Rational Saccharide Extension by Using the Natural Product Glycosyltransferase LanGT4

Dirk Hoffmeister,[a, b] Monika Weber,[a] Gerald Dräger,[c] Koji Ichinose,[d] Clemens Dürr,[a] and Andreas Bechthold*[a]

Introduction

Glycosylated natural-product polyketides, mainly streptomycete metabolites, have attracted attention since they exhibit potent antibacterial as well as anticancer activity.[1] Much effort has been made to engineer polyketide backbones, including late tailoring steps, such as the transfer of modified sugars.[2] Surprisingly, saccharide extension reactions have so far been investigated to only a much smaller extent, although for a number of bioactive oligosaccharide-conjugated natural products, among them mithramycin and vancomycin, extending glycosyltransferases (GTs) have been identified.[3, 4] However, none of those GTs available has so far been used to elongate natural products' saccharide chains beyond the wild-type length. Here, we report the in vivo conversion of a natural-product trisaccharide into an artificial tetrasaccharide side chain. As catalyst, we chose the glycosyltransferase LanGT4, which functions in the pathway to the anticancer drug landomycin A (**1**, Scheme 1) in *Streptomyces (S.) cyanogenus* S136.[5] Previous work demonstrated that LanGT4 is responsible for the transfer of the unusual NDP-activated 2,3,6-trideoxy-L-*threo*-hexopyranose (L-rhodinose) to the equatorial 3-OH of D-configured deoxysugars.[6] To investigate LanGT4 in vivo, we used *S. fradiae* Tü2717, which produces urdamycins A (**2**, Scheme 1) and B (**3**, Scheme 1).[7]

The first evidence for an altered secondary metabolite spectrum arose from HPLC-MS analyses of crude extracts of *S. fradiae*, expressing *lanGT4*.[8] Two novel metabolites were found with different retention times and molecular ion signals (20.5 min, $m/z = 958$, and 24.2 min, $m/z = 810$), than **2** (14.0 min, $m/z = 844$) and **3** (18.3 min, $m/z = 696$), which constitute the principal compounds in the wild-type *S. fradiae* (Figure 1). However, the UV-visible spectra of these two novel compounds, hereafter referred to as urdamycins U (**4**, Scheme 1) and V (**5**, Scheme 1), respectively, were identical to those of the angucyclic juglon-derived chromophores of **2** and **3**. In both cases, the mass difference with respect to the parent compounds **2** and **3** is 114, which matches exactly the mass after attachment of a trideoxysugar residue. Therefore, we concluded that an additional trideoxyhexose must be present in both new substances. Extensive NMR studies were done, primarily with **5**, since most regions of the aquayamycin aglycon in **4** showed extremely broad signals in its ^{1}H and ^{13}C NMR spectra. The ^{1}H and ^{13}C NMR spectra of compound **5** showed the existence of an urdamycinone B aglycon.[7, 8] Along with the *C*-glycosidically linked β-D-olivose, three more deoxyhexoses were identified in the ^{1}H NMR spectrum. The spin systems of all of these sugars were detected with selective TOCSY experiments and confirmed by Heteronuclear Multiple Quantum Coherence (HMQC) and Bond Correlation (HMBC) data. Analysis of the sugar moieties unambiguously showed two α-L-rhodinoses and one β-D-olivose that were *O*-glycosidically linked. The absolute configuration of the sugar moieties was determined as depicted in Figure 2, also taking into account that streptomycete GTs, like all GTs affiliated with family 1 in the CAZy classification,[9] follow a mechanism inverting the anomeric configuration. Final evidence for the regiochemistry of the glycosidic linkage arose from HMBC spectra, which unambiguously displayed ^3J couplings of each anomeric proton to the corresponding carbon atom of the adjacent sugar moiety. Thus, we could prove that the additional sugar unit is, as anticipated, L-rhodinose, α-(1,3)-glycosidically bound to the equatorial 3‴-OH group of the regular urdamycin trisaccharide. Follow-up NMR analyses of **4** with the same methods as before revealed the presence of a tetrasaccharide, identical to the one found in **5**.

