Inactivation of the *urdGT2* Gene, Which Encodes a Glycosyltransferase Responsible for the C-Glycosyltransfer of Activated D-Olivose, Leads to Formation of the Novel Urdamycins I, J, and K

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Abstract: A targeted search for glycosyltransferase (GT) encoding genes in the gene cluster of the urdamycin A producer *Streptomyces fradiae* Tü2717 resulted in the discovery of *urdGT2*, a GT encoding gene located approximately 7 kb downstream of the minimal polyketide synthase (PKS) encoding genes. Subsequent inactivation of this gene created a mutant strain, which produces completely different metabolites than the wild-type strain, consisting of the three new urdamycins I, J, and K. Their structures provide new insight into the important C-glycosyl-transfer step of the urdamycin biosynthetic pathway. The structures indicate that the corresponding gene product UrdGT2 catalyzes the C-glycosyl transfer of activated D-olivose to an angucyclinone precursor, which already bears the angular 12b-OH group. The structures of the new urdamycins could not have arisen without the involvement of substrate flexible post-PKS modifying genes, i.e., glycosyltransferases and oxidoreductases. This work proves that targeted gene disruption experiments can lead to novel biologically active "unnatural" natural products, which arise through a formerly nonactivated shunt pathway. This approach is especially fruitful in work toward antitumor drugs. Urdamycin J shows a good anticancer activity in in vitro tests.

Introduction

Urdamycin A (**1**, Chart 1), an angucycline type antibiotic[1,2] and anticancer agent,[3] is the principal product of *Streptomyces fradiae* Tü2717. It consists of aquayamycin (**2**, Chart 1), which contains a C-glycosidically linked D-olivose, and three additional O-glycosidically linked deoxysugars, two L-rhodinoses, and another D-olivose. Compound **2** is the most common "aglycon"[4] among the angucycline group of antibiotics, and thus it is an intermediate in several biosynthetic pathways, including the urdamycin biosynthesis.[5,6] Another important product of *S. fradiae* Tü2717 is the ring B aromatic compound urdamycin B (**5**, Chart 1). Our earlier work has shed little light on the formation of the C-glycosidic moiety in **1**, **2**, and **5**. The use of blocked mutants obtained through mutagen treatment led to the accumulation of shunt product 104-1 (**6**) and rabelomycin (**7**, Chart 1). Thus, it was concluded[2,6] that a tetracyclic intermediate **8** (or **9**, Chart 1) is the most likely substrate for the C-glycosyltransferase, **9** being identical with or an isomer of the recently isolated compound UWM6.[7] This work was directed toward the identification of glycosyltransferases (GTs) involved in the urdamycin biosynthesis, the recognition of their substrates, and the creation of novel urdamycins through targeted disruption of GT encoding genes.

Results

The genes of the biosynthesis of **1** which encode the minimal polyketide synthase (PKS) and some adjacent genes had already been identified.[8] To search for genes involved in the deoxysugar biosynthesis and the glycosyl-transfer steps, we used DNA probes encoding a key enzyme involved in the deoxysugar biosynthesis, NDP-glucose 4,6-dehydratase, which catalyzes the conversion of NDP-glucose to NDP-4-keto-6-deoxy-glucose,[9−14]

