Digitoxosyltetracenomycin C and Glucosyltetracenomycin C, Two Novel Elloramycin Analogues Obtained by Exploring the Sugar Donor Substrate Specificity of Glycosyltransferase ElmGT

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Our explorations of glycosyltransferase ElmGT from *Streptomyces olivaceus* Tü 2353, which shows an interesting flexibility regarding its sugar donor substrate, were extended toward various previously unexplored sugar co-substrates. The studies revealed that ElmGT, which normally transfers L-rhamnose to 8-demethyltetracenomycin C as a crucial biosynthetic step in elloramycin biosynthesis, is also able to process an activated non-deoxygenated sugar, NDP-D-glucose, as well as NDP-L-digitoxose, which is the first example of an NDP-L-sugar co-substrate of ElmGT possessing an axial 3-OH group. The structures of the resulting novel elloramycin analogues of these experiments, 8-demethyl-8-L-digitoxosyltetracenomycin C (**4**) and 8-demethyl-8-D-glucosyltetracenomycin C (**7**), were elucidated mainly by ^{1}H and ^{13}C NMR spectroscopy and by mass spectrometry.

Glycosyltransferases are appealing enzymes for drug discovery programs applying a combinatorial biosynthesis approach, since glycosyltransferases are responsible for the attachment of sugar moieties, often deoxysugars, which add important features to the shape and the stereoelectronic properties of a molecule and often play an essential role in the biological activity of many natural product drugs.[1−4] It has been shown recently that many glycosyltransferases have naturally an unexpected inherent substrate flexibility either toward their acceptor substrate (usually alcohols or phenols) or regarding their (deoxy)sugar donor co-substrates, sometimes even to both.[5−7]

The elloramycins[8−10] and tetracenomycins[11,12] form a distinct group of tetracyclic aromatic polyketides, which is remotely related to the anthracyclines and tetracyclines. ElmGT[7] is a glycosyltransferase (GT) that connects L-rhamnose with 8-demethyltetracenomycin C (**1**) to form the glycosylated late intermediate **2** in elloramycin (**3**) biosynthesis, which then only needs three more *O*-methylation steps at the deoxysugar as well as one at the aglycone moiety (12a-position) to finish the biosynthesis of the antitumor agent elloramycin (**3**, Figure 1).[9] The corresponding gene *elmGT* was isolated from cosmid 16F4 that contains a 25 kb DNA region of this cluster including all genes necessary for the formation of the polyketide aglycone moiety of elloramycin as well as the methyltransferases necessary to methylate all OH groups of the sugar moiety. The tremendously relaxed specificity of ElmGT toward its NDP-sugar donor substrate became obvious after heterologous expression of cosmid 16F4 into various host strains.[7,9,10,13−15] ElmGT was able to glycosylate its acceptor substrate 8-demethyltetracenomycin C (**1**) with L-rhamnose, L-rhodinose, L-olivose, D-olivose, D-mycarose, and even a disaccharide consisting of two D-olivose moieties (for structures, see Supporting Information). In all these experiments the NDP-sugar co-substrates were provided by the host strains, while ElmGT, and not one of the host GTs, was responsible for the linkage of the various sugar building blocks.[7] The only "shortcoming" of ElmGT with respect to its broad substrate specificity toward its NDP-sugar donor substrate was that it could not transfer 4-keto-sugars, such as 4-keto-L-rhamnose.[9]

In this note we report further investigations of ElmGT toward its NDP-sugar donor substrate. We were interested whether ElmGT can handle (i) L-sugars with an axial 3-OH group and (ii) nondeoxysugars. To test these alternatives, the following general approach was used. *Streptomyces lividans* served as host strain, into which two plasmids were heterologously expressed, namely, (i) cosmid 16F4, which contains all genes necessary for the biosynthesis of 8-demethyltetracenomycin C (**1**), the GT encoding gene *elmGT*, and three methyltransferase encoding genes, and (ii) different plasmid constructs directing the biosynthesis of NDP-L-sugars. Upon cultivation of the different recombinant strains, formation of novel glycosylated derivatives was analyzed by HPLC and, if appropriate, compounds were isolated and structures were elucidated.

