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A Simple Strategy for Glycosyltransferase-Catalyzed Aminosugar Nucleotide Synthesis

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Abstract

A set of 2-chloro-4-nitrophenyl glucosamino/xylosaminosides were synthesized and assessed as potential substrates in the context of glycosyltransferase-catalyzed formation of the corresponding UDP/TDP-α-D-glucosamino-/xylosaminosugars and single vessel transglycosylation reactions with a model acceptor. This study highlights a robust platform for aminosugar nucleotide synthesis and reveals OleD Loki as a proficient catalyst for U/TDP-aminosugar synthesis and utilization.

Keywords

glycobiology; enzyme; glycoside; glycosylation; glycorandomization; carbohydrate

Aminosugars are ubiquitous in nature where they serve as functionally and/or structurally important building blocks for a range of biologically relevant glycoconjugates including peptidoglycans,[1] glycosaminoglycans,[2] aminoglycosides,[3] glycoproteins,[4] and glycosylated natural products (Scheme 1).[5] A unique feature of aminosugars is their enhanced solubility and potential for ionic interactions by virtue of the inherent positive charge under normal physiological conditions.[6] Within this context, aminosugar conjugation has been reported to improve the unconjugated parental compound's basicity,[7] pharmacological properties,[8] and/or even alteration of mechanism.[9] Aminosugar conjugation can be accomplished via either chemical[10] or glycosyltransferase (GT)-catalyzed strategies,[11] the latter of which typically depends upon the availability of suitable aminosugar nucleotide donors. Yet, the reported syntheses of aminosugar nucleotides via chemical,[12] enzymatic,[13] or chemoenzymatic[14] strategies still typically are restricted to

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multi-step, low yielding processes and few, if any, are directly orthogonal to downstream GT-catalyzed reactions. A simple robust strategy directly compatible with downstream aminosugar nucleotide utilizing processes would therefore be considered advantageous.

We recently reported simple aromatic glycosides to serve as efficient donors in glycosyltransferase-catalyzed reactions for both sugar nucleotide formation (*i.e.*, the 'reverse' of a conventional GT-catalyzed reaction) and transglycosylation wherein the use of 2-chloro-4-nitrophenyl glycoside donors also offered a convenient colorimetric screen to enable the directed evolution of enhanced GTs with broad substrate permissivity.[15] Herein we describe an interrogation of two of the most permissive glycosyltransferase prodigy from these prior studies (OleD TDP-16 – a P67T/S132F/A242L/Q268V variant;[15a,16] OleD Loki – a P67T/I112P/T113M/S132F/A242I variant[15b]) for their abilities to catalyze the production of variant aminopentose and/or aminohexose nucleotides in the presence of the corresponding 2-chloro-4-nitrophenyl aminosugar donors and TDP/UDP. This study reveals OleD Loki to catalyze the conversion of 6 out of 7 simple D-glucosamino- and D-xylosamino-glycoside donors into their corresponding UDP/TDP-aminosugar nucleotides and also to utilize 6-azido/acetylamino-D-glucoside donors. Using 4-methylumbelliferone as a model acceptor, this study also highlights the efficient nucleotide-mediated single vessel transglycosylation with 5 of the 6 representative OleD Loki aminosugar substrates. In addition to providing a convenient strategy for novel aminosugar nucleotides, this work also sets the stage to assess the potential for OleD-catalyzed aminosugar conjugation to a range of bioactive natural products and drugs.[17]

For this study, seventeen 2-chloro-4-nitrophenyl azidosugar and aminosugar glycoside donors were synthesized using a simple four-step synthesis (bromination, glycosylation,[18] deprotection, and reduction) from peracylated (acetyl or benzoyl) azidosugars with an overall average yield of 23%. The synthesized 2-chloro-4-nitrophenyl glucosamino/xylosaminosides were subsequently converted to their corresponding hydrochloride salts in an average yield of 94% (Scheme 2, Table S1). In all cases, the corresponding 2-chloro-4-nitrophenyl glycosides were confirmed as the desired β-anomers. Additional analogs generated during synthetic methods development and included in this study include (2-chloro-4-nitrophenyl)-α-L/β-D-arabinoside (**18, 18d**), (2-chloro-4-nitrophenyl)-6-deoxy-6-*N*-acetylamino-β-D-glucoside (**19**), and (2-chloro-4-nitrophenyl)-2-deoxy-2-amino-α-D-glucoside (20) (Figure 1, Figure S1).

