc.[7] In order to effectively use glycosyltransferases to prepare complex oligosaccharides for biological study, efficient methodologies are required to access sugar nucleotide substrates. Although chemoenzymatic strategies to prepare sugar nucleotides are emerging,[8,9] and several new

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(6) Abbreviations: UDP, uridine 5′-diphosphate; GDP, guanosine 5′-diphosphate; CMP, cytidine 5′-monophosphate; Glc, glucose; Gal, galactose; GlcNAc, *N*-acetyl-glucosamine; GalNAc, *N*-acetylgalactosamine; Man, mannose; Fuc, fucose; NeuAc, *N*-acetylneuraminic acid.
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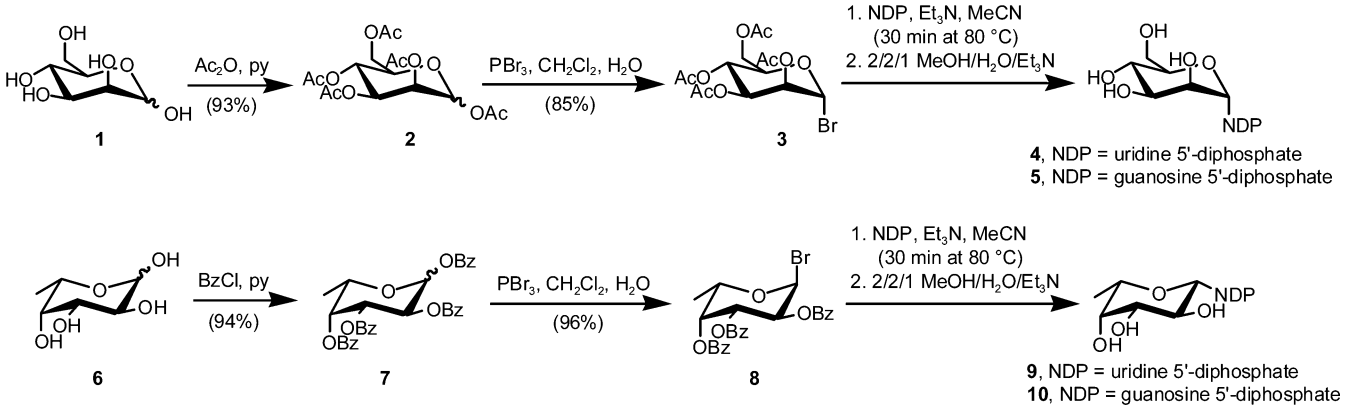
Scheme 1. Stereoselective Synthesis of Sugar Nucleotides via Direct Displacement of Acylated Glycosyl Bromides



in vitro approaches have recently been described to help circumvent this requirement,[10,11] chemical synthesis remains a robust and versatile method to prepare these important substrates.

The majority of chemical syntheses of sugar nucleotides involve the coupling of sugar 1-phosphates with activated nucleoside 5′-monophosphates. Although some common sugar 1-phosphates are commercially available, others must be prepared via multistep syntheses. The nucleoside 5′-monophosphate is frequently activated as a morpholidate,[12,13] and the coupling reaction with the sugar 1-phosphate typically takes several days and results in only moderate yields.[14,15,16] More recently, the use of an *N*-methylimidazolide nucleoside 5′-monophosphate donor has improved reaction times (2 h),[17] but overall yields remain moderate and tedious purifications often result. Purifications are often difficult because of the dinucleoside diphosphate (NppN) byproducts that result from the self-condensation of activated nucleoside 5′-monophosphates and, as a result, purification protocols vary greatly from laboratory to laboratory.[17−19]