To assay the first alternative (i.e., L-sugars with an axial 3-OH group), we used pLNB43. This is a pLN2[16] derivative that contains all L-oleandrose genes from the oleandomycin producer *Streptomyces antibioticus*, except *oleY* (encoding the *O*-methyltransferase),[17] which was substituted by *eryBIII* (encoding the 3*C*-methyltransferase of the L-cladinose building block of eythromycin A), and *oleU* (encoding the 4-ketoreductase), which was replaced by the analogous gene *eryBIV* (encoding the 4-ketoreductase of L-cladinose biosynthesis) of the erythromycin producer *Saccharopolyspora erythraea*. We anticipated the following possibilities for the outcome of this experiment:

(a) The "sugar plasmid" leads to the construction of activated L-mycarose; thus the experiment should yield L-mycarosyltetracenomycin C.

(b) The erythromycin C-methyltransferase EryBIII fails, and the activated sugar encoded by plasmid pLNB43 yields either NDP-L-digitoxose or NDP-L-olivose or both, depending how the stereochemistry for the 3-position is controlled

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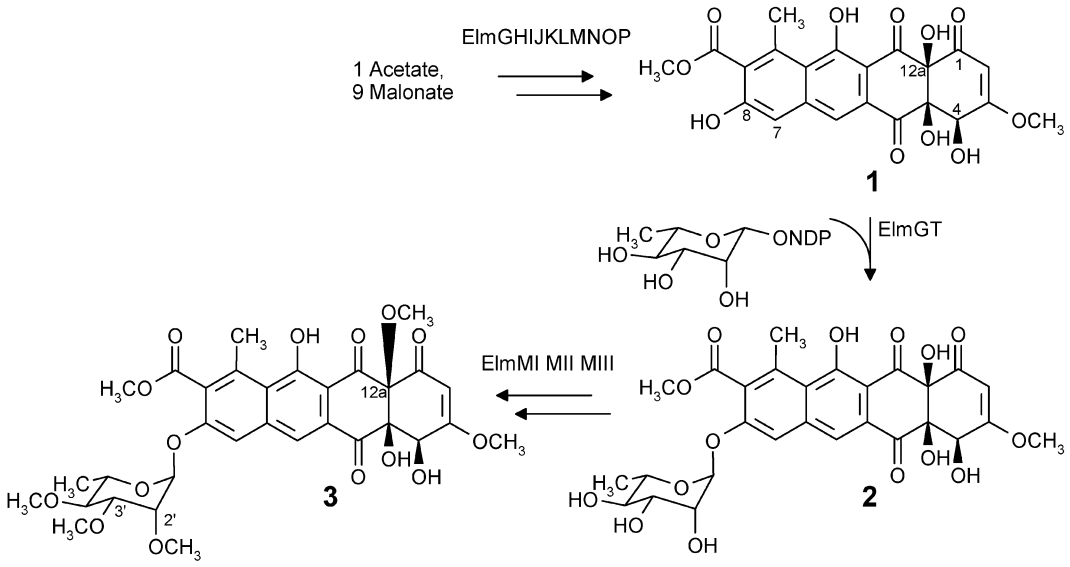


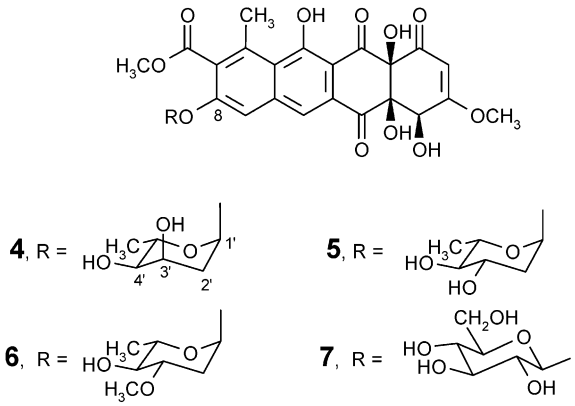
Figure 1. Glycosyltransfer step catalyzed by ElmGT within the elloramycin biosynthesis: **1**, 8-demethyltetracenomycin C; **2**, L-rhamnosyltetracenomycin C; **3**, elloramycin A.

(does EryBIII contribute at least to the stereochemical outcome or not at all, does EryBIV influence the 3-stereochemistry?). Thus the experiment should yield L-olivosyltetracenomycin C (a known product from a previous experiment) or L-digitoxosyltetracenomycin C, or both.