Three enzymes (wtOleD, OleD TDP-16, and OleD Loki) were selected to compare in the context of their potential to produce aminosugar nucleotides from the 2-chloro-4-nitrophenyl glycoside panel described above. Of these, TDP-16[16] and Loki[15b] are engineered variants of the *Streptomyces antibioticus* macrolide glucosyltransferase (wtOleD).[19] Standard conditions (10 μM OleD variant, 2 mM UDP or 5 mM TDP, 2 mM 2-chloro-4-nitophenyl glycoside, 50 mM Tris-HCl, pH 8.0, final volume of 100 μL, 25μC, 12 h followed by the RP HPLC analysis) were utilized to compare the turnover across the entire panel of enzyme/glycoside combinations. Figure 1 highlights the outcome of this cumulative study and reveals OleD Loki to have the broadest capacity for aminosugar conversion with all but one targeted free aminosugar donor (3-deoxy-3-amino-β-D-xyloside **17**) leading to appreciable product (≥ 50%) in the presence of either UDP or TDP (Figure 1). An overall preference for

glucosides (rank order of 6-NH_2 ≈ 4-NH_2 ≈ 2-NH_2 > 3-NH_2) over xylosides (rank order of 4-NH_2 ≈ 2-NH_2) was observed with no apparent difference between the donor free base and the corresponding hydrochloride salt (Table S2, Figure S2, S3, S4, S5). By comparison, both wtOleD and OleD TDP-16 were notably worse than OleD Loki with one exception (6-deoxy-6-azido-β-D-glucoside **2**), a previously reported substrate of TDP-16,[15a] where TDP-16 was found to slightly outperform OleD Loki in this endpoint assay. In addition, OleD Loki displayed notable improvement with additional non-native donors beyond the scope of the targeted aminosugar series including 6-deoxy-6-*N*-acetylamino-β-D-glucoside **19** and slight improvement with α-L-arabinoside **18** - both analogs generated during the course of synthetic methods development. Intriguingly, both wtOleD and OleD TDP-16 outperformed OleD Loki with β-D-glucoside **1**. As UDP-glucose is the native substrate of wtOleD,[19] this assessment suggests OleD Loki to offer a unique divergence in sugar specificity from wtOleD prodigy studied to date.

In the context of aminosugar nucleotide synthesis, this OleD catalyzed reversible reaction provides a noteworthy alternative to the synthesis of aminosugar nucleotides and compares favorably to prior precedent. For example, as comparison, prior chemical syntheses of the UDP-2-deoxy-2-amino-α-D-glucose and UDP-6-deoxy-6-amino-α-D-glucose from peracetylated azidosugar precursors required 6 steps with overall yields ranging from 4.5 – 20% and a lengthy (up to 5 days) key conjugation reaction between peracetylated azido-α-D-glucoside-1-phosphates and UMP-morpholidate.[20,21] The prior chemenzymatic syntheses of NDP-2-deoxy-2-amino-, 3-deoxy-3-amino-, 4-deoxy-4-amino-, and 6-deoxy-6-amino-α-D-glucose have also previously been accomplished via the use of an engineered α-D-glucose-1-phosphate thymidylyl-transferases (RmlA) with overall yields ranging from 5–24% (including up to 7 chemical transformations to provide the requisite aminosugar-α-1-phosphate substrates).[22] The current strategy affords the desired UDP/TDP-aminosugars in 7%–28% yield (including the simple four-step synthesis from peracylated azidosugars). Furthermore, given OleD Loki was evolved to also efficiently utilize ADP, CDP, and GDP,[15b] the current study suggests the potential to also employ OleD Loki for the corresponding syntheses of ADP-, CDP-, and/or GDP-aminosugars.

To assess the direct compatibility of this approach with a downstream coupled sugar nucleotide utilizing processes,[23] we examined the ability of the coupled OleD Loki-driven system to mediate the glycosylation of a model acceptor 4-methylumbelliferone **54** (Figure 2). The advantage of 4-methylumbelliferone as a surrogate acceptor is its inherent fluorescence. Specifically, glycosylation of the 4-methylumbelliferone C7-OH extinguishes fluorescence, thereby enabling a highly sensitive fluorescent-based continuous GT assay.[24] To set the stage for this assessment, the UDP concentration was first optimized in the context of the coupled reaction to afford the greatest transglycosylation output (*i.e.*, the best 4-methyumbelliferone glycoside formation) in the presence of (2-chloro-4-nitrophenyl)-2-deoxy-2-amino-β-D-glucoside **5** as a representative aminosugar donor (Figure 2B, 2C). The optimization series [10 μM OleD Loki, 1 mM 4-methylumbelliferone **54**, 1 mM 2-deoxy-2-amino-β-D-glucoside **5** and variant UDP (0.1 – 1.5 mM) in 50 mM Tris-HCl, pH 8.0 with a final volume of 100 μL] revealed 0.1 eq UDP as the optimal relative concentration to support the coupled transglycosylation process. Using this optimized protocol, the coupled

