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The *C*-Glycosyltransferase UrdGT2 Is Unselective toward D- and L-Configured Nucleotide-Bound Rhodinoses

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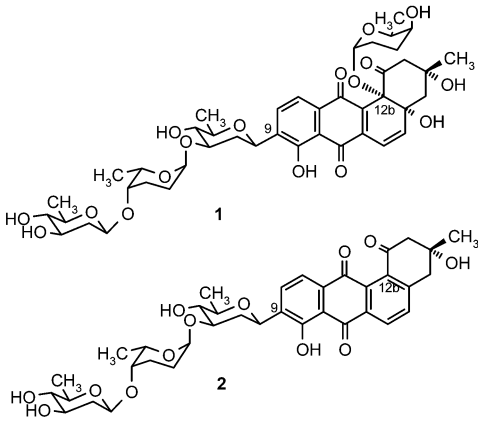
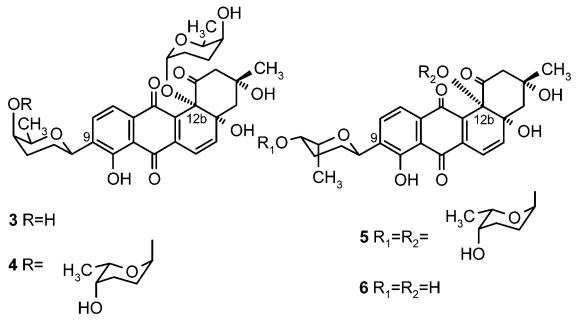
Over the past years it has become more and more obvious that glycosylation affects bioactivity or selectivity of natural products, e.g., anticancer drugs or antibiotics.[1,2] Therefore, altering the glycosylation patterns has become a focus within natural product chemistry and pharmaceutical sciences. The past couple of years saw an encouraging number of investigated wild-type or engineered glycosyltransferases revealing different degrees of enzymatic promiscuity toward their substrates.[3] However, none of these catalysts is able to establish a *C*-glycosidic bond so that biosynthetic chemists are still left without an enzymatic tool for that kind of structural element. In this communication we report the versatility of UrdGT2, a procaryote glycosyltransferase from *Streptomyces (S.) fradiae* Tü2717, the producer of the angucyclic antitumor drugs urdamycin A (**1**) and B (**2**).[4] Both are glycosidic compounds with interesting modes of sugar attachment since they show an unusual *C*-glycosylation at carbon 9. The urdamycin A backbone offers a second glycosylation target at the tertiary OH group at C-12b. For urdamycins and a number of other angucycline glycosides, bioactivity is well documented.[4,5]



Initially, genetic work proved UrdGT2 to be responsible for the transfer of 2,6-dideoxy-D-glucose (D-olivose) from its nucleoside diphosphate (NDP)-activated form to C-9 of the urdamycin polyketide backbone, forming a *C*-glycosidic bond.[6] Further in vivo work showed that UrdGT2 can also attach D-olivose as well as D-mycarose to premithramycinone, a biosynthetic precursor of the aureolic acid antitumor drug mithramycin.[7] Despite promising progress in the synthesis of nucleotide-activated deoxygenated sugars,[8] investigations on glycosyltransferases still suffer from the drawback that the sugar donor substrates are, in many cases, not available for in vitro work. To overcome these limitations, at least in part, we reverted to an in vivo system. Previously, we had investigated the biosynthetic routes to NDP-D-olivose and NDP-L-rhodinose, demonstrating that they share a common pathway starting with D-glucose-1-phosphate which is converted to NDP-4-keto-2,6-dideoxy-D-glucose in a multistep process. Three enzymes, UrdQ, UrdZ1, and UrdZ3, are then specifically involved in NDP-l-rhodinose formation. A NDP-4-keto-2,6-dideoxyglucose ketoreductase, UrdR, is catalyzing the final step in NDP-D-olivose biosynthesis. *S. fradiae* RN-435, a mutant lacking UrdR, was shown to produce NDP-rhodinose of both D- and L-configuration side by side, proven by the biosynthesis of urdamycin M (**3**).[9] Here, we report the isolation and structural elucidation of novel urdamycin derivatives, now referred to as urdamycins R (**4**) and S (**5**), which both display a *C*-glycosidically linked rhodinose moiety at 9-position, but differ with respect to the configuration of this sugar moiety.



A first hint on the enzyme's unusually broad capability of *C*-glycoside formation was provided by HPLC−UV and LC−MS investigations.[10] They revealed two chromatographically separated compounds, **4** and **5**, with retention times of 9.25 and 10.5 min, respectively, both with a molecular mass of $m/z = 698$ and the typical urdamycin A UV−vis spectrum. This mass is consistent with the known angucyclic polyketide backbone and three trideoxyhexoses. To a lesser extent, the corresponding metabolic twins with an urdamycin B-type backbone were also found, whose masses ($m/z = 550$) indicate only two trideoxyhexoses, which was expected, since the urdamycin B polyketide lacks the tertiary alcohol group at C-12b as a glycosylation site. Therefore, these two compounds lack the 12b-bound sugar moiety, but carry the same disaccharides consisting of two trideoxysugars. We proposed that in one of these structures the sugar at C-9 might be a D-rhodinose, as was found for **3**; the other sugar's identity and stereochemistry remained to be elucidated.

