

# Exploiting the Reversibility of Natural Product Glycosyltransferase-Catalyzed Reactions

Changsheng Zhang,<sup>1</sup> Byron R. Griffith,<sup>1</sup> Qiang Fu,<sup>2</sup> Christoph Albermann,<sup>1\*</sup> Xun Fu,<sup>1</sup> In-Kyoung Lee,<sup>1†</sup> Lingjun Li,<sup>2</sup> Jon S. Thorson<sup>1‡</sup>

Glycosyltransferases (GTs), an essential class of ubiquitous enzymes, are generally perceived as unidirectional catalysts. In contrast, we report that four glycosyltransferases from two distinct natural product biosynthetic pathways—calicheamicin and vancomycin—readily catalyze reversible reactions, allowing sugars and aglycons to be exchanged with ease. As proof of the broader applicability of these new reactions, more than 70 differentially glycosylated calicheamicin and vancomycin variants are reported. This study suggests the reversibility of GT-catalyzed reactions may be general and useful for generating exotic nucleotide sugars, establishing *in vitro* GT activity in complex systems, and enhancing natural product diversity.

Glycosyltransferases (GTs) constitute a superfamily of ubiquitous enzymes that attach carbohydrate moieties to biological molecules (1) and thus play a role in the biosynthesis of oligosaccharides (2), glycosaminoglycans (3), glycopeptides (4), and glycosylated anticancer and anti-infective agents (5). These enzymes are generally perceived as unidirectional catalysts that drive the formation of glycosidic bonds from nucleotide diphosphate

sugar (NDP-sugar) donors and aglycon acceptors (6). In contrast, we report that GTs involved in the biosynthesis of anticancer (the enediyne calicheamicin, CLM) and antibiotic (the glycopeptide vancomycin, VCM) natural product-based drugs catalyze reversible, bidirectional reactions. Specifically, the four GTs tested (CLM CalG1 and CalG4 and VCM GtfD and GtfE) were found to catalyze three new reactions: (i) the synthesis of exotic NDP-sugars from glycosylated natural

products, (ii) the exchange of native natural-product glycosides with exogenous carbohydrates supplied as NDP-sugars, and (iii) the transfer of a sugar from one natural product backbone to a distinct natural-product scaffold. As proof of the broader applicability of these new reactions, the GT-catalyzed production of >70 differentially glycosylated CLM variants and a VCM analog bearing both a handle for chemical diversification and a rare amino sugar are also reported.

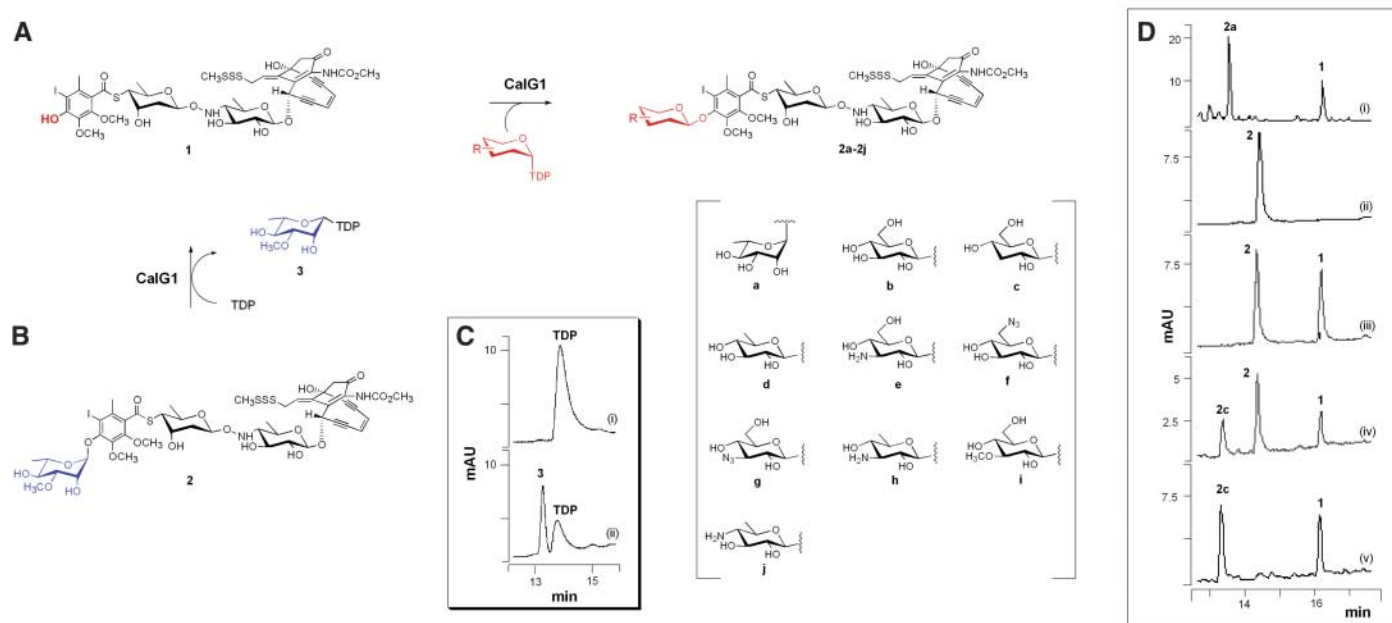
The *calG1* gene was amplified from the genomic DNA of the CLM producer, *Micromonospora echinospora*, and overexpressed in *Escherichia coli*, and the recombinant CalG1 was purified to homogeneity (fig. S1) (7, 8). Incubation of the aglycon 1 with the surrogate substrate thymidine diphosphate (TDP)- $\beta$ -L-rhamnose (Fig. 1A) in the presence of CalG1