In attempts to improve the synthesis and purification of sugar nucleoside diphosphates, the direct coupling of various glycosyl donors with nucleoside 5′-diphosphates has been explored. Examples of glycosyl donors used in this approach include benzylated glycosyl bromides,[19] trimethylsilylated glycosyl iodides,4 2-(1,2-*trans*-glycopyranosyloxy)-3-methoxypyridines (MOP glycosides),[20] and triethylsilylated and benzylated epoxides derived from glycals.[21,22] These methodologies have generally resulted in similarly moderate yields in the key coupling step but eliminate the need for sugar 1-phosphates, using instead more easily accessible glycosyl donors. Purification of these reaction mixtures is often more straightforward as residual nucleoside 5′-diphosphates can easily be degraded to their respective nucleoside bases and inorganic phosphate using alkaline phosphatase before passing reaction mixtures through a reversed-phase or ion-exchange column.[4] The major drawback to these direct coupling approaches is the lack of stereoselectivity obtained in the coupling of glycosyl donors with nucleoside 5′-diphosphates. In the majority of cases α/β selectivities are approximately 1/1 and even in cases where couplings are more selective it is difficult to predict which diastereomer will predominate. It has been suggested that α/β diastereomeric mixtures of sugar nucleotides are not problematic since glycosyltransferases are believed to select for the required diastereomer and not be significantly inhibited by sugar nucleotides of opposite anomeric configuration.[4,19] This being said, it is obviously advantageous to develop synthetic routes with high levels of stereocontrol to improve the yields of desired sugar nucleotide diastereomers, and to allow kinetic studies without any possibility of interference from unwanted diastereomers.

Herein, we present the first stereocontroled synthesis of four sugar nucleotides using a direct coupling approach. Using this methodology, UDP-α-D-Man, GDP-α-D-Man, UDP-β-L-Fuc, and GDP-β-L-Fuc were efficiently prepared in only four synthetic steps from their respective reducing sugars (Scheme 1). Through the use of acyl protecting groups, neighboring group participation was employed resulting in the exclusive preparation of sugar nucleotides with the desired anomeric configurations (Figure 1). Yields, determined by both mass and UV absorbance, are presented along with key NMR chemical shifts and coupling constants in Table 1. Although the assignment of L-fuco-linked

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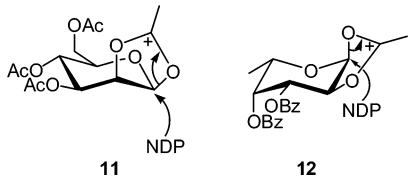

11 12

Figure 1. Influence of neighboring group participation on the trajectory of NDP attack for D-manno- (**11**) and L-fuco-configured (**12**) acylated sugars.

products as β configured sugar nucleotides was straightforward from large vicinal H1″−H2″ J values, the anomeric configuration of D-manno-linked sugar nucleotides was more difficult to assign. As with L-rhamnose, both α and β anomers of glycosides derived from D-mannose have similar small H1″−H2″ J values differing by ∼1 Hz. To unambiguously determine the anomeric configuration of D-manno-containing products, the ^{13}C1−H1 J values[23−25] of all sugar nucleotides was determined. The measured ^{13}C1−H1 J values (Table 1) were consistent with literature expectations for α and β-linked glycosides.

This synthetic methodology begins with D-mannose (**1**) and L-fucose (**6**), which were first protected with acetyl and benzoyl groups, respectively, to produce acylated compounds **2** and **7**. Both acetyl and benzoyl groups were employed to investigate the generality of this approach with respect to acyl protecting groups. Second, a bromo substituent was easily installed at the anomeric center using phosphorus tribromide to give α-linked glycosyl bromides **3** and **8**, which were obtained in stereopure form due to the anomeric effect.

Prior to the coupling reaction, nucleoside 5′-diphosphates were converted to free acids by passing through a column of Amberlite IR-120 (H^+) ion-exchange resin. The free acids were subsequently titrated with an aqueous solution of tetrabutylammonium hydroxide solution (40% w/v) to pH 6 and lyophilized. Care should be taken during the titration because titrations to pH 5 and 7 have resulted in product degradation and reduced nucleophilicity/lower yields, respectively, in coupling reactions.[21,22]