(c) The erythromycin ketoreductase EryBIV fails, and plasmid pLNB43 directs the biosynthesis of a 4-ketosugar, either with an equatorial or an axial OH group in 3-position, either with or without the 3-*C*-methyl group (depending on EryBIII). Thus the result of the experiment may also be 4-keto-L-olivosyltetracenomycin C, 4-keto-L-mycarosyltetracenomycin C, or 4-keto-L-digitoxosyltetracenomycin C.

The product spectrum of *S. lividans* (16F4, pLNB43) yielded one major product and one minor product. The latter was identified as 8-demethyl-8-L-olivosyltetracenomycin C (**5**, 0.2 mg/L).[9] The main product was elucidated to be 8-demethyl-8-L-digitoxosyltetracenomycin C (**4**, 2 mg/L). This was evident from the HRESMS, showing molecular ions at *m*/*z* 611.1359 ($M + Na^+$, 100%) and 589.1550 ($M + H^+$, 10%), consistent with a molecular formula of $C_{28}H_{28}O_{14}$, and from the NMR data. The ^{13}C NMR showed signals expected for the aglycone moiety and six extra signals consistent with one deoxyhexose moiety. Analysis of the ^1H NMR revealed that the sugar is α-glycosidically linked to a phenolic oxygen atom (δ_H of 1′-H: 5.97 d, $J = 3.5$ Hz) and that it is a 2,6-dideoxysugar with an equatorial 4-OH and an axial 3-OH group. This stereochemistry was deduced from the signal patterns of 2′-H_a (δ_H 2.20 ddd, $J =$ 15, 3.5, 3.5 Hz) and 2′-H_e (δ_H 2.28 ddd, $J = 15$, 3.0, 1.0 Hz), which both show only small couplings with 3′-H (after D_2O exchange: δ_H 4.04 ddd, $J = 3.5$, 3.0, 3.0 Hz). The 4′-H signal (after D_2O exchange: δ_H 3.27 dd, $J = 9.5$, 3.0 Hz) showed one small (to 3′-H) and one large coupling (to 5′-H), thus indicating its axial position. The HMBC spectrum revealed coupling between 1′-H and C-8, proving the attachment of the sugar moiety to the phenolic 8-*O*-atom.

We attempted to search for minor non-deoxysugar-containing compounds, since at least activated D-glucose is ubiquitous and thus is also produced by *S. lividans*. If ElmGT is able to handle activated non-deoxysugars, then D-glucosyltetracenomycin C might be formed as a minor side product of the experiments. When analyzing extracts from different recombinant strains harboring cosmid 16F4 and different "sugar plasmids", we frequently noticed that



there was a minor HPLC peak in ethyl acetate extracts of these strains. We also found that, in some recombinant strains, the size of this peak greatly increased when the culture supernatants were directly analyzed (without organic solvent extraction) by HPLC. Its yield was particularly high in *S. lividans* containing cosmid 16F4 and plasmid pRHAM[18] or derivatives.[13] This new product was isolated and elucidated as 8-demethyl-8-D-glucosyltetracenomycin C (**7**, 3.5 mg/L). The HRESMS shows molecular ions at *m*/*z* 643.1298 (100%, $M + Na^+$) and at *m*/*z* 621.1483 (30%, $M + H^+$), both in agreement with a molecular formula of $C_{28}H_{28}O_{16}$. The NMR spectra indicated the presence of an additional sugar moiety which was, considering the chemical shifts of its six ^{13}C NMR signals and its ^1H NMR signals, not a deoxysugar. The coupling constants of the sugar showed its β-glycosidic linkage and are in agreement with the glucose stereochemistry. A cross signal of the HMBC spectrum between 1′-H and C-8 proved the linkage of this D-glucose moiety to the 8-position.

The results described here prove for the first time that ElmGT is able to process (i) the nondeoxygenated activated sugar NDP-D-glucose and (ii) NDP-L-digitoxose, i.e., an activated L-sugar, whose 3-OH is axial. The results also show that ketoreductase EryBIV, which normally reduces NDP-4-keto-L-mycarose, was able to act on a substrate lacking the equatorial 3-*C*-methyl group. Since the natural substrate of EryBIV also has an axial 3-OH group, it is likely that EryBIV is responsible for this step. It is somewhat surprising that EryBIII is unable to *C*-methylate