system was subsequently examined in the context of seven 2-chloro-4-nitrophenyl glycoside donors including the five established aminosugar donors for aminosugar nucleotide synthesis (amino-β-D-glucosides **3, 5, 7, 9** and 4-deoxy-4-amino-β-D-xyloside **13**), 6-deoxy-6-azido-β-D-glucoside **2** and β-D-glucoside **1** (Figure 2D). Overall, the trends for transglycosylation generally paralleled that for sugar nucleotide formation highlighted in Figure 1 with a general preference for hexose over pentose congeners and 2-/6-amination favored over the corresponding 3-/4-substitution. Importantly, this assessment confirms that the OleD-catalyzed NDP-aminosugar production strategy can be directly coupled to the downstream sugar nucleotide-utilizing applications.

Inspired by the ability of diverse simple 'activated' donors to modulate the thermodynamics of GT-catalyzed reactions, this work highlights the first systematic interrogation of the most proficient/permissive OleD variants in the context of aminosugar nucleotide formation and utilization. This study revealed OleD Loki to slightly outperform OleD TDP-16 in nearly all standard endpoint assays conducted and to serve as an efficient catalyst for the production of 12 out of 14 targeted UDP/TDP-α-D-glucosamino-/xylosaminosugars from a series of simple 2-chloro-4-nitrophenyl glucosamino/xylosaminoside donors. In addition, OleD Loki also enabled the subsequent production of the corresponding set of model 4-methylumbelliferone glucosamino/xylosaminosides in a series of model UDP-mediated transglycosylation reactions. As such, this work notably highlights an efficient platform for UDP-/TDP-(and potentially ADP-/GDP-/CDP-) aminosugar production that is directly orthogonal to subsequent sugar nucleotide-dependent reactions relevant to a range of glycoconjugation and glycobiology applications.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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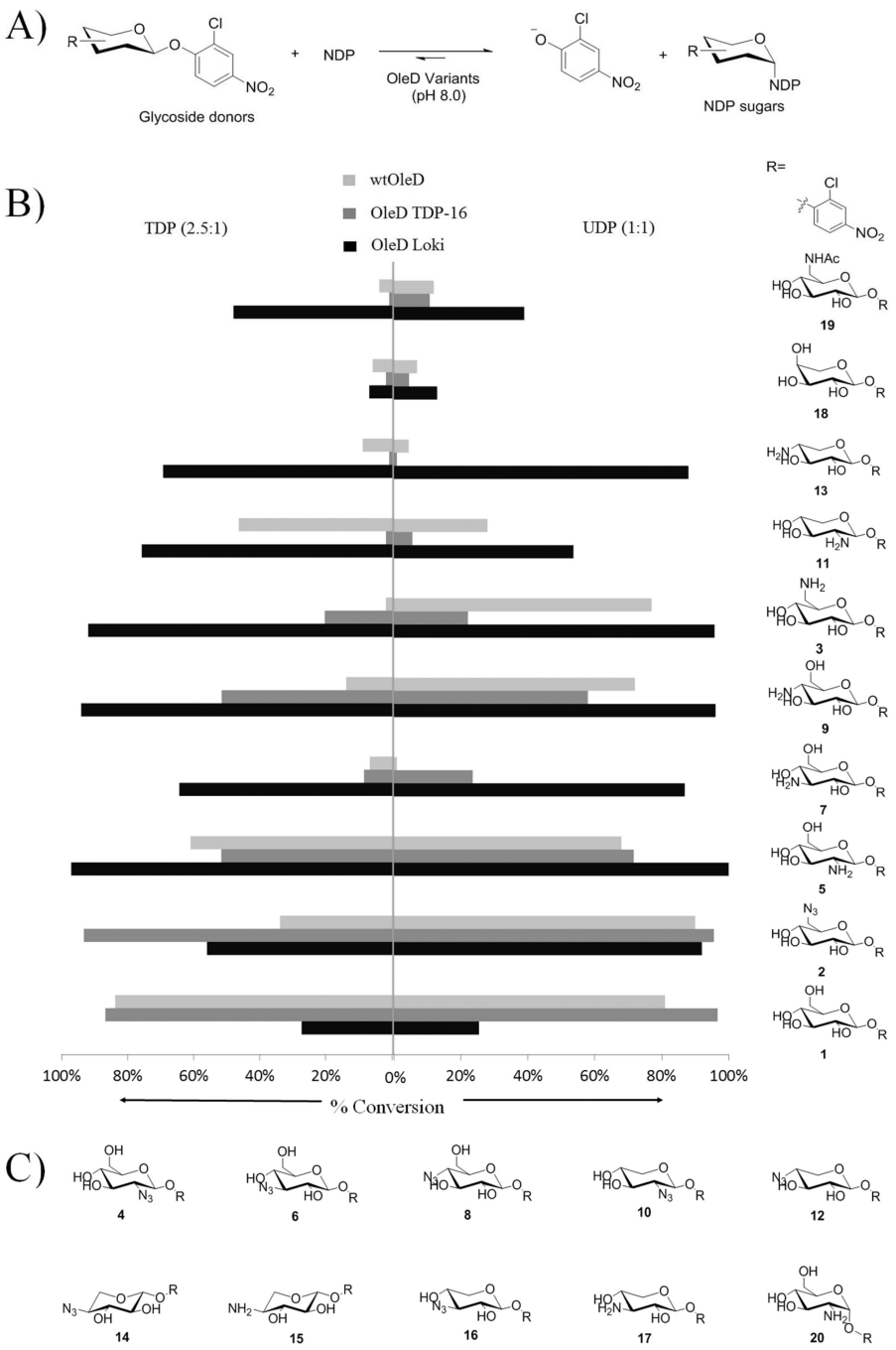