<sup>1</sup>Laboratory for Biosynthetic Chemistry, Pharmaceutical Sciences Division, School of Pharmacy, National Cooperative Drug Discovery Group Program, University of Wisconsin (UW)—Madison, 777 Highland Avenue, Madison, WI 53705–2222, USA. <sup>2</sup>School of Pharmacy and Department of Chemistry, University of Wisconsin, 777 Highland Avenue, Madison, WI 53705–2222, USA.

\*Present address: Institute of Microbiology, University of Stuttgart, Allmandring 31, 70569 Stuttgart, Germany.

†Present address: Korea Research Institute of Bioscience and Biotechnology, Yuseong, Daejeon 305-333, Korea.

‡To whom correspondence should be addressed. E-mail: jsthorson@pharmacy.wisc.edu



**Fig. 1.** *In vitro* CalG1-catalyzed reactions. (A) The CalG1-catalyzed transfer of unnatural sugars to the acceptor 1. The TDP-sugars corresponding to glycosides 2c to 2j were enzymatically generated as previously described, TDP- $\beta$ -L-rhamnose (for 2a) was prepared via chemical synthesis, and TDP- $\alpha$ -D-glucose (for 2b) was obtained from a commercial source. (B) CalG1-catalyzed reverse glycosyltransfer and sugar exchange reactions. In the first step, the terminal 3'-O-methylrhamnose unit of 2 (CLM  $\alpha_3$ , 1 of 10 CLMs produced by *M. echinospora*) was transferred to TDP, yielding 1 and TDP-3-O-methyl- $\beta$ -L-rhamnose [3, see also (C) and (D)]. The subsequent sugar exchange involved the transfer of unnatural sugars (from exogenous NDP-sugars) to 1 to give compounds 2a to 2j. (C) Anion-exchange HPLC of CalG1-catalyzed 3 formation:

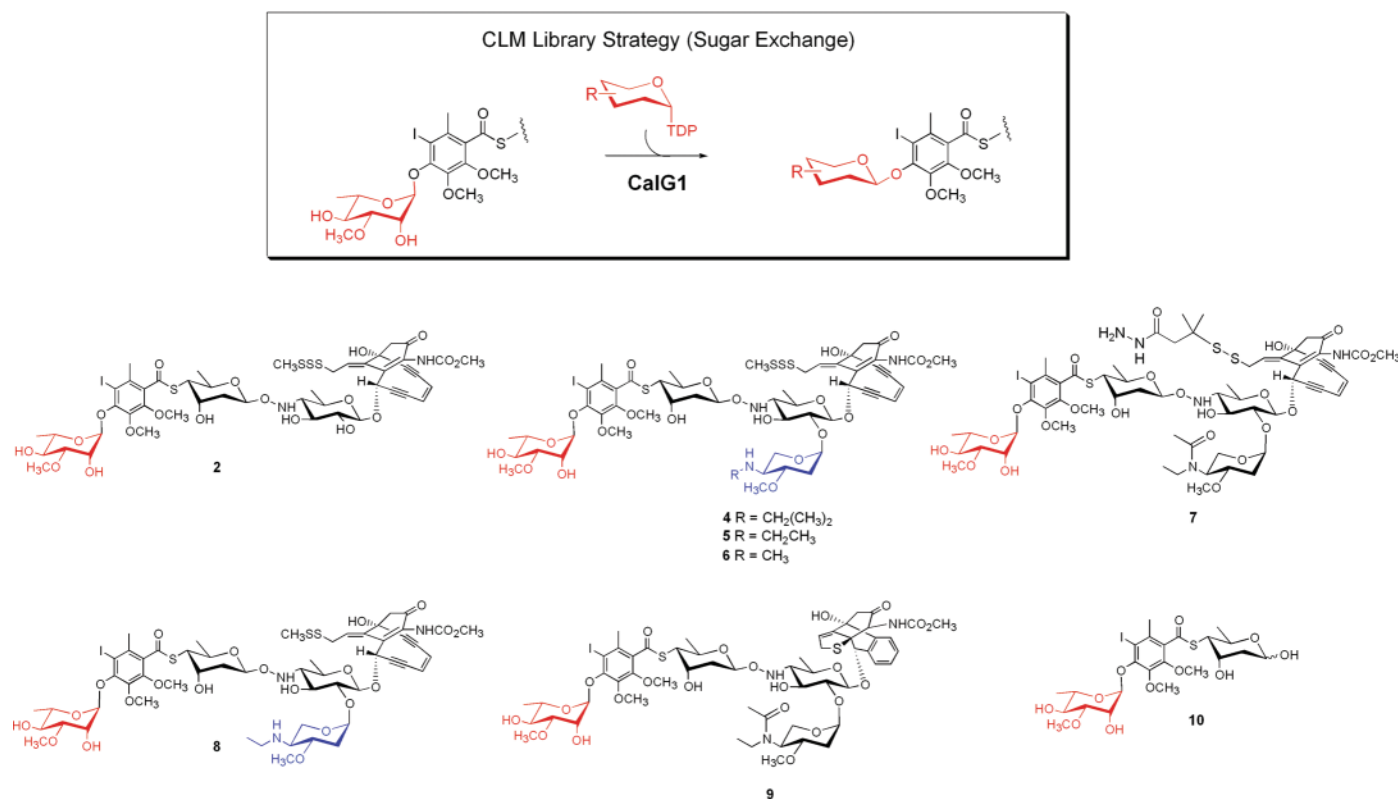
(i) control with 50  $\mu$ M 2 and 100  $\mu$ M TDP [see also (D), ii] and (ii) 50  $\mu$ M 2, 100  $\mu$ M TDP, and CalG1 [see also (D), iii]. The new peak at 13 min. was isolated and identified as 3 by MS/MS (fig. S5). AU, absorbance units. (D) Reverse-phase HPLC of CalG1-catalyzed reactions: (i) 50  $\mu$ M 1, 300  $\mu$ M TDP- $\beta$ -L-rhamnose, and CalG1; (ii) reverse glycosyltransfer control with 50  $\mu$ M 2 and 100  $\mu$ M TDP [see also (C), i]; (iii) 50  $\mu$ M 2, 100  $\mu$ M TDP, and CalG1 [see also (C), ii]; (iv) 50  $\mu$ M 2, 300  $\mu$ M TDP-3-deoxy- $\alpha$ -D-glucose, and CalG1 (sugar exchange); and (v) 50  $\mu$ M 1, 300  $\mu$ M TDP-3-deoxy- $\alpha$ -D-glucose, and CalG1. All CalG1 assays were performed in a total volume of 100  $\mu$ l in tris-HCl buffer (10 mM, pH = 7.5) containing 1 mM of MgCl<sub>2</sub> and 10  $\mu$ M CalG1 with incubation at 30°C for 3 to 12 hours. HPLC parameters are described in the Materials and Methods.

led to the formation of a new product (Fig. 1D, i), characterized as **2a** by liquid chromatography–mass spectrometry (LC-MS). Consistent with CalG1 as the requisite rhamnosyltransferase in CLM biosynthesis, no product was observed when CalG1 was replaced with other GTs in this assay. Also, substitution of TDP- $\alpha$ -L-rhamnose for TDP- $\beta$ -L-rhamnose in the CalG1 assay yielded no product, consistent with CalG1 functioning as a stereospecific inverting GT. A diverse library of 22 TDP sugars (Materials and Methods) was used to probe the NDP-sugar specificity of CalG1 (Fig. 1A and fig. S2). Nine additional TDP-sugar substrates were converted to their corresponding CLM glycosides, **2b** to **2j** (Fig. 1A), in percent conversions of 27 to 62% (fig. S3). LC coupled to tandem MS (LC-MS/MS) of products **2b** and **2d** revealed fragmentation patterns consistent with attachment of the sugar to the aromatic ring of the substrate and were highly consistent with the fragmentation of naturally occurring standard CLM variants  $\alpha_3^1$  (**2**) and  $\gamma_1^1$  (**5**) (Fig. 2 and fig. S4). Cumulatively, these studies designated CalG1 as the CLM rhamnosyltransferase, capable of flexibility toward diverse TDP-D- and TDP-L-sugar donors.