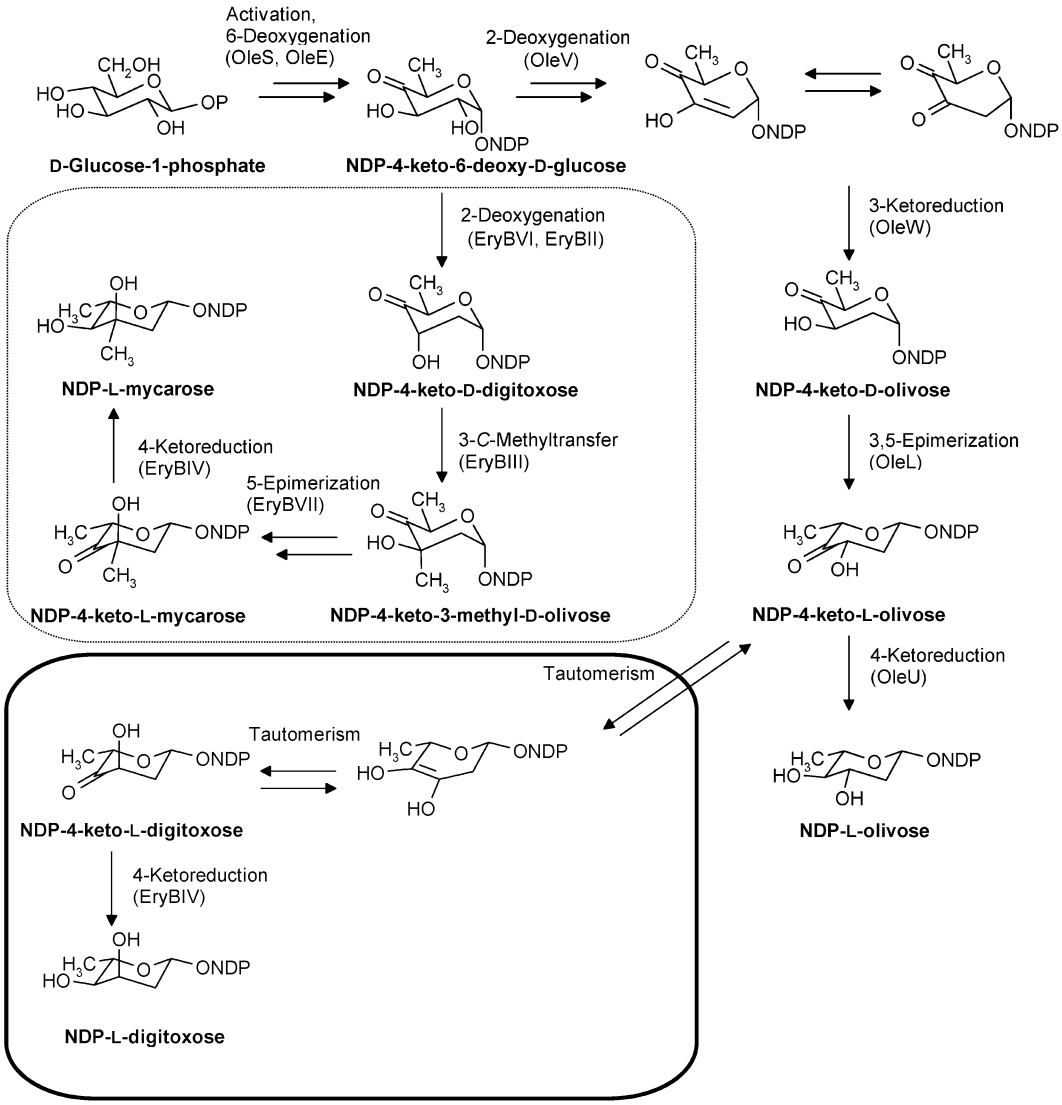


Figure 2. Biosynthetic pathways to NDP-L-olivose (in the oleandomycin producer *S. antibioticus*) and suggested pathways to NDP-L-mycarose in *S. erythraea* (upper, dotted box) and to NDP-L-digitoxose in *S. lividans* (16F4, pLNB43) or *S. lividans* (16F4, pLNBIV) (lower, bold solid box).