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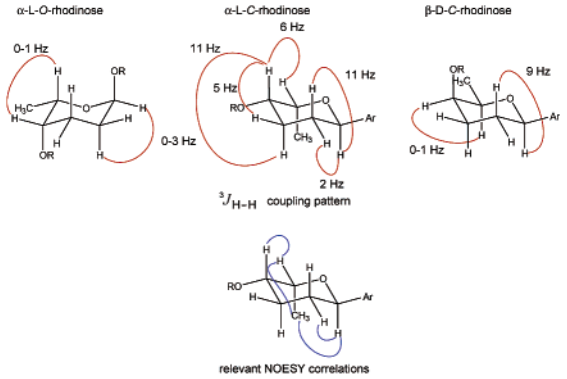

Figure 1. $^3J_{H-H}$ coupling patterns and NOESY correlations for discrimination of *O*- and *C*-glycosidically attached rhodinoses in **4** and **5**.

From a 10 L-fermentation of *S. fradiae* RN-435 we purified 12 mg of pure substance **4** and **5**, respectively. NMR analyses revealed that both **4** and **5** contain two α-L-*O*-glycosides and one *C*-glycoside. All α-L-*O*-glycosidic moieties in **4** and **5** were identified as α-L-rhodinoses, proven by the typical H−H-coupling pattern depicted in Figure 1. Also the *C*-glycosidically bound sugar in **5** was identified as α-L-rhodinose that, however, appears in this structure in $^4C_1$ conformation which is atypical for L-rhodinose, with an equatorial aromatic system at C-1, an equatorial OH group at C-4, and an axial CH_3 group at C-5. This was concluded from the $^3J_{H-H}$ coupling data showing two large di-axial $^3J_{H-H}$ couplings ($1\text{-}2_{ax}$, $3_{ax}\text{-}4$), and two axial−equatorial couplings (3_{eq}-4, 4-5). Further proof of this conformation was obtained from NOESY data showing the correlations shown in Figure 1 ($1\rightarrow 2_{eq}$, 1→6, 5→6, 5→4). All these NMR correlations were also verified with the corresponding spectra of 12b,4'-diderhodinosyl-urdamycin S (**6**) obtained by acidic hydrolysis of (**5**), which were free of the "background noise" of the two *O*-glycosidically bound rhodinose moieties.

The *C*-glycosidic sugar moiety in **4** was identified as a *β*-D-rhodinose, after analyzing the H−H coupling pattern and selective NOESY data as well. For both structures **4** and **5** the NMR data also confirm one rhodinose moiety to be attached to the 12b-OH group, indicated by the significantly downfield shifted 6-H_3-signals (δ 0.50 and 0.55 for **4** and **5**, respectively), due to the location of this rhodinose's CH_3 group in the anisotropy cone of the quinone chromophore.

In summary, the structural analyses unequivocally demonstrate that both NDP-activated L- and D-rhodinose are suitable donor substrates for UrdGT2 to set up a *C*-glycosidic system at C-9 of the urdamycin polyketide backbone. Since HPLC peak areas prove an equal biosynthetic rate, we conclude that UrdGT2 is not stereoselective with respect to the configuration at 4- and 5-positions of the sugar donor substrate, in addition to the earlier found flexibility regarding the 3-position of the sugar donor substrate and the acceptor molecule.[7] Targeted gene inactivation of *urdGT2* clearly proved that *S. fradiae* does not harbor a second transferase capable of transferring L-rhodinose, or other sugars present in the cell, to the urdamycin backbone at C-9, since all metabolites found in that mutant strain lacked any sugar at that particular position.[6] Hence, we can rule out that a cryptic transferase outside the urdamycin biosynthetic locus accounts for the observed lacking stereospecificity.

Interestingly, elongation to a disaccharide was only accomplished with L-rhodinose. Thus, UrdGT1c, the glycosyltransferase responsible for the transfer of the second unit in the trisaccharide of **1** and **2**[10] was found to be limited to L-rhodinose, although its D-configured isomer was present. However, this transferase seems to be flexible toward the acceptor substrate, since it can establish both α-(1−3)-*O*-glycosidic bonds under wild-type conditions as well as α-(1−4)-*O*-glycosidic bonds regardless of the acceptor sugar's stereochemistry as shown during this study.

The rationale behind this study on UrdGT2 was to provide more insight into a *C*-glycosyltransferase, important yet under-investigated tools for combinatorial drug lead diversification. This enzyme's *C*-glycosylation target, a carbon in ortho position to a phenolic OH group of a juglon-like chromophore, is a common structural element found with numerous natural products. Since many of them are bioactive as well, further studies on UrdGT2 have to reveal a far-ranging flexibility on the acceptor side to demonstrate its versatility in drug development.

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Supporting Information Available: NMR data for compounds **4**, **5**, and **6**, and experimental procedures (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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