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Articles

Carbocyclic Substrate Analogues Reveal Kanosamine Biosynthesis Begins with the α-Anomer of Glucose 6-Phosphate

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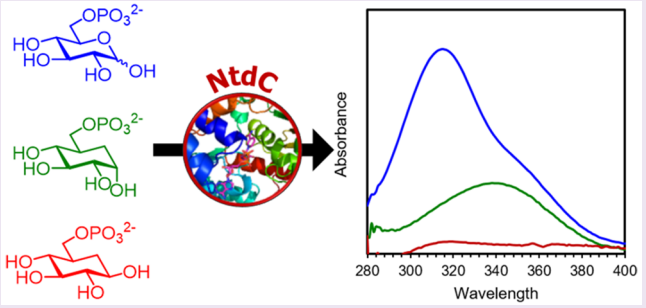
ABSTRACT: NtdC is an NAD-dependent dehydrogenase that catalyzes the conversion of glucose 6-phosphate (G6P) to 3-oxo-glucose 6-phosphate (3oG6P), the first step in kanosamine biosynthesis in *Bacillus subtilis* and other closely-related bacteria. The NtdC-catalyzed reaction is unusual because 3oG6P undergoes rapid ring opening, resulting in a 1,3-dicarbonyl compound that is inherently unstable due to enolate formation. We have reported the steady-state kinetic behavior of NtdC, but many questions remain about the nature of this reaction, including whether it is the α-anomer, β-anomer, or open-chain form that is the substrate for the enzyme. Here, we report the synthesis of carbocyclic G6P analogues by two routes, one based upon the Ferrier II rearrangement to generate the carbocycle and one based upon a Claisen rearrangement. We were able to synthesize both pseudo-anomers of carbaglucose 6-phosphate (C6P) using the Ferrier approach, and activity assays revealed that the pseudo-α-anomer is a good substrate for NtdC, while the pseudo-β-anomer and the open-chain analogue, sorbitol 6-phosphate (S6P), are not substrates. A more efficient synthesis of α-C6P was achieved using the Claisen rearrangement approach, which allowed for a thorough evaluation of the NtdC-catalyzed oxidation of α-C6P. The requirement for the α-anomer indicates that NtdC and NtdA, the subsequent enzyme in the pathway, have co-evolved to recognize the α-anomer in order to avoid mutarotation between enzymatic steps.



Kanosamine, 3-amino-3-deoxy-D-glucose, is an amino-sugar known to inhibit the growth of a variety of bacterial and fungal species, in particular, those that lead to plant pathogenesis.[1,2] It is also a precursor to more complex antibiotics, such as kanamycin, rifamycins, and ansamycins.[1,3,4] Biosynthetic pathways of kanosamine have been found in a variety of bacterial species, such as *Bacillus pumilus*,[5] *Bacillus cereus*,[6] *Amycolatopsis mediterranei*,[4,7] and *Bacillus subtilis*. In *B. subtilis*, kanosamine is synthesized in three steps from glucose 6-phosphate (G6P) catalyzed by the enzymes NtdC, NtdA, and NtdB, as shown in Figure 1a.[8]

The first step in this pathway, catalyzed by NtdC (a glucose-6-phosphate 3-dehydrogenase), converts G6P to 3-dehydro-D-glucose 6-phosphate, also known as 3-oxo-glucose 6-phosphate (3oG6P) through an NAD-dependent oxidation. While dehydrogenases are ubiquitous in nature, this is the only known enzyme to catalyze this particular reaction on the C3 hydroxyl of a phosphorylated reducing sugar, and in fact, there are only a handful of reports of 3-keto reducing sugars in the literature.[9−12] There is a notable similarity of this reaction to the mechanism of glycosyl hydrolase family 4, a group of 6-phospho glycoside hydrolases that oxidize the C3 hydroxyl of G6P glycosides with NAD to facilitate hydrolysis of the glycoside, and the resulting enzyme-bound 3oG6P is reduced back to G6P by NADH prior to the product release.[13−15] Because of the rapid mutarotation of reducing sugars, 3-keto sugars can generate the corresponding 1,3-dicarbonyl in the acyclic form, and as a result, the C2 proton becomes much more acidic. Under mildly basic conditions, the 1,3-dicarbonyl then rapidly enolizes, resulting in a strongly UV-absorbing species (Figure 1b), and indeed, 3oG6P displays a strong UV absorbance at 310 nm.[8] Because of this rapid enolate formation, the absorbance due to NADH at 340 nm is masked by that of the enolate at 310 nm when monitoring the reaction spectrophotometrically.