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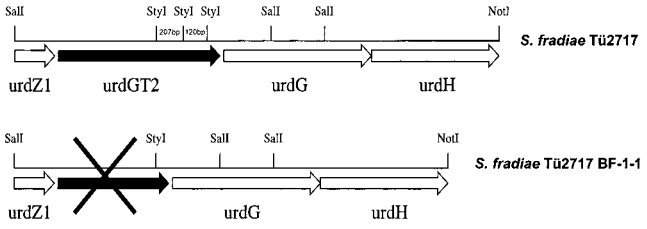
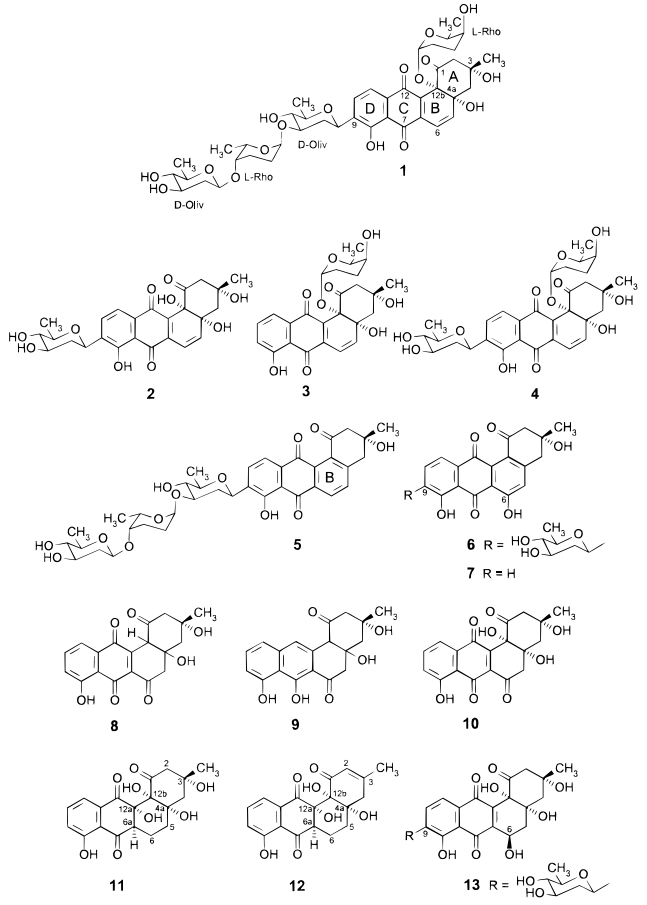


Figure 1.

Chart 1



and therefore should be also involved in the biosyntheses of the deoxysugars of **1**. Applying our recently developed PCR method to clone such 4,6-dehydratase genes,[15] we obtained a PCR fragment using chromosomal DNA isolated from *S. fradiae* as a template. The deduced amino acid sequence of this fragment revealed homology to known NDP-glucose 4,6-dehydratases. The PCR fragment was further used as a probe to screen a cosmid library. A 3.7-kb DNA fragment containing DNA hybridizing to the probe was sequenced, and four new ORFs (*urdZ1*, *urdGT2*, *urdG*, *urdH*) located approximately 7 kb downstream from the PKS genes (Figure 1) were identified. Comparison of the deduced amino acid sequence of the corresponding products of these ORFs with database sequences indicated that three of the corresponding enzymes (UrdZ1, UrdG, UrdH) are involved in the deoxysugar biosynthesis,[11,16] while UrdGT2 showed significant similarity to known glycosyltransferases (e.g., 46% homology to ORF14 from *S. violaceoruber* Tü22,[17] 55% homology to LanGT2 from *S. cyanogenus*).[18]

To assign the specific function of the *urdGT2* product, a targeted gene inactivation experiment was performed through an in-frame deletion in the chromosomal *urdGT2* gene by homologous double-crossover recombination with plasmid pSP-urdGT2d, in which a 327bp *Sty*I fragment of *urdGT2* was deleted (Figure 1). The chemical structures of the products of the resulting mutant *S. fradiae* Tü2717 BF-1-1 were analyzed using NMR spectroscopy and mass spectrometry methods. A transformation of BF-1-1 with *urdGT2* on a 1.88-kb *Sal*I fragment ligated into the expression vector pEM4[19] (=pBF-EM4-1) verified that only the glycosyltransferase UrdGT2 was affected, since in *S. fradiae* Tü2717 BF-1-1 (pBF-EM4-1) the **1** production was reinstalled.

Mutant BF-1-1 accumulates two major products, urdamycins I (**11**, ca. 12 mg/L) and J (**12**, ca. 12 mg/L), and one minor compound, urdamycin K (**3**, ca. 1 mg/L) (Chart 1). The major products **11** and **12** are novel angucyclinones. Urdamycin I (**11**) possesses the molecular formula $C_{19}H_{20}O_8$ (376 g/mol), which could be deduced from the FAB MS, whereas urdamycin J (**12**) is its 2,3-dehydration product ($C_{19}H_{18}O_7$, 358 g/mol). Both contain the tertiary OH groups in the 4a- and 12b-positions and an unprecedented 12a-OH group. The typical 5,6-double bond (see **1**) is replaced with a saturated C−C bond. All three new structural features are unprecedented among the known urdamycins. Urdamycin K[20] (**3**) resembles blocked mutant product 100-2 (**4**, Chart 1)[6] except for the C-glycosidically bound D-olivose moiety, which is missing here and in **11** and **12**, as indicated by the aromatic ABC systems (9-H, 10-H, 11-H). The 12b-linked L-rhodinose moiety of **3** can be unambiguously recognized by its typical ^{1}H NMR signals, in particular the upfield-shifted 6′-H_3 group (δ 0.58). The structures of urdamycin I and J were confirmed through 2D long-range couplings (HMBC, see Table 1), while the configurations at C-3 (in **3** and **11**) and C-4a and C-12b in **3**, **11**, and **12** were assigned from biosynthetic evidence (i.e., they should have the same configurations as deduced for the other urdamycins, such as **1**), and the centers at C-6a (*S*) and C-12a (*S*) in **11** and **12** followed from the 2D-NOESY correlation and the H,H-couplings of 6a-H.[20]