In an experiment designed to further verify the regiospecificity of CalG1, CLM  $\alpha_3^1$  (**2**) (Fig. 1B) and TDP-3-deoxy- $\alpha$ -D-glucose were co-incubated with CalG1 under standard conditions. Because the CalG1 glycosylation site in **2** is occupied by 3'-O-methylrhamnose (Fig. 1B), no reaction was expected. However, two new products were observed, subsequently identified by LC-MS as **1** and the corresponding 3-deoxyglucoside, **2c** (Fig. 1D, iv and v). Analysis of control reactions led to the conclusion that this transformation involved a TDP-dependent reverse glycosyltransfer. Specifically, co-incubation of **2** with TDP yielded **1** only in the presence of CalG1 (Fig. 1D, ii and iii, and fig. S5A), and analysis of the same “reverse” reaction by anion-exchange high performance liquid chromatography (HPLC) (Fig. 1C) unveiled the production of TDP-3-O-methyl- $\beta$ -L-rhamnose (**3**) (Fig. 1B and fig. S5) in substantial quantity, which was absent in the control assay. Thus, CalG1 efficiently excised the native CLM 3'-O-methylrhamnosyl unit in the presence of TDP (to provide **1** and TDP sugar **3**) and, in the presence of a slight excess of exogenous TDP-3-deoxyglucose, ultimately catalyzed the formation

of **2c**. Such CalG1-catalyzed in situ “sugar exchange” might offer an expeditious method for substituting the CLM 3'-O-methylrhamnose with other natural or unnatural sugars. To test this idea, we assayed CLM derivatives (Fig. 2)  $\alpha_3^1$  (**2**),  $\beta_1^1$  (**4**),  $\gamma_1^1$  (**5**), and  $\delta_1^1$  (**6**); dimethyl hydrazide (DMH) Nac  $\gamma$  (**7**),  $\gamma_2^1$  (**8**), and Nac  $\epsilon$  (**9**); and “fragment III” (**10**) (**9**) in CalG1-catalyzed reactions with the 10 established CalG1 TDP-sugar substrates. In every case, the desired sugar-exchanged product was observed by HPLC (figs. S6 and S10) with an average sugar exchange conversion of 60% for the eight CLM aglycons in the presence of purified TDP- $\alpha$ -D-glucose or TDP- $\beta$ -L-rhamnose. Notably, this simple set of assays led to the CalG1-catalyzed production of a CLM library exceeding 70 members (**2a** to **2j**, **4a** to **4j**, **5a** to **5j**, **6a** to **6j**, **7a** to **7j**, **8a** to **8j**, **9a** to **9j**, and **10a** and **10b**) (figs. S7 and S8) and thereby highlights the combinatorial power of GT-catalyzed sugar exchange.

Given that GT-catalyzed sugar exchange activity proceeds via an established NDP-sugar intermediate, we hypothesized that GTs could also be used to harvest an exotic sugar from one natural-product scaffold and transfer it to a different



**Fig. 2.** Strategy for the construction of a CLM library by CalG1-catalyzed sugar exchange. The general strategy involved the CalG1-mediated exchange of the natural 3'-O-methylrhamnose (highlighted in red) in CLMs  $\alpha_3^1$  (**2**),  $\beta_1^1$  (**4**),  $\gamma_1^1$  (**5**), and  $\delta_1^1$  (**6**) and DMH Nac  $\gamma$  (**7**),  $\gamma_2^1$  (**8**), and Nac  $\epsilon$  (**9**) with sugars supplied via the 10 established CalG1 NDP-sugar substrates (fig. S2A). In addition, fragment III (**10**) was also converted to the rhamnoside and glucoside to cumulatively provide 72 diversely functionalized CLM derivatives. For this study, CLMs **2** and **4**, **5**, and **6** are natural metabolites, whereas **7**, **8**, **9**, and **10** are chemically modified CLM derivatives. A typical

CalG1 sugar exchange reaction contained 50  $\mu$ M aglycon (**2** and **4** to **10**), 300  $\mu$ M NDP sugar, and 10  $\mu$ M CalG1 in a total volume of 100  $\mu$ l in tri-HCl buffer (10 mM, pH = 7.5) containing 1 mM of MgCl<sub>2</sub> at 30°C for 3 hours. HPLC parameters are described in the Materials and Methods, and chromatograms for representative reactions are provided in fig. S6. The structures of all library members are illustrated in fig. S7, and conversion rates are provided in figs. S8 and S10. It should also be noted that CalG4 can excise the aminopentosyl units (highlighted in blue) from **4**, **5**, **6**, and **8** for sugar or aglycon exchange.

aglycon in a single reaction. This permutation of GT catalysis would avoid the often-complex synthesis of highly functionalized NDP-sugars (10). The assays designed to test this idea contained CalG1, a putative 3'-*O*-methylrhhamnose donor—4, 5, 6, 7, 8, or 10 (Fig. 2)—TDP, and the representative acceptor 1. In each case, the simultaneous excision and in situ transfer of 3'-*O*-methylrhhamnose from each respective donor to 1 was observed, yielding the expected 3'-*O*-methylrhhamnosylated product 2 (fig. S9). In comparison, controls lacking either CalG1 or TDP gave only starting materials. Thus, in situ aglycon exchange reactions can extend the potential diversity accessible by CalG1.