We have previously reported the kinetics of NtdC by itself and with the next enzyme in the pathway, NtdA, which converts 3oG6P to kanosamine 6-phosphate through a glutamate-coupled PLP-dependent transamination.[16] We have observed that the equilibrium of both the NtdC reaction and the NtdC−NtdA-coupled reaction lies heavily toward G6P, and as a result, we have been unable to isolate the 3oG6P

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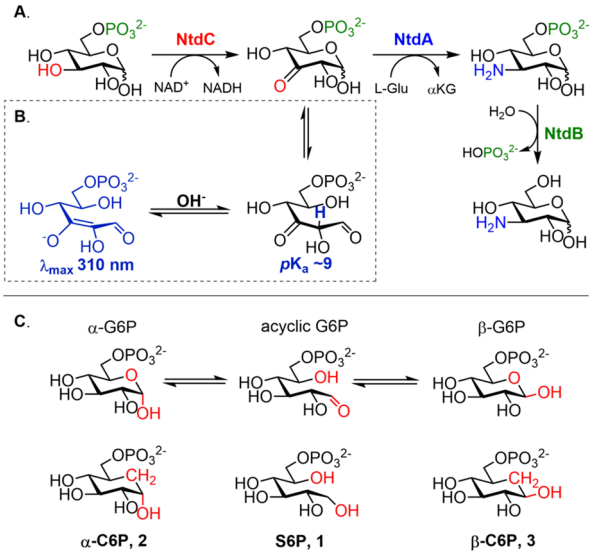

Figure 1. (a) Kanosamine biosynthesis in *B. subtilis*. (b) Enolate formation of 3oG6P when released into solution. (c) Mutarotation of G6P and synthetic analogues as mimics of the various forms of G6P.

product. Additionally, we have not been able to synthesize this compound, likely due to rapid degradation.

We seek a complete understanding of this unusual enzymatic function, and unfortunately, we have been unable to generate a crystal structure of NtdC (with or without G6P). The rapid mutarotation of G6P in solution means that all forms of the sugar are easily accessible on the time scale of the enzymatic reaction (Figure 1c), meaning that we do not know the nature of the substrate form recognized by the enzyme. Furthermore, we have observed no activity with any substrates that bear substitutions at C1, such as methyl or UDP glycosides, suggesting this position is crucial for substrate binding to the active site. This prompted us to synthesize carbocyclic analogues of G6P (α- and β-carbaglucose 6-phosphate, C6P), which can mimic the cyclic forms of the substrate, as well as an acyclic analogue (sorbitol 6-phosphate, S6P) to mimic the open chain form (Figure 1c). Additionally, since these carbocyclic compounds cannot undergo the same ring opening as G6P, the stereochemistry at the pseudo-anomeric position remains fixed, allowing us to determine the anomeric preference of NtdC.

RESULTS AND DISCUSSION

Synthesis of Sorbitol 6-Phosphate. S6P (**1**) was synthesized in one step in an 80% yield from G6P using $NaBH_4$. After purification using anion exchange chromatography, ^{11}B NMR showed contaminating boric acid present in the sample, which was then further removed as trimethyl borate by stirring with methanol under vacuum.

Synthesis of α-C6P and β-C6P via Ferrier II Rearrangement. We first chose to synthesize carbocyclic analogues **2** and **3** using the well-known Ferrier-II carbocyclization reaction and readily accessible glucose precursors, similar to what Ko et al. reported to generate carbocyclic glucose 1-phosphate.[17] The Ferrier substrate was synthesized in four steps starting from commercially available methyl 4,6-*O*-benzylidene-α-D-glucopyranoside (**4**, Scheme 1). Benzylation at C2 and C3 and subsequent regioselective cleavage of the 4,6-benzylidene[18] afforded tri-*O*-benzyl derivative **6** in an excellent yield. The primary alcohol at C6 was converted to an iodide under Appel conditions in quantitative yields, and the elimination of hydrogen iodide with a base produced exo-methylene derivative **8**.

The elimination proved difficult under a variety of conditions, giving undesired isomerization of the exo-methylene **8** to the thermodynamically favored endo-alkene **9**. Most commonly, DBU has been used in DMF at 70−80 °C,[17,19−21] and while these conditions are reported to produce the desired exo-methylene in good yields, our experience has shown minimal success. At best, we were able to isolate the desired product in a 44% yield; however, significant isomerization was still observed. In one instance, we were able to isolate a significant quantity of a second product, which was confirmed by NMR and mass spectrometry to be a DBU nucleophilic adduct (Supporting Information, compound **S27**). Using fresh KOtBu in THF,[22] we were able to achieve very good yields quite rapidly under much milder conditions. Note, however, that this reaction was highly sensitive to moisture, making it difficult to reproduce.

The Ferrier II carbocyclization of compound **8** was carried out with palladium chloride in aqueous dioxane, which proceeded in a 75% yield and produced an inseparable mixture of pseudo-α and -β diastereomers in a 3:1 ratio, consistent with a previous report.[17] The two isomers could be separated upon conversion to the silyl ethers **11a** and **11b**; however, degradation of the α-isomer was observed, resulting in a different ratio of products. This degradation was also observed when we performed this reaction with pure pseudo-α diastereomer of **10**.

