Surprising Bacterial Nucleotidyltransferase Selectivity in the Conversion of Carbaglucose-1-phosphate

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Herein we report the first synthesis of the carbocyclic version of the most common naturally occurring sugar-1-phosphate, glucose-1-phosphate, and its evaluation with a bacterial and a eukaryotic sugar nucleotidyltransferase. In contrast to results with the eukaryotic enzyme, the carbocyclic glucose-1-phosphate serves as a substrate for the bacterial enzyme to provide the carbocyclic uridinediphosphoglucose. This result demonstrates not only the first chemoenzymatic strategy to this class of glycosyltransferase inhibitors but also the possibility of using sugar nucleotidyltransferases in vivo to convert prodrug forms of glycosyltransferase inhibitors. In addition, we report several general microwave-assisted reactions that serve to accelerate the synthesis of carbasugars for further studies.

Sugars mediate a large variety of protein—protein and cell—cell interactions implicated in disease states, thereby making carbohydrate-based therapeutics attractive.[1] The drive to understand the molecular determinants of these carbohydrate binding interactions as well as the search for more chemically and biochemically stable sugar derivatives has led to the synthesis of a variety of analogues that replace the glycosidic oxygen with sulfur or carbon.[2] In contrast, the effect of substitution of the ring oxygen on the conformations and biological activity of pyranose sugars has been largely neglected, in part because of the difficulty in obtaining these analogues. The small amount of existing biological data outside glycosidase inhibitors has shown sulfur versions of activated nucleotidediphosphosugars to be poor substrates for glycosyltransferases,[3] whereas the carbocyclic versions serve as inhibitors of these enzymes.[4] These substrates are difficult to synthesize chemically but led us to consider strategies to form such analogues biologically. However, no data are available for the tolerance of sugar nucleotidyltransferases to ring oxygen substitutions.

The ubiquitous Leloir pathway glycosyltransferases require activated sugars that are produced by sugar nucleotidyltransferases (Figure 1). The latter enzymes have been proposed as possible antibiotic targets,[5a,5g,6] but facile screening assays and knowledge of differences in carbohydrate substrate recognition between bacteria and humans are needed to design compounds with the necessary selectivity. Presently, only a few structures of sugar nucleotidyltransferases are known.[5] No sequence homology between enzymes of similar function from eukaryotes and prokaryotes is apparent; therefore, differences in substrate recognition and turnover could be expected for exploitation in antibiotic design.

Discovery of the tolerance of sugar nucleotidyltransferases to carbocyclic sugar substrates first required the synthesis of carba-sugar-1-phosphates. Several strategies have been applied to the synthesis of carbasugars, including radical cyclizations, conversion of quinic acid or bacterial metabolites, zirconium-mediated ring contractions, ring-closing metathesis, Cope rearrangements, and anionic or transition metal-mediated cyclizations.[7,4b] The Ferrier rearrangement is the most common approach to carbasugars, but
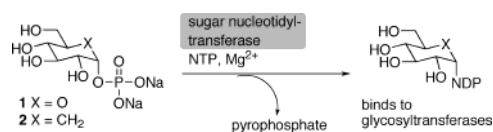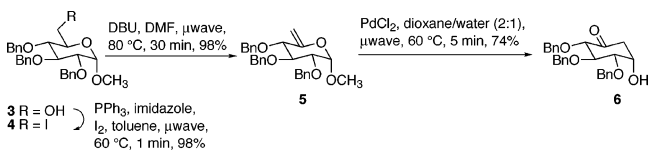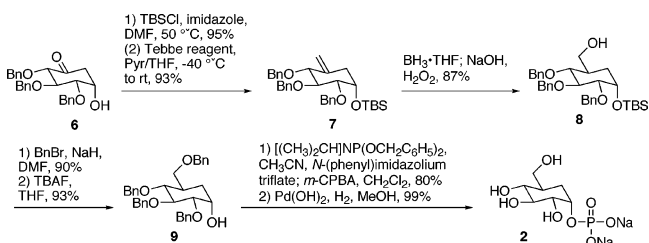

Figure 1. Sugar nucleotidyltransferases catalyze the formation of activated nucleotide diphosphate sugars that are substrates for, when X = O, but inhibitors of, when X = CH$_2$, Leloir pathway glycosyltransferases.