The past couple of years have seen significant advances in oligosaccharide synthesis, for example strategies for the synthesis of challenging 2-deoxy-β-glycosidic linkages,[10] solid-phase approaches that may enable future automation,[11] and chemoenzymatic preparation by using engineered glycosynthases.[12] However, none of these embraces an aglycon backbone of pharmaceutically relevant drugs. Therefore, GTs involved in secondary metabolism, such as glycoside antibiotics, maintain or even increase their value as tools for drug diversification.

In summary, the implications of our present work are twofold. First, the notion of GTs as fairly flexible backbone-tailoring tools in drug development can be extended to the so far little-known transferases involved in saccharide-elongating steps. Moreover, the results hint at iteratively acting natural-product GTs; the details of how the landomycin A hexasaccharide biosynthesis proceeds is still not well understood since the corresponding biosynthetic gene locus harbors only four GT genes.[5] This and previous work demonstrate that the capability of LanGT4 includes adding the second and fourth elements of a polyketide-borne saccharide.[6] During landomycin biosynthesis, however, L-rhodinoses are transferred as third and sixth residues, in both cases through an α-(1,3)-linkage to an equatorial hydroxyl group. We assume that LanGT4 is highly flexible with respect to

[a] *Dr. D. Hoffmeister, M. Weber, C. Dürr, Prof. Dr. A. Bechthold*
Albert-Ludwigs-Universität, Institut für Pharmazeutische Wissenschaften
Stefan-Meier-Straße 19, 79104 Freiburg (Germany)
Fax: (+49) 761-203-8383
E-mail: andreas.bechthold@pharmazie.uni-freiburg.de

[b] *Dr. D. Hoffmeister*
The University of Wisconsin, School of Pharmacy
777 Highland Avenue, Madison, WI 53705 (USA)

[c] *Dr. G. Dräger*
Universität Hannover, Institut für Organische Chemie
Schneiderberg 1b, 30167 Hannover (Germany)

[d] *Dr. K. Ichinose*
The University of Tokyo, Graduate School of Pharmaceutical Sciences
Hongo, Bunkyo-ku, 113-0033 Tokyo (Japan)

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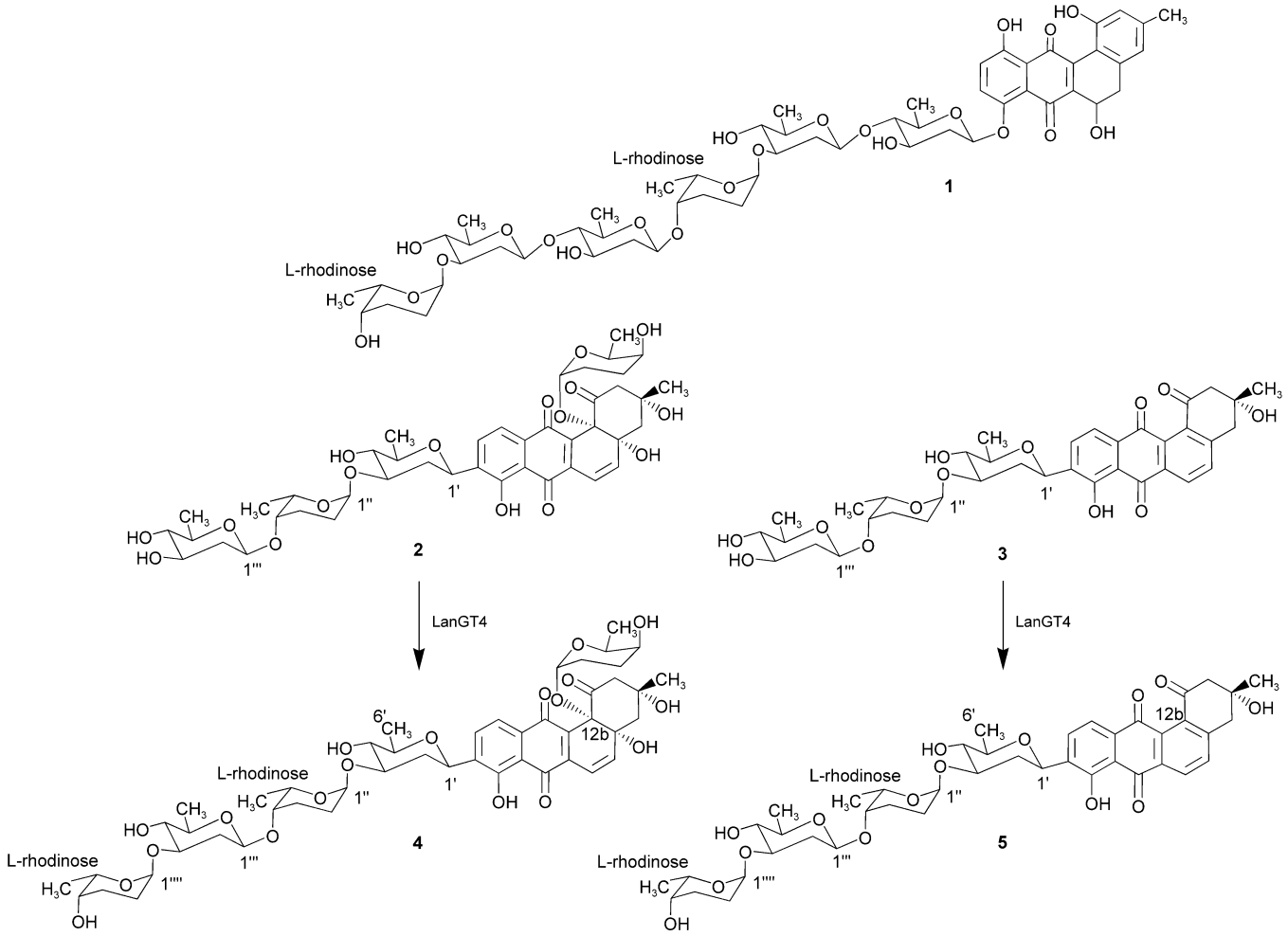