Homologation of **11** at C5 using the Tebbe reagent produced exo-methylene derivative **12** in good yields (Scheme 1). Interestingly, homologation under Wittig conditions afforded only a 51% yield with the pseudo-α-isomer, resulting in two elimination by-products being formed, with the loss of either the C3-OBn (15%) or C1-OTBS (29%); this was not observed with the pseudo-β isomer. Hydroboration-oxidation of **12** led predominantly to the undesired *ido* configuration at C5 (**13**) in contrast to results reported by Ko et al.[17] Careful inspection of both the ^1H and 2D NMR spectra for **13a**, which matches that obtained by Ko, showed a small coupling constant (5 Hz) of H4 to H5, suggesting that CH_2OH is in the axial orientation (Figure 2a) and not the equatorial orientation as previously reported.[17] We were able to convert this product back to the desired *gluco* configuration (**14**) in three steps by oxidation to the corresponding aldehyde, isomerization to the low-energy isomer under basic conditions,[22] and reduction back to the alcohol. Examination of the NMR spectra of this product showed a clear, large H4/H5 coupling constant of 11 Hz, indicative of the *gluco* configuration (Figure 2b).

Phosphorylation with diphenyl phosphoryl chloride afforded C6P derivatives **15a** and **16** in good yields. The TBS protecting group of the β isomer was easily cleaved upon acidic workup, while the TBS group of the α-isomer proved difficult to deprotect under the same conditions. Interestingly, the use of TBAF to deprotect the TBS group of **15a** resulted in fluorine substitution at phosphorus and not silyl deprotection, as we had expected (Supporting Information, compound **S28**). Final deprotection of both the benzyl and phenyl groups required two separate hydrogenolysis steps, with the diphenyl phosphate deprotection proceeding in lower yields. Overall, α-C6P and β-C6P were obtained in 7 and 1% yields, respectively, in 14 steps.

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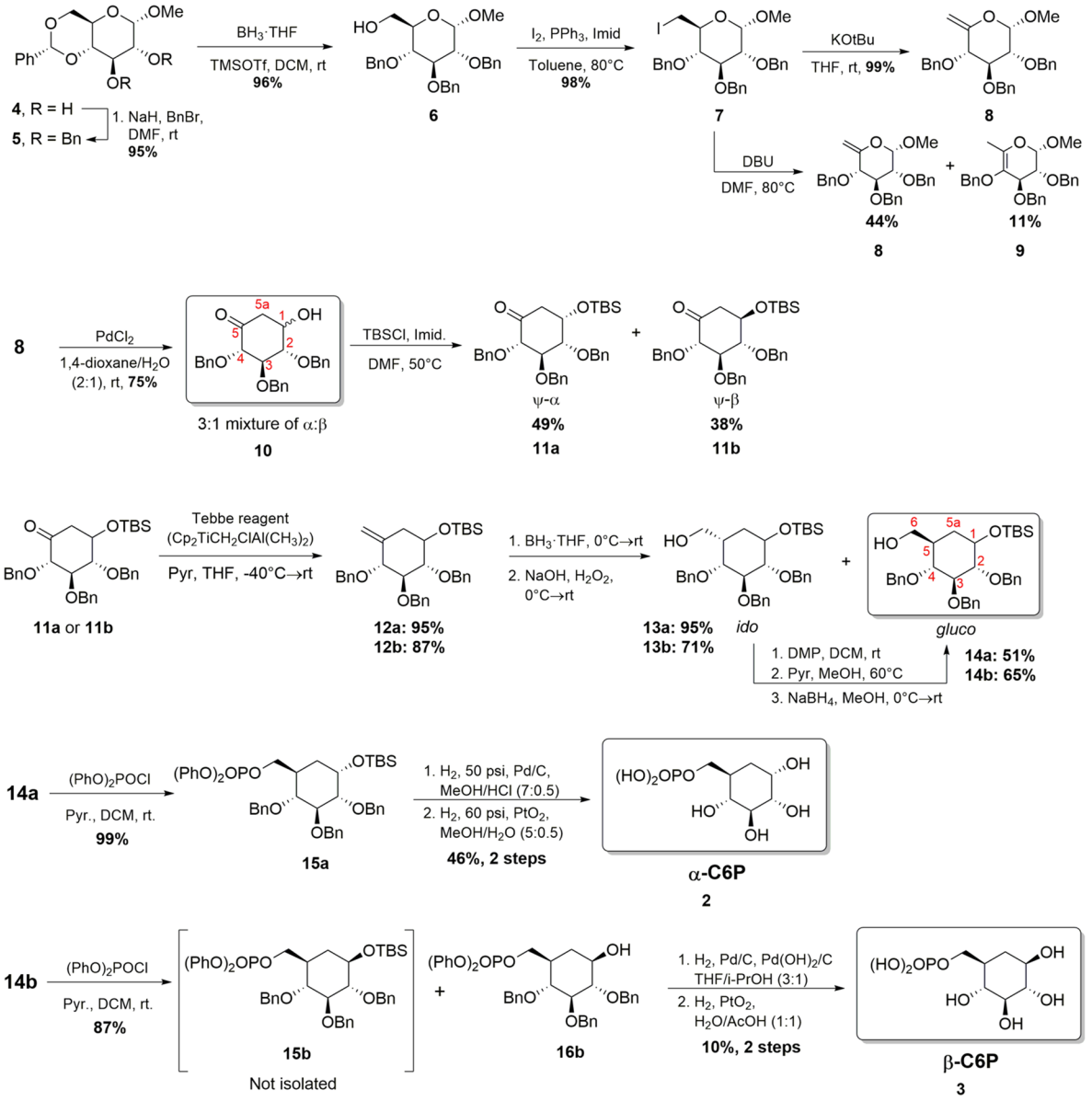
Scheme 1. Synthesis of α-C6P and β-C6P via Ferrier II Rearrangement[a]