Scheme 1

Scheme 2

mercury is often used and the rearrangements require hours. Many reaction times, especially for transition metal-mediated reactions, can be significantly shortened with microwave assistance.[8] Indeed, we found that not only could the Ferrier rearrangement be carried out in less time and with higher yields in the presence of palladium dichloride but that the synthesis of the necessary precursor **5** could also be hastened by the application of microwave-assisted reactions rather than conventional heating (Scheme 1 and Supporting Information). Iodination[9] of the selectively protected glucose precursor **3** took place in a minute; the subsequent elimination reaction took place in a half hour under microwave irradiation without any competing side reactions. The development of this series of microwave-assisted reactions significantly shortened the time to form the core carbocyclic structure from a protected D-glucose, thereby providing a route that should be applicable to a variety of other sugars for studies with sugar nucleotidyltransferases.

To complete the synthesis of the desired carbaglucose-1-phosphate (**2**), the free hydroxyl group of the Ferrier product **6** was silylated and methylation with Tebbe's reagent yielded the corresponding *exo*-methylene derivative **7** (Scheme 2). Hydrobo-ration/oxidation of alkene **7** resulted in alcohol **8** with the desired equatorial configuration in 87% overall yield. Alcohol protection by benzylation followed by silyl protecting group removal produced free hydroxyl **9**. Treatment of **9** with dibenzyl diisopropyl phos-phoramidite in the presence of a catalytic amount of *N*-(phenyl)-imidazolium triflate[10] produced a phosphite intermediate that was

oxidized in situ to the phosphate. Global debenzylation afforded the desired carbasugar **2**.

With the desired carbocyclic analogue in hand, we next compared its interactions with representative bacterial and eukaryotic sugar nucleotidyltransferases. A major obstacle in testing nonnatural substrates and inhibitors with this class of enzymes has been the lack of a rapid assay to determine kinetic parameters for a variety of compounds; however, the recent development of an electrospray ionization mass spectrometry (ESI-MS)-based assay[11] circumvents these difficulties. The carbasugar **2** was first incubated with a glucose-1-phosphate uridylyltransferase from *Escherichia coli*, which is known to also accept thymidine triphosphate and is homologous to a range of bacterial sugar nucleotidyltransferases.[12] Surprisingly, the analogue was turned over to produce the carbocyclic version of UDP-glucose. In fact, carbasugar **2** exhibited K_m values (17 $\pm$ 2 μM) similar to those of the natural substrate **1** (12 $\pm$ 2 μM). However, a lower turnover rate meant that k_{cat}/K_m values (s^{-1} μM^{-1}) were 0.0020 for the analogue compared to 1.45 for the natural substrate. In contrast, the corresponding sugar nucleotidyltransferase from yeast, which is also homologous to the human enzyme, showed no evidence for carbocyclic UDP-glucose formation even with 5-fold higher enzyme concentrations.

These data provide the first evidence that carbocyclic sugar analogues can serve to inhibit the class of enzymes that provide sugar nucleotide donors to glycosyltransferases, which make compounds such as the cyclic glucans that render some bacteria resistant to standard antibiotics.[13] The relatively weak inherent affinity of glycosyltransferase substrates has been a large hurdle in the design of potent and, most importantly, selective inhibitors for this class of enzymes. This difficulty stems in part from the fact that a large portion of the protein binding energy of these charged sugar substrates comes from the phosphates and not from the carbohydrate itself. However, the incorporation of a catalytic step in addition to a binding step can create a more prominent distinction between prokaryotes and eukaryotes. Differences in substrate turnover that have been exploited in the design of cancer drugs (cancer cells often upregulate enzymes that convert pro-drugs)[14] also can serve as a potential strategy to increase the selectivity of drugs targeted for carbohydrate biosynthetic pathways. Compounds can be designed to make use of not only the inherent differences in bacterial versus eukaryotic substrate binding pockets but also the differences in substrate turnover. Finally, we have shown that sugar nucleotidyltransferases provide means for the facile chemoenzymatic synthesis of carbocyclic versions of activated sugars for further studies of the effects of this substitution on the conformations and properties of carbasugars and for cocrystallization studies with glycosyltransferases and their respective glycosyl acceptors.

Supporting Information Available: Experimental details for the synthesis of **2** with ¹H and ¹³C NMR spectra, details of the mass spectrometry analysis, including calibration curves, and Michaelis−Menten plots (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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