The reversibility of the CalG1-catalyzed sugar exchange and aglycon exchange transformations described above raised the question as to whether other GT systems would exhibit similar behavior. Thus, three additional GT-catalyzed reactions were examined for reversibility: those of CalG4 (the putative CLM aminopentosyltransferase), GtfD, and GtfE (the VCM vancosaminyl- and glucosyltransferase, respectively) (7, 11–13). CalG4 was produced in a similar fashion as CalG1 (fig. S1) (7, 8). In the presence of TDP,

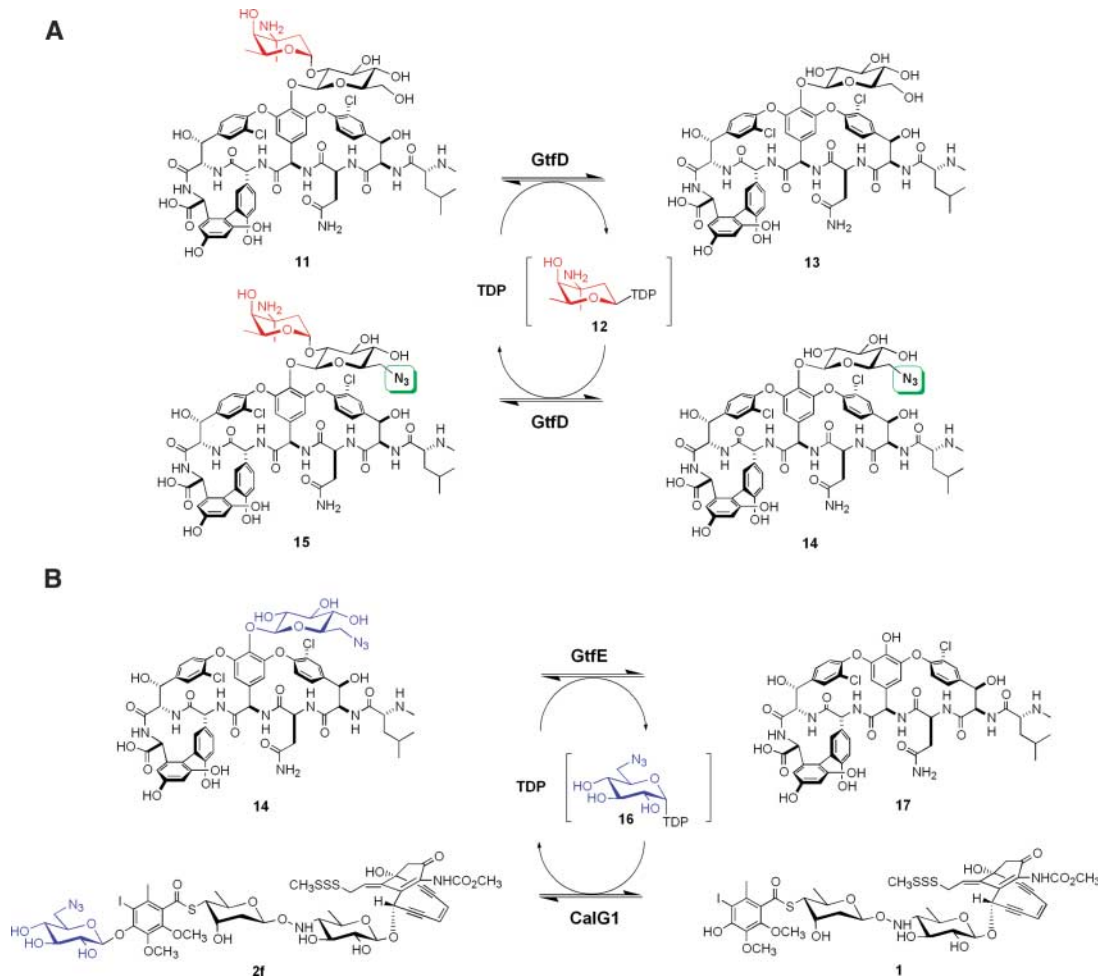
CalG4 catalyzed the excision of the aminopentose sugar moiety (Fig. 2, highlighted in blue) from sugar donor CLM derivatives 4, 5, 6, and 8 (fig. S11). CalG4 also catalyzed in situ aglycon exchange, transferring the excised aminopentoses from donors 4, 5, 6, and 8 to the exogenous aglycon acceptor 1 in the presence of TDP with conversions ranging from 19 to 69% (fig. S12). In comparison, controls lacking TDP (even in the presence of alternative NDPs) or CalG4 gave only starting materials. Besides identifying CalG4 as the aminopentosyltransferase involved in CLM biosynthesis, these results confirm that, in contrast to the previously proposed uridine diphosphate (UDP) sugar pathways (14), CLM aminopentose biosynthesis proceeds via a TDP-sugar pathway. Additionally, this study demonstrates that the reversibility of GT catalysis is not unique to the CalG1 reaction.