[a]Numbering of the carbocyclic ring is indicated in red for compounds **10** and **14**.

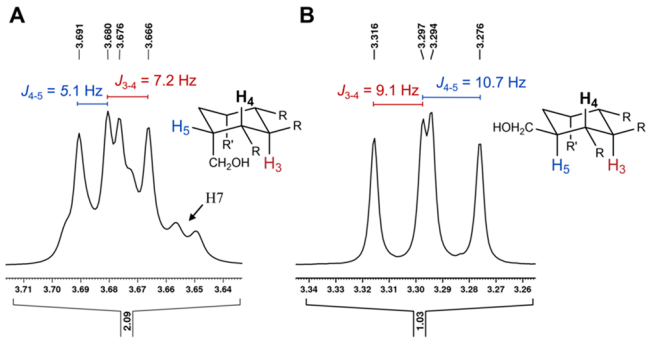


Figure 2. (a) ^{1}H NMR signal of H4 from compound **13a** showing large coupling to H3 and small coupling to H5, indicative of the CH_2OH in the axial position. (b) ^{1}H NMR signal of H4 from compound **14a** showing large coupling to both H3 and H5, indicative of the CH_2OH in the equatorial position. R═OBn; R′═OTBS.

NtdC Activity with Acyclic and Carbocyclic Analogues. Having all three G6P analogues (S6P, α-C6P, and β-C6P) in hand, we tested their activity with NtdC. α-C6P, while comparatively slower than G6P (Figure 3a), proved to be a good substrate with NtdC (Figure 3c), while no reaction was observed with β-C6P and S6P (Figure 3b and 3d), even at higher substrate concentrations or varied pH. Under identical conditions, α-C6P was oxidized at approximately 30% the rate of G6P, considerably better than the next best substrate of NtdC, D-glucose (<1%). Additionally, no absorbance at 310 nm was observed, as expected, due to the inability to form the 1,3-dicarbonyl product. Preliminary kinetics at 1 mM NAD revealed an apparent K_m of 0.9 mM for α-C6P, approximately 20-fold higher than G6P (43 μM) (Supporting Information, Figure S6). Additionally, a significantly lower apparent k_{cat} was observed for α-C6P (0.8 s^{-1}) compared to G6P (4.1 s^{-1}).

Synthesis of α-C6P via Claisen Rearrangement. Having discovered that α-C6P is a good substrate for NtdC,

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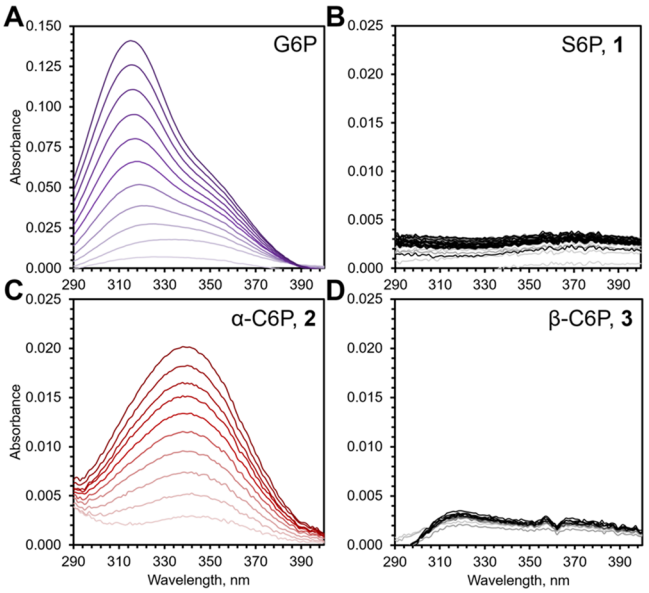

Figure 3. Absorbance traces of the NtdC reaction with (a) G6P, (b) S6P, (c) α-C6P, and (d) β-C6P. Each experiment contained 1 mM NAD, 1 mM substrate, 100 mM Amp-HCl pH 9.5, and 29 nM NtdC. Wavelength scans were taken every 10 s for a total reaction time of 5 min.