NDP-4-keto-L-olivose, since this activated L-sugar (or its 3-epimer[19]) is discussed as its natural substrate.[20−22] However, NDP-4-keto-D-digitoxose (=2,6-dideoxy-D-glycero-4-hexulose) was recently established as sugar donor substrate for the EryBIII analogue TylC3,[23,24] which catalyzes exactly the same *C*-methylation step in the formation of the NDP-L-mycarose building block within tylosin biosynthesis. These findings also suggest an alternative sequence of events, i.e., 2-deoxygenation and 3-*C*-methylation prior to 5-epimerization, for NDP-L-mycarose biosynthesis in *S. erythraea* (see Figure 2, upper dotted box). Since our experiment seems to render EryBIII unnecessary for the formation of NDP-L-digitoxose, we also used plasmid pLNBIV,[16] a pLN2 derivative containing *eryBIV* but lacking *eryBIII*. This plasmid was heterologously expressed into *S. lividans* together with cosmid 16F4, and the results were essentially the same as in the experiment described above: production of mainly **4** (2 mg/L) along with smaller amounts of **5** (0.2 mg/L). In addition, the production of 8-demethyl-8-L-oleandrosyltetracenomycin C (**6**, 0.1 mg/L) was also observed as a consequence of the participation of the *elmMII O*-methyltransferase encoding gene in cosmid 16F4. These follow-up experiments prove that EryBIII does not contribute to the generation of L-digitoxosyltetracenomycin C (**4**), which probably means that the stereochemistry in the 3′-position of this metabolite is controlled solely by ketoreductase EryBIV. It is likely that both NDP-sugars, NDP-4-keto-L-olivose and NDP-4-keto-L-digitoxose, are in tautomeric equilibrium (see Figure 2). Since the latter is the better substrate for EryBIV due to its axial 3-OH group (like in its natural suggested substrate NDP-4-keto-L-mycarose), digitoxosyltetracenomycin C (**4**) is always the major product of these experiments involving the *eryBIV* gene, while L-olivosyltetracenomycin C (**5**) is always the minor product.

Experimental Section

General Experimental Procedures. CD spectra were recorded on a AVIV circular dichroism spectropolarimeter (model 60DS) in a 1 cm cell. UV spectra were recorded on a Beckman DU 650 spectrophotometer, IR spectra were obtained from pure samples on KCl disks in a Mattson Genesis II FT-IR spectrometer, and NMR spectra were recorded in acetone-d_6 or CD_3OD on a Varian Inova 400 NMR instrument at 400 MHz for ^1H and at 100.7 MHz for ^{13}C, using 1D spectra and 2D homo- and heteronuclear correlation experiments (^1H, ^{13}C, DEPT, H,H-COSY, HSQC, and HMBC). HRESMS was carried out using a VG Trio-3 triple quadrupole mass spectrometer with a 4000 amu mass range, equipped with a dual APCI/electrospray ionization source. HPLC was performed on a

Waters HPLC system (Delta 600, M32 add-on single system, with a photodiode array detector model 996), using the columns and guard columns described below.

Bacterial Strains and Plasmids. *Streptomyces lividans* TK21 was used as transformation host. Growth was carried out on trypticase soy broth (TSB; Oxoid) or R5A medium.[25] For sporulation, cells were grown for 7 days at 30 °C on agar plates containing medium A.[25] Plasmids pLN2 and pLNBIV were previously described.[16] pLNB43 is a pLNBIV derivative in which the *oleY* gene was removed from pLNBIV by digestion with *Nhe*I and *Pac*I and then replaced by the *eryBIII* gene using the same restriction sites. When antibiotic selection was required, 2.5 μg/mL (liquid medium) or 25 μg/mL (solid medium) of thiostrepton or apramycin was used.

Cultivation and Fermentation. Spores of *S. lividans* (16F4, pLNB43) were initially grown in TSB medium for 24 h at 30 °C and 250 rpm. This seed culture was used to inoculate (at 2.5%, v/v) seven 2 L Erlenmeyer flasks, each containing 400 mL of R5A medium,[25] and incubated for 6 days under the above conditions. A seed culture of *S. lividans* (16F4, pRHAM) was prepared as above and used to inoculate (at 2.5%, v/v) three 2 L Erlenmeyer flasks each containing 400 mL of R5A medium, which were grown for 60 h.