To extend these studies beyond enediyne scaffolds, we overexpressed and purified the VCM GTs GtfD and GtfE as previously described (11). Similar to the CLM GTs, GtfD catalyzed the excision of L-vancosamine from the parent sugar donor VCM (11) to form pseudoaglycon 13 (Fig. 3A). In a separate aglycon

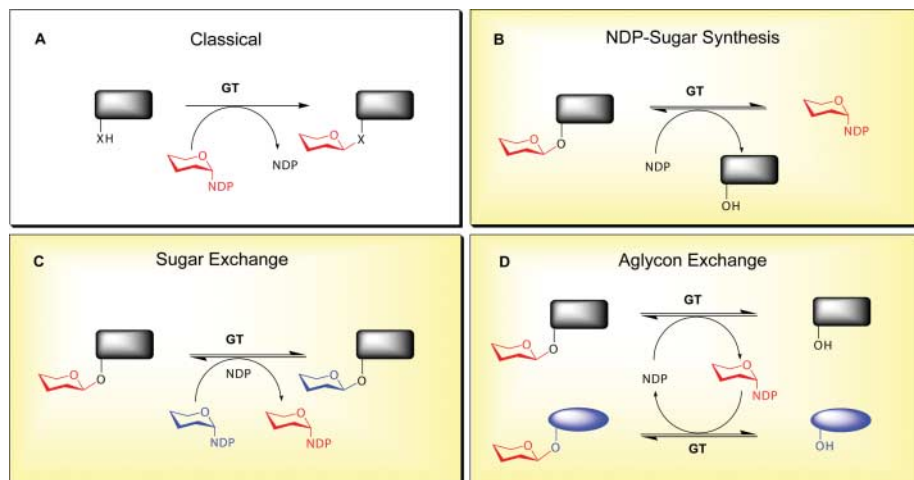
exchange reaction, GtfD catalyzed the transfer of L-vancosamine from 11 to the unnatural acceptor 14 (13) to give 15, a VCM analog containing both a sugar-appended azido handle for chemoselective ligation and a vancosaminyl moiety (27% conversion) (Fig. 3A and fig. S13). Likewise, the glucosyltransferase GtfE could also catalyze sugar excision from both 13 and the unnatural sugar donor 14. Consistent with an equilibrium only moderately favoring the glycoside product in the GtfE-catalyzed reaction, the equilibrium constant ( $K_{eq}$ ) was determined to be 4.5 (fig. S14). GtfE could also participate in aglycon exchange, as revealed by the GtfE-catalyzed generation of unnatural NDP-sugar 16 for CalG1-catalyzed glycosyltransfer to the enediyne acceptor 1 in a tandem, one-pot, GtfE-CalG1-catalyzed aglycon exchange reaction (Fig. 3B). With an overall conversion of 48%, this transformation highlights the potential of two-GT systems to mediate aglycon exchange between compounds from different natural product classes (fig. S15).

The exploitation of GT-catalyzed reaction reversibility may facilitate the use of glycosylation as a tool to modulate the activity of

**Fig. 3. VCM GT-catalyzed reverse and aglycon exchange reactions. (A)** GtfD-catalyzed aglycon exchange reaction to provide 2'-vancosaminyl-6'-azidoglucosyl-VCM (15). The TDP-β-L-vancosamine (12) for this reaction was generated in situ by a GtfD-catalyzed reverse glycosyltransfer and subsequently transferred to the unnatural 6-azidoglucose-containing derivative 14 to give compound 15 in 27% conversion (fig. S13). The reaction was performed in a total volume of 100 μl in tricine-NaOH buffer [75 mM Tricine, pH = 9.0, 2.5 mM MgCl<sub>2</sub>, 2.5 mM tris (2-carboxy ethyl)-phosphine, and 1 mg/ml bovine serum albumin (BSA)] containing 100 μM 11, 100 μM 14, 1 mM TDP, and 12 μM GtfD. **(B)** A two-component GT-catalyzed aglycon exchange reaction using two diverse natural product scaffolds. In this one-pot reaction, TDP-6-azido-α-D-glucose (16, provided by GtfE-catalyzed reverse glycosyltransfer from sugar donor 14) served as the NDP-sugar donor for the CalG1-mediated attachment of 6-azidoglucose to CLM 1, yielding 2f in 48% conversion (fig. S14). A typical reaction contained 100 μM 14, 50 μM 1, 100 μM TDP,



10 μM GtfE, and 10 μM CalG1 in a total volume of 100 μl in tris-HCl buffer (10 mM, pH = 7.5) containing 1 mM of MgCl<sub>2</sub> at 30°C for 3 hours. For Fig. 3, detailed assay and HPLC parameters and chromatograms are provided in the Materials and Methods.