we wanted to gather a more complete kinetic profile with this substrate. Upon an examination of our previous synthetic scheme, we were discouraged by several low-yielding steps, unwanted isomerizations and by-products, and the loss of nearly half our overall yield as the unwanted β isomer. This prompted us to pursue an alternative synthetic route. Several other carbocyclic sugar analogues have been previously synthesized using a Claisen rearrangement approach, which centers on a key thermal [3,3]-sigmatropic rearrangement of a homologated glycal, as shown in Figure 4.[23−26]

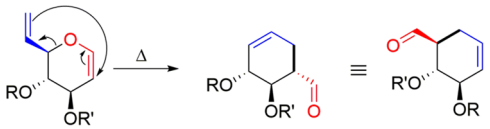

Figure 4. Thermal Claisen rearrangement to generate carbocyclic sugars.[26]

Starting from commercially available triacetyl D-glucal, **17**, we were able to deacetylate C6 regioselectively using lipase from *Candida rugosa* in good yields (Scheme 2).[27] We envisioned oxidation and homologation of **18** would generate the desired alkene; however, we were unable to isolate the desired product from this procedure, likely due to the lability of the acetyl protecting groups. Instead, the C6−OH of **18** was silylated with TBSCl in a 90% yield, and the acetyl groups replaced with benzyl groups in two steps and 64% overall yield. Deprotection of the TBS with TBAF proceeded cleanly, and subsequent oxidation and Wittig homologation, based on procedures reported by Gao et al., gave methylene **23** in a 63% yield over two steps.[24] The Claisen rearrangement of **23** was carried out in diphenyl ether at 210 °C according to reported procedures,[23,25] and immediate reduction with $NaBH_4$ generated carbaglucal analogue **24** in 69% yield. The desired configuration at C5 was confirmed by ^{1}H NMR, with a large H4/H5 coupling constant of 11 Hz (Supporting Information). This was then phosphorylated with tetrabenzyl pyrophosphate in DMF to give fully benzyl-protected carbaglucal **25** (Scheme 2).

cis-Dihydroxylation of compound **24** was previously reported to proceed stereoselectively with osmium tetraoxide, without any added chiral auxiliaries,[23,24] and we expected the same selectivity with **25**. Indeed, we observed only one product formed, in 90% yield (Scheme 2). ^{1}H NMR of **26** showed a large coupling between H3 and H2 (9 Hz) to confirm that C2−OH is equatorial, and the small coupling between H2 and H1 (3 Hz) confirmed that C1−OH is axial. Finally, hydrogenolysis with Pd/C in MeOH/AcOH at 50 psi of H_2 afforded α-C6P in a quantitative yield.

The Claisen rearrangement route offers significant advantages over the Ferrier II route in our experience. Overall, we were able to synthesize α-C6P on a larger scale, with double the overall yield (15%) in three fewer steps. Additionally, all steps were easily purified by column chromatography, with little or no by-products observed; several steps in this route allowed for two-step transformations to be conducted without purification, such as the conversion of diacetate **19** to dibenzyl **21**, the tandem oxidation and Wittig olefination to generate **23**, and the Claisen rearrangement and reduction in one-pot to give carbaglucal **24**. The final deprotection required only one step and proceeded very cleanly such that no further purification was needed on the final product. The final product was converted to the monosodium salt by careful titration with NaOH. In our experience, handling the monosodium salt is far easier, as it is less hygroscopic than the free acid.

Kinetics of NtdC with α-C6P. Our initial kinetics of NtdC with α-C6P revealed an apparent K_m of 0.9 mM and a k_{cat} of 0.8 s^{-1}. To gain a complete understanding of the kinetics with this substrate, we obtained a series of saturation curves by varying the concentration of C6P at various fixed concentrations of NAD. The data was fit to a variety of two-substrate kinetic models, including ping-pong, sequential, and rapid equilibrium ordered mechanisms (Supporting Information, eq S1−5, Scheme S1−4). As expected, the best fit obtained was with a sequential bisubstrate mechanism (eq S1, Figure 5), the same as previously observed for NtdC with G6P.[16] No cooperativity was observed, and the inclusion of substrate inhibition terms did not improve the quality of the fit. The kinetic constants are summarized in Table 1 and are compared with those reported for G6P.[16]

An examination of the calculated kinetic constants revealed a remarkably high catalytic turnover (k_{cat} of 2.0 s^{-1}), only half that of the natural substrate, G6P. While the replacement of the ring oxygen with methylene is approximately isosteric, the capacity to form polar or hydrogen-bonding interactions is lost, altering the binding in the active site. This is reflected in the increased K_m of both substrates in the reaction of α-C6P, indicating that the endocyclic oxygen makes a contribution to the energy of the ternary complex and its conversion to products.