Isolation of 4 and 7. The cultures were centrifuged, and the supernatants were filtered and applied to a solid-phase extraction cartridge (Supelclean LC-18; 10 g; Supelco). The cartridge was eluted with MeOH and H_2O, using a linear gradient from 0 to 100% MeOH in 60 min, at 10 mL/min, taking fractions every 5 min. Fractions containing the product **4** or **7**, as assessed by HPLC analysis,[26] were dried in vacuo and redissolved in a small amount of MeOH/DMSO (1:1). The resulting products were purified by preparative HPLC using a μBondapak C_{18} radial compression cartridge (PrepPak Cartridge; 25 by 100 mm; Waters). An isocratic elution with a mixture of MeOH and H_2O (4:6), at 10 mL/min, allowed the separation of **7** as a pure peak; 21 mg of **7** was isolated as an amorphous yellow solid. For the isolation of **4**, the extracted material was chromatographed as above, but using acetonitrile and 0.1% trifluoroacetic acid in H_2O (27:73) as a mobile phase. The purified compound was diluted 4-fold with H_2O, applied to a solid-phase extraction column (Sep-Pak Vac, Waters), washed with H_2O to eliminate trifluoroacetic acid, and finally eluted with MeOH and dried in a vacuum; 27.8 mg of **4** was isolated as an amorphous yellow solid.

Characterization of 8-Demethyl-8-L-digitoxosyltetracenomycin C (4): CD (MeOH) λ_{extr} (Θ^{20}) 383 (49200), 313 sh (6800), 289 (−11600), 260 (2700), 213 (12300) nm; UV (MeOH) λ_{max} (ϵ) 502 (1900), 435 (12000), 411 (13600), 286 (32500), 244 sh (28900) nm; (MeOH−HCl) λ_{max} (ϵ) 406 (15700), 388 (14600), 286 (51300), 238 (33100), 209 (24000) nm; (MeOH−NaOH) λ_{max} (ϵ) 507 (3200), 440 (14700), 422 sh (13400), 260 (35800), 205 (93800) nm; IR ν_{max} (KCl) 3373, 2956, 2358, 1713, 1671, 1596, 1436, 1365, 1228, 1101, 1058, 960, 901, 832, 631 cm^{-1}; ^{1}H NMR (acetone-d_6, 400 MHz) δ 14.03 (1H, s, O*H*-11), 7.98 (1H, s, H-6), 7.74 (1H, s, H-7), 5.97 (1H, d, J = 3.5 Hz, H-1′), 5.76 (1H, s, O*H*-12a), 5.61 (1H, s, H-2), 5.13 (1H, s, O*H*-4a), 5.05 (1H, d, J = 6.0 Hz, H-4), 4.93 (1H, d, J = 6.0 Hz, O*H*-4), 4.04 (1H, dddd, J = 6.5, 3.5, 3.0, 3.0 Hz, H-3′), 3.95 (3H, s, OCH_3-9), 3.93 (1H, dq, J = 9.5, 6.0 Hz, H-5′), 3.83 (3H, s, OCH_3-3), 3.71 (1H, d, J = 7.0 Hz, O*H*-4′), 3.34 (1H, d, J = 6.5 Hz, O*H*-3′), 3.27 (1H, ddd, J = 9.5, 7.0, 3.0 Hz, H-4′), 2.83 (3H, s, CH_3-10), 2.28 (1H, ddd, J = 15.0, 3.0, 1.0 Hz, H_{eq}-2′), 2.20 (1H, ddd, J = 15.0, 3.5, 3.5 Hz, H_{aq}-2′), 1.17 (3H, d, J = 6.0 Hz, CH_3-5′); ^{13}C NMR (acetone-d_6, 100.7 MHz) δ 198.1 (s, C-12), 194.2 (s, C-5), 190.8 (s, C-1), 175.4 (s, C-3), 168.4 (s, *C*O-9), 167.9 (s, C-11), 155.7 (s, C-8), 141.2 (s, C-5a), 138.6 (s, C-10), 130.8 (s, C-6a), 129.4 (s, C-9), 121.9 (s, C-10a), 121.3 (d, C-6), 111.7 (d, C-7), 110.3 (s, C-11a), 100.1 (d, C-2), 96.3 (d, C-1′), 83.7 (s, C-12a), 73.2 (d, C-4′), 70.7 (d, C-4), 67.1 (d, C-3′), 66.0 (d, C-5′), 57.4 (q, O*C*H$_3$-3), 53.2 (q, O*C*H$_3$-9), 36.2 (t, C-2′), 21.2 (q, *C*H$_3$-10), 18.3 (q, C-6′); HRESMS *m*/*z* 611 ([M + Na]$^+$, 611.1359, calcd for $C_{28}H_{28}O_{14}Na$, 611.1377, 100), 589 ([M + H]$^+$, 589.1550, calcd for $C_{28}H_{29}O_{14}$, 589.1557, 10).