Scheme 1. *Chemical structures of the natural products Landomycin A (**1**) from* S. cyanogenus *S136, Urdamycins A (**2**) and B (**3**) as principal metabolites of* S. fradiae *Tü2717, and the novel Urdamycins U (**4**) and V (**5**).*

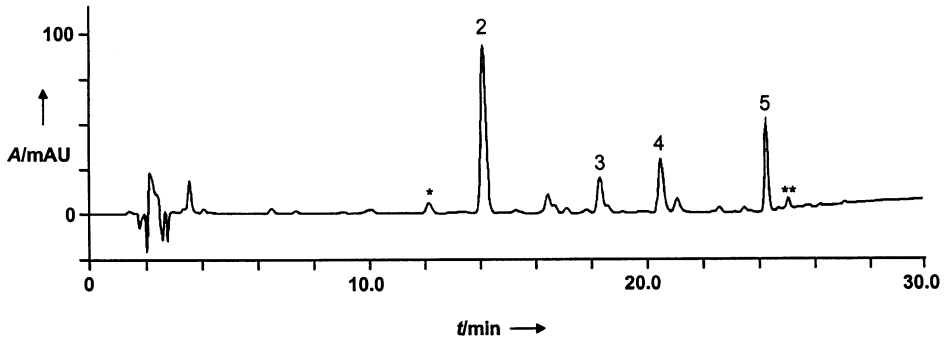

Figure 1. *HPLC chromatogram of a crude extract from* S. fradiae *Tü2717 after* lanGT4 *overexpression. Traces of urdamycins not mentioned in the text are marked by asterisks: * indicates urdamycin G (the disaccharide precursor of 2), and ** indicates an unidentified derivative of **5**, presumably with a hexasaccharide chain.*

the acceptor saccharide length. Therefore, we propose that both rhodinosylations in landomycin A biosynthesis are accomplished by this unusual GT. Consequently, LanGT4 represents a tool for saccharide extensions in a predictable way.