CONCLUSION

The production of antimicrobial compounds such as kanosamine by environmental bacteria such as *Bacillus spp.* is important in applications such as managing plant diseases.[28] The manipulation of kanosamine production and the application or repurposing of kanosamine biosynthesis enzymes in synthetic biology demand a comprehensive

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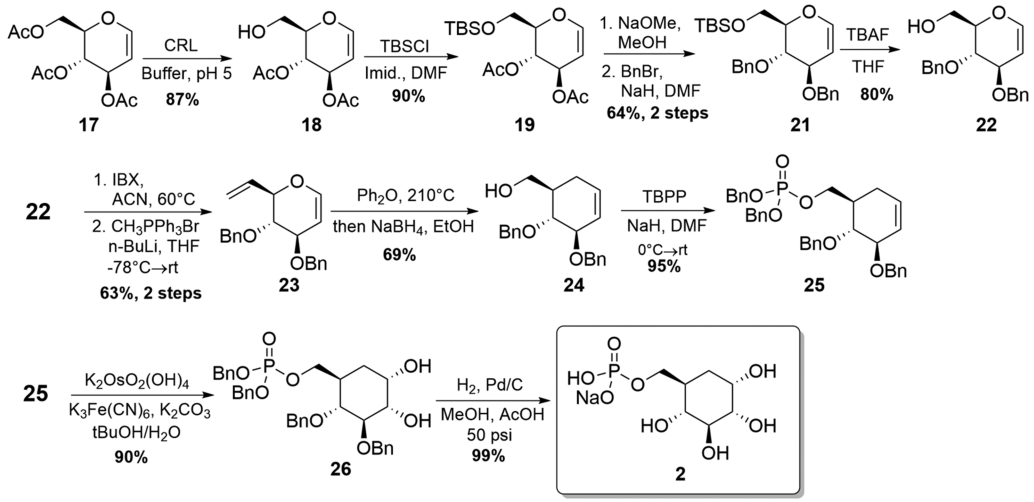
Scheme 2. Synthesis of α-C6P via Claisen Rearrangement



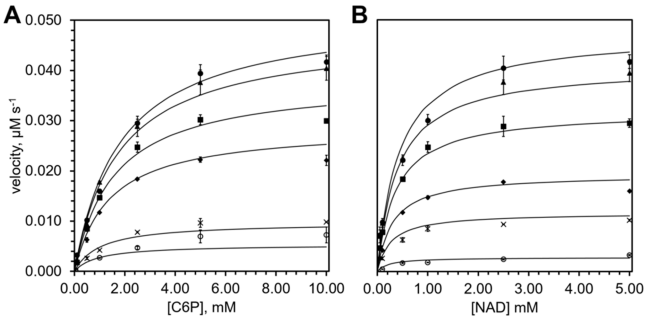


Figure 5. Kinetics of NtdC with C6P. (a) Varied [C6P] at fixed [NAD]: 5.00 mM (●), 2.50 mM (▲), 1.00 mM (■), 0.50 mM (♦), 0.10 mM (×), and 0.05 mM (○). (b) Varied [NAD] at fixed [C6P]: 10.0 mM (●), 5.00 mM (▲), 2.50 mM (■), 1.00 mM (♦), 0.50 mM (×), and 0.10 mM (○). Reactions were performed in 100 mM Tris-HCl pH 9, with 29 nM NtdC. Each data point represents duplicate experiments. Solid lines represent the global fit of the data to eq S1.

Table 1. Kinetic Data of the NtdC Reaction with G6P and α-C6P

	G6P[a]	C6P	
K_{NAD}	0.040 ± 0.004	0.48 ± 0.06	mM
K_{sugar}	0.043 ± 0.004	1.9 ± 0.2	mM
K_i^{NAD}	0.9 ± 0.1	0.2 ± 0.1	mM
k_{cat}	4.1 ± 0.1	2.0 ± 0.1	s^{-1}
[b]k_{cat}/K_{NAD}	(10 ± 1) × 10^4	(0.41 ± 0.03) × 10^4	M^{-1} s^{-1}
[b]k_{cat}/K_{sugar}	(9.6 ± 0.8) × 10^4	(0.10 ± 0.07) × 10^4	M^{-1} s^{-1}

[a]See ref 16. [b]Calculated from a global fitting of the kinetic data to eq S1, rearranged to treat V_{max}/K_a or V_{max}/K_b as single parameters.

understanding of these enzymes. We have synthesized three carbohydrate analogues in order to mimic three forms of G6P in solution to test as substrates with NtdC. An analogue of the open-chain form, S6P, was synthesized in one step by $NaBH_4$ reduction of G6P. Both carbocyclic analogues, α-C6P and β-C6P, were synthesized in 14 steps (in 7 and 1% overall yield, respectively) using a Ferrier II carbocyclization approach to the carbaglucose core, following a previous report.[17] Unfortunately, the hydroboration-oxidation of Ferrier products **12a** and **12b** led almost exclusively to the opposite stereochemistry than reported. While the NMR spectra of our compounds matched that of their work, we have been able to show that the reported stereochemistry at C5 is in fact incorrect, and the previous report describes the synthesis of the carbocyclic analogue of L-idose.[17]

Having synthesized all three substrate analogues, we were able to test their activity with NtdC and our results revealed that α-C6P is a good substrate for the enzyme, while no enzyme activity was observed with the other two compounds. Synthesis of α-C6P was improved upon using a synthetic scheme centering on a Claisen rearrangement to generate the desired carbocycle and avoid the formation of the inactive β-isomer. This approach offered an improved overall yield (15%) in fewer steps (11 steps), and was amenable to scaling up. We examined the kinetics of NtdC with α-C6P, which revealed a relatively high k_{cat} (2.0 ± 0.1) and a K_m of 1.9 mM for C6P, nearly 50 times that of G6P. Additionally, the K_m of NAD increased 10-fold.