**Fig. 4.** Schematic of glycosyltransferase catalysis. **(A)** The classical GT-catalyzed sugar transfer from an NDP-sugar donor to an acceptor to form a glycosidic bond. Although the acceptor nucleophile in these reactions is most often oxygen ( $X=O$ ), GTs are also known to catalyze the formation of *N*-, *S*- and even *C*-glycosidic bonds. **(B)** NDP-sugar synthesis via reverse glycosyltransfer. **(C)** The GT-catalyzed sugar exchange reaction to exchange native natural product sugar appendages with alternative sugars supplied as exogenous NDP-sugars. **(D)** A generalized scheme for an aglycon exchange reaction wherein a sugar is excised from one natural product (as an NDP-sugar) and subsequently attached to a distinct aglycon acceptor. In this reaction, the interchange of aglycons from a single natural product class is generally accomplished via one GT, whereas the interchange of aglycons from different compound classes requires multiple GTs.

therapeutically important natural products (5). For example, before this work, only two methods for differentially glycosylating CLMs were available: pathway engineering and total synthesis. Whereas the former has proven to be a powerful derivatization tool for certain natural products (15), the stringent genetic limitations of the CLM-producing *M. echinospora* has rendered this approach impractical (7). Alternatively, reworking previously reported CLM syntheses to provide efficient divergent routes to the >70 CLM analogs reported herein is also likely impracticable (16–18). With respect to rare NDP-sugars, the demonstrated in situ generation of TDP- $\beta$ -L-vancosamine (12) (Fig. 3A) herein is an advance over reported synthetic methods that required seven linear steps to achieve an overall yield of less than 7%, originating from the same starting material, VCM (10). The CLM-derived TDP-3-*O*-methyl- $\beta$ -L-rhamnose (3) (Fig. 1B) and the three TDP-*N*-alkylaminopentoses (derived from donors 4, 5, 6, and 8) (Fig. 2 and fig. S11) have not been previously synthesized, and therefore direct comparisons to other synthetic routes are not possible (19–21).

Although Glaser and Brown described the reversibility of the native chitin synthetase reaction in one of the first reports of in vitro GT activity (22), the perception of GT catalysis has remained one of unidirectionality, transforming NDP-sugar and aglycon substrates into glycoside products (Fig. 4A) (23–28). In contrast, this study uncovered reversibility in reactions catalyzed by both previously uncharacterized GTs (CalG1 and CalG4) and well-studied GTs (GtfD and GtfE) (11–13). Consistent with an equilibrium only

moderately favoring glycoside formation ( $K_{eq} = 4.5$  for GtfE), these model GT-catalyzed reactions could be modulated via simple adjustments in relative substrate concentrations. Glycosyltransferase reversibility could be exploited to synthesize valuable rare NDP-sugars (Fig. 4B), exchange one sugar on a core scaffold for another (Fig. 4C), or transfer sugars from one scaffold to another (Fig. 4D), suggesting GT catalysis to be of greater versatility and utility than was previously appreciated.

#### References and Notes

- Breton, L. Snajdrova, C. Jeanneau, J. Koca, A. Imbert, *Glycobiology* **16**, 29R (2006).
- P. Sears, C.-H. Wong, *Science* **291**, 2344 (2001).
- P. L. Deangelis, L. C. Oatman, D. F. Gay, *J. Biol. Chem.* **278**, 35199 (2003).
- M. Wacker *et al.*, *Science* **298**, 1790 (2002).
- B. R. Griffith, J. M. Langenhan, J. S. Thorson, *Curr. Opin. Biotechnol.* **16**, 622 (2005).
- K. M. Koeller, C. H. Wong, *Chem. Rev.* **100**, 4465 (2000).
- J. Ahlert *et al.*, *Science* **297**, 1173 (2002).
- Analysis of the CLM  $\gamma_1^1$  biosynthetic gene cluster revealed four putative GT-encoding genes, *calG1*, *calG2*, *calG3*, and *calG4*, implicating a distinct GT for each sugar attachment (7). The *calG1* and *calG4* genes were expressed and purified to near homogeneity (fig. S1), with overall yields of 10 to 15 mg per liter of culture, as described in the Materials and Methods.
- S. Walker, R. Landovitz, W. D. Ding, G. A. Ellestad, D. Kahne, *Proc. Natl. Acad. Sci. U.S.A.* **89**, 4608 (1992).
- M. Oberthür, C. Leimkuhler, D. Kahne, *Org. Lett.* **6**, 2873 (2004).
- H. C. Losey *et al.*, *Biochemistry* **40**, 4745 (2001).
- C. T. Walsh, H. C. Losey, C. L. Freely Meyers, *Biochem. Soc. Trans.* **31**, 487 (2003).
- X. Fu *et al.*, *Nat. Biotechnol.* **21**, 1467 (2003); and references cited therein.
- T. Billign, E. M. Shepard, J. Ahlert, J. S. Thorson, *ChemBioChem* **3**, 1143 (2002).
- S. Blanchard, J. S. Thorson, *Curr. Opin. Chem. Biol.* **10**, 263 (2006).