Characterization of 8-Demethyl-8-D-glucosyltetracenomycin C (7): CD (MeOH) λ_{extr} (Θ^{20}) 381 (53100), 372 (42800), 358 (46500), 284 (−700), 212 (27300) nm; UV (MeOH) λ_{max} (ϵ) 505 (2000), 437 (9500), 417 (9300), 263 (25400), 208 (24200) nm; (MeOH−HCl) λ_{max} (ϵ) 405 (9200), 390 (8700), 285 (34700), 237 (24400), 208 (21000) nm; (MeOH−NaOH) λ_{max} (ϵ) 441 (10700), 261 (27300), 203 (116900) nm; IR ν_{max} (KCl) 3337, 2942, 2050, 1706, 1663, 1595, 1366, 1227, 1066, 981, 940, 786, 830, 636 cm^{-1}; ^{1}H NMR (CD$_3$OD, 400 MHz) δ 8.03 (1H, s, H-6), 7.67 (1H, s, H-7), 5.61 (1H, s, H-2), 5.20 (1H, d, J = 7.0 Hz, H-1′), 4.89−4.90 (1H, m, H-4), 3.97 (3H, s, OCH_3-9), 3.94−3.97 and 3.67−3.74 (2H, m, CH_2-5′), 3.82 (3H, s, OCH_3-3), 3.59−3.65 (1H, m, H-5′), 3.43−3.55 (1H, m, H-3′), 3.43−3.55 (1H, m, H-2′), 3.38 (1H, dd, J = 9.0, 9.0 Hz, H-4′), 2.84 (3H, s, CH_3-10); ^{13}C NMR (CD$_3$OD, 100.7 MHz) δ 198.3 (s, C-12), 193.2 (s, C-5), 176.1 (s, C-3), 169.7 (s, *C*O-9), 156.7 (s, C-8), 141.8 (s, C-5a), 138.4 (s, C-10), 130.8 (s, C-9), 129.7 (s, C-6a), 123.1 (s, C-10a), 122.2 (d, C-6), 113.1 (d, C-7), 110.6 (s, C-11a), 101.9 (d, C-1′), 100.8 (d, C-2), 84.7 (s, C-12a), 78.6 (d, C-5′), 78.3 (d, C-3′), 74.9 (t, C-2′), 71.4 (d, C-4′), 70.9 (d, C-4), 62.7 (t, C-6′), 57.7 (q, O*C*H$_3$-3), 53.4 (q, O*C*H$_3$-9), 21.3 (q, *C*H$_3$-10); HRESMS *m*/*z* 643 ([M + Na]$^+$, 643.1298, calcd for $C_{28}H_{28}O_{16}Na$, 643.1275, 100), 621 ([M + H]$^+$, 621.1483, calcd for $C_{28}H_{29}O_{16}$, 621.1456, 30).