Figure 1.

Comparison of catalysts in 2-chloro-4-nitrophenyl glycoside driven sugar nucleotide syntheses. A) General reaction scheme. B) Maximum observed percentage conversion of (U/T)DP to (U/T)DP-sugars by OleD Loki (dark), TDP-16 (light dark) and wtOleD (gray) ($n \geq 2$, S. D. < 8%) (See Supplement methods). C) Additional 2-chloro-4-nitophenyl glycosides tested for which no products were observed within the sensitivity limits of the assay.

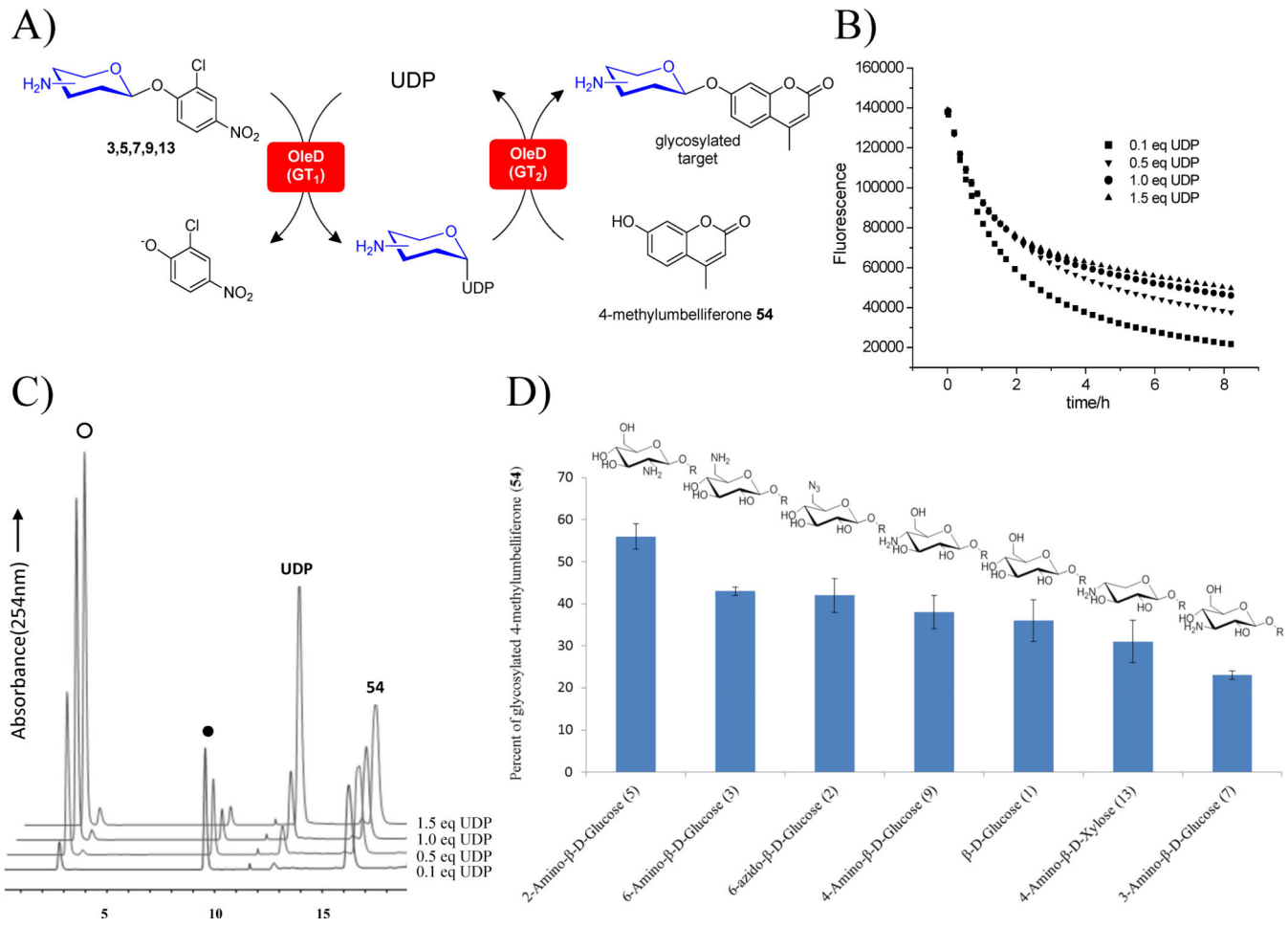
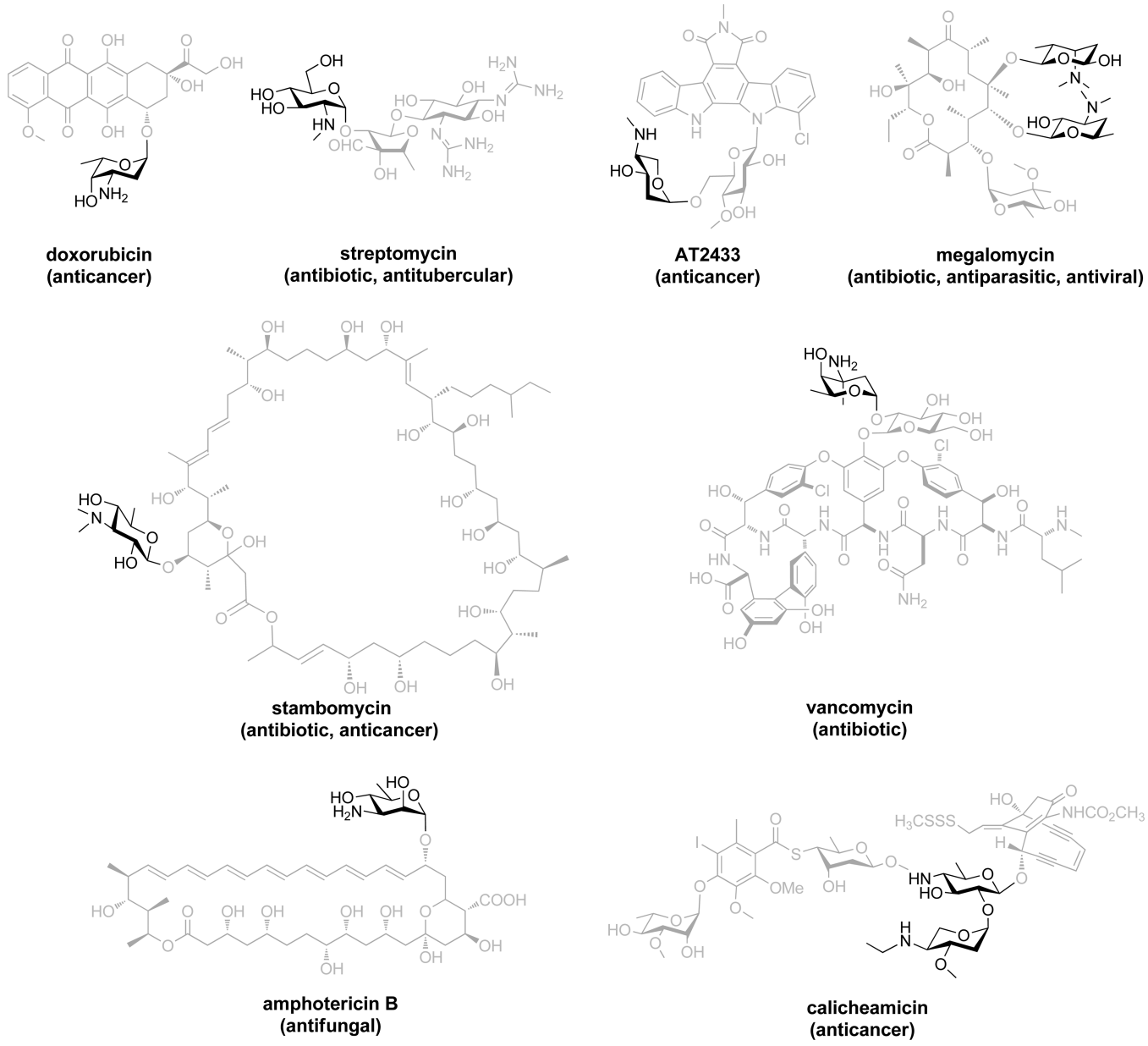