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(16) *urdZ1* resembles genes coding for 3,5-epimerases in deoxysugar biosyntheses;[11] however, this gene is incomplete in the 3.7-kB fragment. *urdG* encodes an NDP-hexose pyrophosphorylase, with the highest resemblance (=66% identical amino acids) to LanG.[18] *urdH* encodes an NDP-hexose dehydratase and shows the highest resemblance to LanH (75% identical amino acids).[18]

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(20) Both compounds showed a strong positive NOE effect between 12a-OH and 4a-OH and a weaker one between 4a-OH and 12b-OH. **11** also shows an NOE effect between 12a-OH and 6a-H. The two coupling constants (5, 2 Hz) of 6a-H indicate its equatorial position. The sharpness of the tertiary 12a- and 12b-OH signals and the 2D-NOE spectra indicatees either a water molecule fixed between 12a-OH and 12b-OH through hydrogen bonds or a rapid exchange of the OH groups with residual water. The assumed conformations, i.e., ring A of **11** in twist boat, of **12** in half chair, and ring B in both compounds in chair conformation, are based on MM3* force field calculations.

Table 1. NMR Data for Urdamycins I (**11**) and J (**12**)[a,b]

	^{13}C δ, m[c]		^{1}H δ, m[c] (J_{H-H})[d]		HMBC correlations	
position no.	**11**	**12**	**11**	**12**	**11**	**12**
1	209.4 s	197.5 s	–	–		
2	54.8 t	123.8 d	2.85 dd(15,2.5) 3.12 d (15)[e]	6.10 d (1)	1,3,4,12b 1,3,13	4,12b,13
3	74.6 s	166.7 s	OH:[f] 1.80s	–		
4	42.7 t	41.6 t	1.98 dd (15,2.5) 3.06 d (15)[e]	2.38 d (19.5) 3.34 dd (19.5,1)	2,3,4a,12b 3,12b,13	1,2,3,4a,5,12b,13 2,3
4a	81.0 s	74.1 s	OH:[f] 1.80 s	OH:[f] 2.46 s		
5	33.3 t	32.5 t	1.84 ddd (13.5,4,3)[e,g,h] 183 ddd (13.5,13,4)[e,g,h]	1.79 ddd (13.5,4,3) 1.94 ddd (13.5,13,4)	 4,4a,6,6a,12b	1,4,4a,6,6a,12b 3,4,4a,6,6a,12b
6	17.8 t	18.4 t	2.05 dddd (14,13,5,3)[e] 2.61 dddd (14,4,3,2)	2.03 dddd (14,13,5,4) 2.67 dddd (14,4,3,2)	5,6a,7 12a	4a,5,6a,7,12a 4a,5,6a,7,7a,12a
6a	52.8 d	52.2 d	3.09 dd (5,2)[e]	3.08 dd (5,2)	5,6,12,12a,12b	5,6,7,12,12a,12b
7	199.2 s	199.5 s	–	–		
7a	120.6 s	120.7 s	–	–		
8	160.7 s	160.6 s	OH:[f] 11.80s	OH:[f] 11.80 s	7a,8,9,10	7,7a,9,10
9	125.7 d	125.7 d	7.33 dd (8,8)[e,g,h]	7.33 dd (7,2)[g]	8,11	7,8,11,11a
10	136.0 d	135.9 d	7.59 dd (8,8)[e,g,h]	7.63 dd (7,7)[e,g,h]	8,11a	8,11a
11	119.2 d	119.2 d	7.63 dd (8,1.5)[e,g,h]	7.64 dd (7,2)[e,g,h]	7a,9,12	7a,9,11a,12
11a	133.9 s	134.1 s	–	–		
12	198.3 s	197.7 s	–	–		
12a	81.7 s	82.6 s	OH:[f] 4.43 s	OH:[f] 4.20 s	6a,11a,12a,12	6a,7,11a,12a,12b
12b	78.3 s	79.6 s	OH:[f] 3.93 s	OH:[f] 3.55 s	1,2,12a,12b	1,2,4a,12a,12b
13	30.9 q	25.2 q	1.39 s	2.10 s	2,3,4	1,2,3,4