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Supporting Information Available: NDP-sugar donor substrate flexibility of ElmGT as established in previous experiments. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Hallis, T. M.; Liu, H. W. *Acc. Chem. Res.* **1999**, *32*, 579−588.
(2) Liu, H. W.; Thorson, J. S. *Annu. Rev. Microbiol.* **1994**, *48*, 223−256.
(3) Trefzer, A.; Salas, J. A.; Bechthold, A. *Nat. Prod. Rep.* **1999**, *16*, 283−299.
(4) Kirschning, A.; Bechthold, A.; Rohr, J. *Top. Curr. Chem.* **1997**, *188*, 1−84.
(5) Künzel, E.; Faust, B.; Oelkers, C.; Weissbach, U.; Bearden, D. W.; Weitnauer, G.; Westrich, L.; Bechthold, A.; Rohr, J. *J. Am. Chem. Soc.* **1999**, *121*, 11058−11062.
(6) Zhao, L. S.; Ahlert, J.; Xue, Y. Q.; Thorson, J. S.; Sherman, D. H.; Liu, H. W. *J. Am. Chem. Soc.* **1999**, *121*, 9881−9882.
(7) Blanco, G.; Patallo, E. P.; Brana, A. F.; Trefzer, A.; Bechthold, A.; Rohr, J.; Mendez, C.; Salas, J. A. *Chem. Biol.* **2001**, *8*, 253−263.
(8) Drautz, H.; Reuschenbach, P.; Zähner, H.; Rohr, J.; Zeeck, A. *J. Antibiot.* **1985**, *38*, 1291−1301.
(9) Rodriguez, L.; Oelkers, C.; Aguirrezabalaga, I.; Brana, A. F.; Rohr, J.; Mendez, C.; Salas, J. A. *J. Mol. Microbiol. Biotechnol.* **2000**, *2*, 271−276.
(10) Decker, H.; Rohr, J.; Motamedi, H.; Zahner, H.; Hutchinson, C. R. *Gene* **1995**, *166*, 121−126.
(11) Weber, W.; Zähner, H.; Siebers, J.; Schröder, K.; Zeeck, A. *Arch. Microbiol.* **1979**, *121*, 111−116.
(12) Egert, E.; Noltemeyer, M.; Siebers, J.; Rohr, J.; Zeeck, A. *J. Antibiot.* **1992**, *45*, 1190−1192.
(13) Patallo, E. P.; Blanco, G.; Fischer, C.; Braña, A. F.; Rohr, J.; Mendez, C.; Salas, J. A. *J. Biol.Chem.* **2001**, *276*, 18765−18774.
(14) Wohlert, S. E.; Blanco, G.; Lombo, F.; Fernandez, E.; Braña, A. F.; Reich, S.; Udvarnoki, G.; Mendez, C.; Decker, H.; Frevert, J.; Salas, J. A.; Rohr, J. *J. Am. Chem. Soc.* **1998**, *120*, 10596−10601.
(15) Decker, H.; Haag, S.; Udvarnoki, G.; Rohr, J. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 1107−1110.
(16) Rodriguez, L.; Aguirrezabalaga, I.; Allende, N.; Braña, A. F.; Mendez, C.; Salas, J. A. *Chem. Biol.* **2002**, *9*, 721−729.
(17) Rodriguez, L.; Rodriguez, D.; Olano, C.; Braña, A. F.; Mendez, C.; Salas, J. A. *J. Bacteriol.* **2001**, *183*, 5358−5363.
(18) Aguirrezabalaga, I.; Olano, C.; Allende, N.; Rodriguez, L.; Braña, A. F.; Mendez, C.; Salas, J. A. *Antimicrob. Agents Chemother.* **2000**, *44*, 1266−1275.
(19) The epimerization of the 3-position next to the 4-keto group is possible due to tautomerism (as shown in Figure 2, bold solid box).
(20) Wohlert, S. E.; Lomovskaya, N.; Kulowski, K.; Fonstein, L.; Occi, J. L.; Gewain, K. M.; MacNeil, D. J.; Hutchinson, C. R. *Chem. Biol.* **2001**, *8*, 681−700.
(21) Summers, R. G.; Donadio, S.; Staver, M. J.; Wendt-Pienkowski, E.; Hutchinson, C. R.; Katz, L. *Microbiol.* **1997**, *143*, 3251−3262.
(22) Gaisser, S.; Böhm, G. A.; Doumith, M.; Raynal, M. C.; Dhillon, N.; Cortes, J.; Leadlay, P. F. *Mol. Gen. Genet.* **1998**, *258*, 78−88.

(23) Chen, H. W.; Agnihotri, G.; Guo, Z. H.; Que, N. L. S.; Chen, X. M. H.; Liu, H. W. *J. Am. Chem. Soc.* **1999**, *121*, 8124−8125.
(24) Chen, H. W.; Zhao, Z. B.; Hallis, T. M.; Guo, Z. H.; Liu, H. W. *Angew. Chem., Int. Ed.* **2001**, *40*, 607−610.
(25) Fernandez, E.; Weissbach, U.; Reillo, C. S.; Braña, A. F.; Mendez, C.; Rohr, J.; Salas, J. A. *J. Bacteriol.* **1998**, *180*, 4929−4937.
(26) Fernandez-Lozano, M. J.; Remsing, L. L.; Quiros, L. M.; Braña, A. F.; Fernandez, E.; Sanchez, C.; Mendez, C.; Rohr, J.; Salas, J. A. *J. Biol. Chem.* **2000**, *275*, 3065−3074.

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