The enzymatic activity with only the α-C6P analogue indicates that NtdC binds and reacts with the α-glucopyranosyl form of G6P during catalysis. It also highlights the importance of the anomeric hydroxyl on substrate binding. Our previous crystallographic studies have revealed that the next enzyme in kanosamine biosynthesis, NtdA, binds the α anomer of K6P exclusively,[29] and our current results on NtdC suggests the same preference. This is consistent with our proposal that NtdC and NtdA have co-evolved to sequester and process the ketone product of the NtdC reaction as it is formed. Production of the wrong anomer would necessitate mutarotation via the acyclic 1,3-dicarbonyl form, and enolate formation could divert the product from the metabolic pathway (Figure 1b). The sequestration or rapid conversion of the keto-intermediate may be a general strategy for the conversion of hydroxyls to amines in enzymatic pathways.[16]

The relatively high activity of NtdC with α-C6P suggests that Ntd enzymes could be used to generate other carbocyclic analogues, such as carbocyclic kanosamine. Looking forward, the incorporation of carbocyclic kanosamine analogues into complex natural products using synthetic biology approaches could yield new and more stable antibiotics.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acschembio.0c00409.

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Full experimental procedures, including synthetic methods, protein purification, and kinetic methods, as well as ^{1}H and ^{13}C NMR spectra for all compounds listed in this manuscript (PDF)

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Notes

The authors declare no competing financial interest.

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ABBREVIATIONS

C6P, carbaglucose 6-phosphate; 3oG6P, 3-oxo-D-glucose 6-phosphate; G6P, D-glucose 6-phosphate; S6P, sorbitol 6-phosphate

REFERENCES

(1) Milner, J. L., Silo-Suh, L., Lee, J. C., He, H., Clardy, J., and Handelsman, J. (1996) Production of kanosamine by *Bacillus cereus* UW85. *Appl. Environ. Microbiol. 62*, 3061−3065.

(2) Janiak, A. M., and Milewski, S. (2001) Mechanism of antifungal action of kanosamine. *Med. Mycol. 39*, 401−408.

(3) Guo, J., and Frost, J. W. (2004) Synthesis of aminoshikimic acid. *Org. Lett. 6*, 1585−1588.

(4) Floss, H. G., Yu, T.-W., and Arakawa, K. (2011) The biosynthesis of 3-amino-5-hydroxybenzoic acid (AHBA), the precursor of mC7N units in ansamycin and mitomycin antibiotics: a review. *J. Antibiot. 64*, 35−44.

(5) Umezawa, S., Shibahara, S., Omoto, S., Takeuchi, T., and Umezawa, H. (1968) Studies on the biosynthesis of 3-amino-3-deoxy-D-glucose. *J. Antibiot. 21*, 485−491.

(6) Kevany, B. M., Rasko, D. A., and Thomas, M. G. (2009) Characterization of the complete zwittermicin a biosynthesis gene cluster from *Bacillus cereus*. *Appl. Environ. Microbiol. 75*, 1144−1155.

(7) Guo, J., and Frost, J. W. (2002) Kanosamine biosynthesis: A likely source of the aminoshikimate pathway's nitrogen atom. *J. Am. Chem. Soc. 124*, 10642−10643.

(8) Vetter, N. D., Langill, D. M., Anjum, S., Boisvert-Martel, J., Jagdhane, R. C., Omene, E., Zheng, H., van Straaten, K. E., Asiamah, I., Krol, E. S., Sanders, D. A. R., and Palmer, D. R. J. (2013) A previously unrecognized kanosamine biosynthesis pathway in *Bacillus subtilis*. *J. Am. Chem. Soc. 135*, 5970−5973.

(9) Fukui, S., and Hayano, K. (1969) Micro methods for determination of 3-ketosucrose and 3-ketoglucose. *Agric. Biol. Chem. 33*, 1013−1017.

(10) Hayano, K., Tsubouchi, Y., and Fukui, S. (1973) 3-Ketoglucose reductase of *Agrobacterium tumefaciens*. *J. Bacteriol. 113*, 652−657.

(11) Volc, J., Sedmera, P., Halada, P., Přikrylova, V., and Daniel, G. (1998) C-2 and C-3 oxidation of D-Glc, and C-2 oxidation of D-Gal by pyranose dehydrogenase from *Agaricus bisporus*. *Carbohydr. Res. 310*, 151−156.

(12) Kojima, K., Tsugawa, W., and Sode, K. (2001) Cloning and Expression of Glucose 3-Dehydrogenase from *Halomonas sp.* α-15 in *Escherichia coli*, *Biochem. Biochem. Biophys. Res. Commun. 282*, 21−27.