The key synthetic step, coupling of the acylated glycosyl bromides with nucleoside 5′-diphosphates, was initially attempted at room temperature in acetonitrile with 3 Å molecular sieves and triethylamine. Triethylamine (1 equiv) was added to reaction mixtures to help neutralize any acid produced from the reaction and limit degradation of the glycosyl bromide. Higher numbers of equivalents of triethylamine produced no further benefit. Reactions at rt were very sluggish, and, after several days, low conversions to product of approximately 5−10% were obtained by integration of ^{31}P{^{1}H} signals. The addition of extra equivalents of glycosyl bromide improved yields only slightly. In an attempt to expediate the reaction rate and improve the yield of the coupling reaction, experiments were conducted wth 1 equiv of glycosyl bromide and 1 equiv of nucleoside 5′-diphosphate at 80 °C in acetonitrile and monitored closely by TLC and HPLC. After 30 min, all glycosyl bromide had been consumed or degraded by TLC and the presence of a sugar nucleotide product was detected by HPLC. It should be noted that the addition of extra equivalents of glycosyl bromide resulted in substantially more degradation of nucleoside 5′-diphosphates and sugar nucleotide products, thus producing lower overall conversions to product as observed by HPLC. A 1/1/1 ratio of glycosyl bromide/nucleoside 5′-diphosphate/triethylamine was therefore found to be optimal in the coupling step.

To facilitate the efficient purification of sugar nucleotide products, reaction mixtures were concentrated and redissolved in H_2O. The pH was then adjusted to 8 using triethylamine and 50 EU[26] of alkaline phosphatase was added to degrade unreacted nucleoside 5′-diphosphates. The degradation process was monitored by HPLC and was typically complete after stirring at rt for approximately 24 h. After concentration, reaction mixtures were redissolved in a 2/2/1 mixture of MeOH/H_2O/Et_3N and stirred overnight (∼16 h) at rt as previously described[27] to deprotect acyl groups present on the monosaccharides. The reaction mixtures were subsequently subjected to purification via C18 ion-pair reversed-phase chromatography using 10 mM tributylammonium bicarbonate as the ion-pair reagent of choice.[28]

We have determined that acetylated and benzoylated glycosyl bromides work equally well to give exclusively the desired anomeric stereochemistry (a 1,2-trans relationship) upon coupling with nucleoside 5′-diphosphates. This coupling procedure also worked well with both pyrimidine and purine bases, illustrating the general applicability of this approach

Table 1. Yields and NMR Characterization of Sugar Nucleotide Products **4**, **5**, **9**, and **10**

product	sugar	nucleoside 5′-diphosphate (NDP)	yield by UV[a,b] (%)	yield by mass[a] (%)	H1″ chemical shift (ppm)	$^3J_{H1''-H2''}$ (Hz)	$^1J_{C1''-H1''}$ (Hz)
4	D-mannose	uridine 5′-diphosphate	29	33	5.49	n/oc	170
5	D-mannose	guanosine 5′-diphosphate	35	38	5.49	n/oc	173
9	L-fucose	uridine 5′-diphosphate	26	31	4.84	8.0	163
10	L-fucose	guanosine 5′-diphosphate	31	35	4.90	8.0	160

[a] Yields are reported for purified products over two steps (coupling and deprotection). [b] UV yields were determined at λ_{max} 261 nm = 1.01×10^4 $M^{-1}cm^{-1}$ for products containing uridine and λ_{max} 253 nm = 1.37×10^4 $M^{-1}cm^{-1}$ for products containing guanosine. [c] Not observed (br d, $^3J_{H1''-P}$ = 8.0 Hz).

with D-mannose and L-fucose, two prominent sugar residues in many biologically relevant oligosaccharides and glycoconjugates.

In summary, this novel methodology, employing the well-known concept of neighboring group participation, represents a simple, efficient procedure for preparing diastereomerically pure α-D-manno- and β-L-fuco-linked sugar nucleoside diphosphates. Further investigations are currently underway to extend this approach to prepare various α-D-manno- and β-L-fuco-linked sugar nucleotide derivatives synthetically modified at any position except C2 for use as glycosyltransferase probes. Similarly, this procedure is also amenable for use in the synthesis of α-D-manno- and β-L-fuco-linked sugar nucleotide derivatives modified at the nucleobase.[29]

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Supporting Information Available: Experimental procedures and spectral data for compounds **2**−**5** and **7**−**10**; ^{1}H, ^{13}C, and $^{31}P\{^{1}H\}$ spectra as well as HPLC traces for sugar nucleotides **4**, **5**, **9**, and **10**. This material is available free of charge via the Internet at http://pubs.acs.org.

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