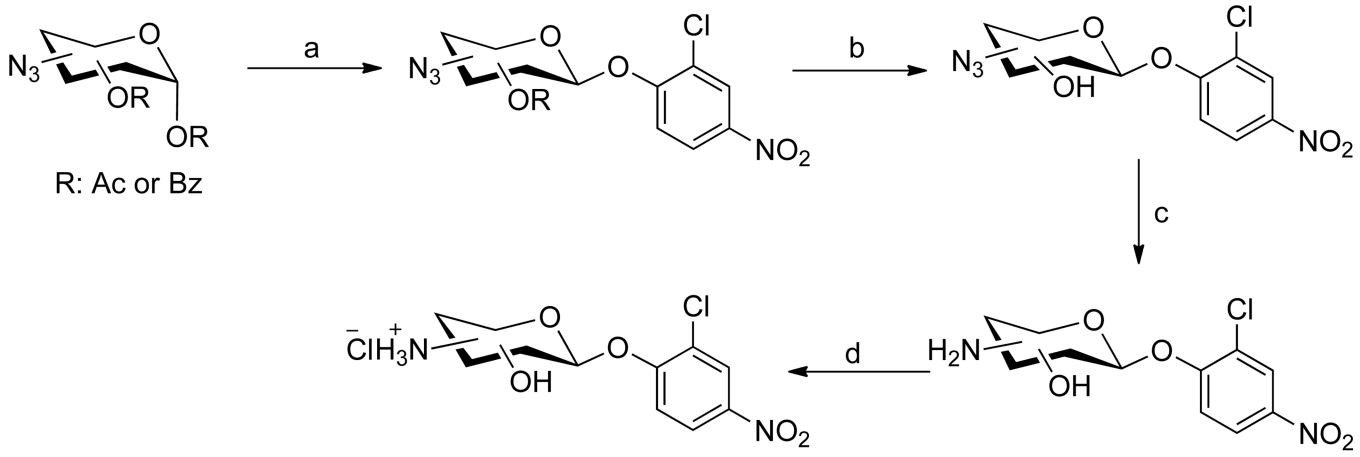
Figure 2.

Single enzyme coupled UDP-mediated transglycosylation reaction of 4-methylumbelliferone **54** with 2-chloro-4-nitrophenyl glucosamino/xylosaminosides. A) General reaction scheme. B) Time course of **5** fluorescence as a measure of reaction progress over 8 h with different UDP concentrations. C) HPLC chromatogram of the final reaction mixtures from panel c after 8 h where the solid circle (●) denotes the **54** glycoside product and (○) represents UDP-2-deoxy-2-amino-α-D-glucose. D) Percentage conversion to **54** glycoside products via OleD Loki-catalyzed NDP-mediated transglycosylation containing 0.1 mM UDP, 1 mM **54**, and 1 mM 2-chloro-4-nitrophenyl glycoside donors (**1, 2, 3, 5, 7, 9, 13**) ($n \geq 2$. S. D. < 5%).



scheme 1.

Representative aminosugar-appended natural products where aminosugars are highlighted in darker shade.



scheme 2.

Synthesis of 2-chloro-4-nitrophenyl aminosugar donors.