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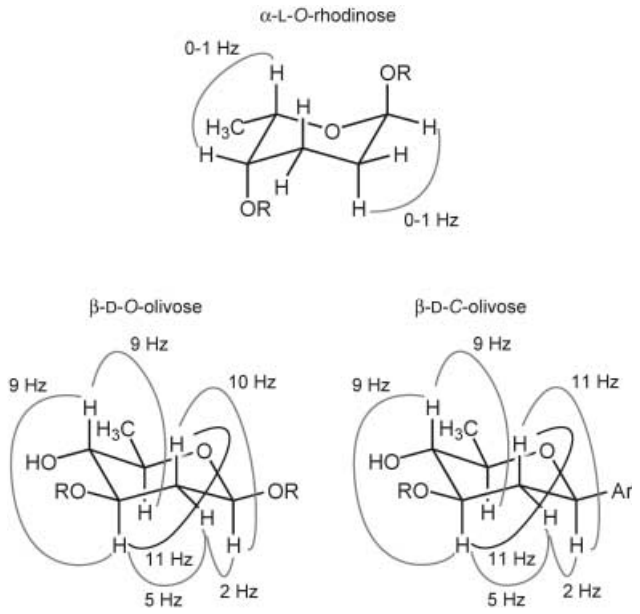

Figure 2. *$^{3}J_{H\text{-}H}$ coupling pattern of the sugar moieties found in **4** and **5**.*

Keywords: biosynthesis · drug design · enzymes · glycosyltransferase · urdamycin

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Expanding the Diversity of Unnatural Cell-Surface Sialic Acids

Sarah J. Luchansky,[b, d] Scarlett Goon,[b, c, d] and Carolyn R. Bertozzi*[a, b]

Novel chemical reactivity can be introduced onto cell surfaces through metabolic oligosaccharide engineering.[1, 2] This technique exploits the substrate promiscuity of cellular biosynthetic enzymes to deliver unnatural monosaccharides that bear bioorthogonal functional groups into cellular glycans. For example, derivatives of *N*-acetylmannosamine (ManNAc) are converted by the cellular biosynthetic machinery into the corresponding sialic acids and subsequently delivered to the cell surface in the form of sialoglycoconjugates (Scheme 1 A). Analogues of *N*-acetylglucosamine (GlcNAc) and *N*-acetylgalactosamine (GalNAc) are also metabolized and incorporated into cell-surface glycans, probably through the sialic acid or GalNAc salvage pathways.[3–6] Furthermore, GlcNAc analogues can be incorporated into nucleocytoplasmic proteins in place of β-*O*-GlcNAc residues.[7] These pathways have been exploited in order to integrate unique electrophiles such as ketones and azides into the target glycoconjugate class. These functional groups can be further elaborated in a chemoselective fashion by condensation with hydrazides[8] or by Staudinger ligation,[9] respectively, thereby introducing detectable probes onto the cell (shown schematically in Scheme 1 B).

We have previously demonstrated that *N*-levulinoylmannosamine (ManLev, **1a**; Scheme 2) is metabolized by cells to *N*-levulinoyl sialic acid (SiaLev, **2a**), which is then appended to glycoconjugates that are ultimately expressed on the cell surface.[3, 8] Increasing the length or steric bulk of the *N*-acyl side chain of **1a** decreases cell-surface expression of the corresponding sialic acids.[10] A rate-determining step in the de novo biosynthesis of unnatural sialic acids appears to be the phosphorylation of ManNAc at the 6-OH by ManNAc 6-kinase.[10] Accordingly, Reutter and co-workers were able to introduce a

[a] *Prof. C. R. Bertozzi*
Departments of Chemistry and Molecular and Cell Biology and Howard Hughes Medical Institute
University of California and Materials Sciences Division
Lawrence Berkeley National Laboratory
Berkeley, CA 94720 (USA)
E-mail: crb@berkeley.edu

[b] *S. J. Luchansky, S. Goon, Prof. C. R. Bertozzi*
Department of Chemistry, University of California – Berkeley
B84 Hildebrand Hall, Berkeley, CA 94720 (USA)
Fax: (+1) 510-643-2628

[c] *S. Goon*
Current address:
Enteric Disease Department, Naval Medical Research Center
Silver Spring, MD 20910 (USA)

[d] *S. J. Luchansky, S. Goon*
These authors contributed equally to this work.

Supporting information for this article is available on the WWW under http://www.chembiochem.org or from the author.