- Nicolaou and co-workers achieved the enantioselective synthesis of CLM  $\gamma_1^1$  in 29 steps with an overall yield of 0.63% (17), whereas Danishefsky and co-workers achieved CLM  $\gamma_1^1$  in 17 steps with an overall yield of 0.67% (18).
- K. C. Nicolaou *et al.*, *J. Am. Chem. Soc.* **115**, 7625 (1993).
- S. A. Hitchcock, M. Y. Chumoy, S. H. Boyer, S. H. Olson, S. J. Danishefsky, *J. Am. Chem. Soc.* **117**, 5750 (1995).
- Advanced synthetic intermediates related to these NDP sugars have been reported. As a point of comparison, the simpler substrate TDP- $\beta$ -L-rhamnose has been prepared by a five-step chemical synthesis with an overall yield of 27% or by a two-step enzymatic method with 62% yield (20). The most advanced intermediate corresponding to the aminopentoses found in the CLMs required 11 linear steps and provided an overall yield of <12% (21).
- K. Marumo *et al.*, *Eur. J. Biochem.* **204**, 539 (1992).
- S.-H. Kim, D. Augeri, D. Yang, D. Kahne, *J. Am. Chem. Soc.* **116**, 1766 (1994).
- L. Glaser, D. H. Brown, *J. Biol. Chem.* **228**, 729 (1957).
- Cardini *et al.* first demonstrated the reversibility of the native sucrose synthetase reaction (24), which has subsequently been exploited to prepare UDP glucose on large scale (25). However, this enzyme is unique among Leloir GTs in that it catalyzes the formation of an unusually high-energy sucrose glycosidic linkage [Gibbs free energy ( $\Delta G^\circ$ ) of  $-29.3$  kJ/mol] (26). The reversibility of a reaction catalyzed by macrolide resistance GT OLeD was implicated by the measurement of its equilibrium constant ( $K_{eq} = 156$ ) (27). Reversibility of the reaction catalyzed by macrolide GT VinC using a threefold molar excess of VinC was also recently reported (28). The reaction catalyzed by the unusually fast plant galactosyltransferase F3GalTase has also been shown to be readily reversible (29).
- C. E. Cardini, L. F. Leloir, J. Chiriboga, *J. Biol. Chem.* **214**, 149 (1955).
- C. Rupprath, T. Schumacher, L. Elling, *Curr. Med. Chem.* **12**, 1637 (2005).
- E. F. Neufeld, W. Z. Hassid, *Adv. Carbohydr. Chem.* **18**, 309 (1963).
- L. M. Quiros, R. J. Carbajo, A. F. Brana, J. A. Salas, *J. Biol. Chem.* **275**, 11713 (2000).
- A. Minami, K. Kakinuma, T. Eguchi, *Tetrahedron Lett.* **46**, 6187 (2005).
- K. D. Miller, V. Guyon, J. N. S. Evans, W. A. Shuttleworth, L. P. Taylor, *J. Biol. Chem.* **274**, 34011 (1999).
- We thank the UW–Madison School of Pharmacy Analytical Instrumentation Facility for analytical support, S. Borisova and H.-w. Liu (University of Texas, Austin) for the gift of TDP- $\alpha$ -L-rhamnose and TDP- $\beta$ -L-rhamnose, C. T. Walsh (Harvard Medical School, Boston, MA) for providing GtfD and GtfE expression clones, Wyeth Research for the generous gift of CLM analogs, and G. Williams and Y. Huang for many helpful discussions. B.R.G. is a postdoctoral fellow of the American Cancer Society (PF-05-016-01-CDD), and Q.F. acknowledges a graduate fellowship sponsored by Merck Research Laboratories, the Analytical Division of the American Chemical Society, and the DuPont Corporate Center for Analytical Sciences. This research was supported in part by NIH grant nos. AI52218, CA84374, and GM70637; National Cooperative Drug Discovery Group grant U19 CA113297 from the National Cancer Institute (to J.S.T.); the UW School of Pharmacy; Wisconsin Alumni Research Foundation; and a NSF CAREER Award (CHE-0449991 to L.L.). L.L. is an Alfred P. Sloan research fellow, and J.S.T. is an H. I. Romnes fellow. J.S.T. has served on the scientific advisory boards of zuChem and Kosan. J.S.T. dedicates this paper to P. G. Schultz on the occasion of Schultz's 50th birthday.

#### Supporting Online Material

www.sciencemag.org/cgi/content/full/313/5791/1291/DC1  
Materials and Methods  
Figs. S1 to S15  
References

16 May 2006; accepted 11 July 2006  
10.1126/science.1130028