(13) Rajan, S. S., Yang, X., Collart, F., Yip, V. L. Y., Withers, S. G., Varrot, A., Thompson, J., Davies, G. J., and Anderson, W. F. (2004) Novel Catalytic Mechanism of Glycoside Hydrolysis Based on the Structure of an NAD^{+}/Mn^{2+}-Dependent Phospho-α-Glucosidase from *Bacillus subtilis*. *Structure 12*, 1619−1629.

(14) Yip, V. L. Y., and Withers, S. G. (2006) Mechanistic Analysis of the Unusual Redox-Elimination Sequence Employed by *Thermotoga maritima* BglT: A 6-Phospho-β-glucosidase from Glycoside Hydrolase Family 4. *Biochemistry 45*, 571−580.

(15) Yip, V. L. Y., Thompson, J., and Withers, S. G. (2007) Mechanism of GlvA from *Bacillus subtilis*: A Detailed Kinetic Analysis of a 6-Phospho-α-glucosidase from Glycoside Hydrolase Family 4. *Biochemistry 46*, 9840−9852.

(16) Vetter, N. D., and Palmer, D. R. J. (2017) Simultaneous Measurement of Glucose-6-phosphate 3-Dehydrogenase (NtdC) Catalysis and the Nonenzymatic Reaction of Its Product: Kinetics and Isotope Effects on the First Step in Kanosamine Biosynthesis. *Biochemistry 56*, 2001−2009.

(17) Ko, K.-S., Zea, C. J., and Pohl, N. L. (2004) Surprising Bacterial Nucleotidyltransferase Selectivity in the Conversion of Carbaglucose-1-phosphate. *J. Am. Chem. Soc. 126*, 13188−13189.

(18) Daragics, K., and Fügedi, P. (2009) Regio- and chemoselective reductive cleavage of 4,6-O-benzylidene-type acetals of hexopyranosides using BH_3·THF−TMSOTf. *Tetrahedron Lett. 50*, 2914−2916.

(19) Ramakrishna, B., and Sridhar, P. R. (2015) Stereoselective synthesis of 1,6-dioxaspirolactones from spiro-cyclopropanecarboxylated sugars: total synthesis of dihydro-pyrenolide D. *RSC Adv. 5*, 8142−8145.

(20) Horváth, A., Ruttens, B., and Herdewijn, P. (2007) Stereoselective synthesis of (−)-ara-cyclohexenyl-adenine. *Tetrahedron Lett. 48*, 3621−3623.

(21) Sato, K.-i., Bokura, M., and Taniguchi, M. (1994) Syntheses of optically active 2,3,6-tri-*O*-benzyl-D-*myo*-inositol, laminitol, and mytilitol from D-glucose. *Bull. Chem. Soc. Jpn. 67*, 1633−1640.

(22) Sun, Y., and Nitz, M. (2012) Syntheses of carbocyclic analogues of α-D-glucosamine, α-D-mannose, α-D-mannuronic acid, β-L-idosamine, and β-L-gulose. *J. Org. Chem. 77*, 7401−7410.

(23) Sudha, A. V. R. L., and Nagarajan, M. (1998) Carbohydrates to carbocycles: an expedient synthesis of pseudo-sugars. *Chem. Commun.* No. 8, 925−926.

(24) Gao, Q., Zaccaria, C., Tontini, M., Poletti, L., Costantino, P., and Lay, L. (2012) Synthesis and preliminary biological evaluation of carba analogues from *Neisseria meningitidis* A capsular polysaccharide. *Org. Biomol. Chem. 10*, 6673−6681.

(25) Stick, R. V., and Stubbs, K. A. (2005) The Synthesis of a New Class of Potential Inhibitors for Glycoside Hydrolases. *J. Carbohydr. Chem. 24*, 529−547.

(26) Frau, I., Bussolo, V. D., Favero, L., Pineschi, M., and Crotti, P. (2011) Stereodivergent synthesis of diastereoisomeric carba analogs of glycal-derived vinyl epoxides: A new access to carbasugars. *Chirality 23*, 820−826.

(27) Filice, M., Guisan, J. M., Terreni, M., and Palomo, J. M. (2012) Regioselective monodeprotection of peracetylated carbohydrates. *Nat. Protoc. 7*, 1783−1796.

(28) Shafi, J., Tian, H., and Ji, M. (2017) *Bacillus* species as versatile weapons for plant pathogens: a review. *Biotechnol. Biotechnol. Equip. 31*, 446−459.
(29) van Straaten, K. E., Ko, J. B., Jagdhane, R., Anjum, S., Palmer, D. R. J., and Sanders, D. A. R. (2013) The Structure of NtdA, a Sugar Aminotransferase Involved in the Kanosamine Biosynthetic Pathway in *Bacillus subtilis*, Reveals a New Subclass of Aminotransferases. *J. Biol. Chem. 288*, 34121−34130.

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