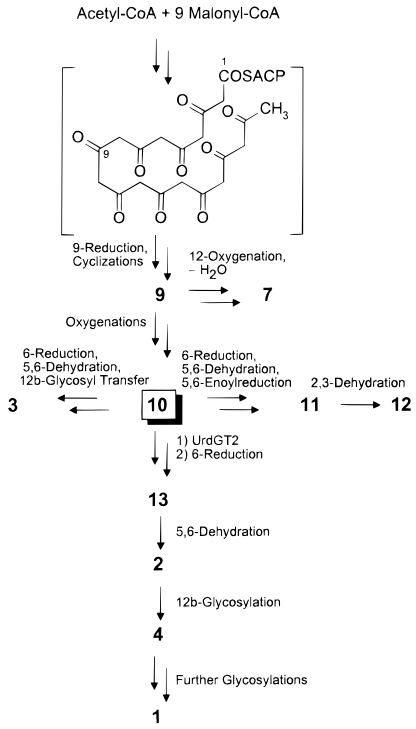
[a] Bruker DMX-500 (B_0 = 11.74 T), $CDCl_3$. [b] Varian VXR-S 400 (B_0 = 9.4 T), $CDCl_3$. [c] Multiplicities from DEPT. [d] Coupling partners confirmed by DQF-COSY. [e] Overlapped by other signal(s). [f] Exchangeable by D_2O. [g] Complicated by higher order effects. [h] *J* determined by computer simulations.

Discussion

By introducing an in-frame deletion into *urdGT2*, negative effects on genes located downstream of *urdGT2* could be avoided. All three products of the mutant BF-1-1 are tetracyclic angucyclinones to which no C-glycosidic moiety is attached. Thus, it can be concluded that UrdGT2 catalyzes the C-glycosyl transfer of an activated D-olivose, the earliest glycosylation step in the urdamycin biosynthesis.[6] The structures of urdamycins I and J suggest that these compounds are shunt products which derive from a hypothetical precursor, such as **10** (Chart 1), that is further converted into **3** or **11** (and on to **12**) through the influence of a substrate flexible glycosyltransferase and oxidoreductases, respectively. In the normal biosynthetic pathway, in which UrdGT2 is not blocked, this putative intermediate **10** is converted via urdamycinone F (**13**, Chart 1) and aquayamycin (**2**) into **1**. Rabelomycin (**7**) was accumulated as a result of a former nontargeted NTG mutation experiment.[6] The NTG mutation obviously also affected other enzymes besides the glycosyltransferase UrdGT2, in particular those responsible for the 12- and 12b-oxygenation steps. Thus, **7** presumably branches off the proposed intermediate UWM6 **9** (Scheme 1).

The 12a-hydroxy group in **11** and **12** might have also suggested that the C-glycosyl-transfer step occurs on a molecule in which the hydroxy group located at C-12a remains from the acetate building block contributing C-12/C-12a. This was tested by feeding [1-^{13}C,$^{18}O_2$]acetate[21,22] to growing cultures of *S. fradiae* Tü2717 BF-1-1. The ^{13}C NMR spectrum of the resulting sample of urdamycin I showed clear upfield shifts at two other carbons bearing a tertiary alcohol group, namely C-3 ($\Delta\delta$ 0.03 ppm) and C-4a ($\Delta\delta$ 0.02 ppm), but not at C-12a.[23] This result suggests that the 12a-hydroxy group is unlikely to be derived from acetate, and therefore it must be introduced by an oxygenase. Since both urdamycin I and J already contain the 12b-OH group, it is most likely that this hydroxy group is introduced prior to the C-glycosylation step, at least in the predominant biosynthetic pathway leading to **1** and **2**. This preference of the C-glycosyltransferases for angucyclinone substrates containing angular OH groups also explains why almost all angucyclines containing a 9-C-glycosyl moiety also bear both angular OH groups at 4a- and 12b-positions.[1,2] *S. fradiae* Tü2717 is an exception in that it also can produce ring B aromatic compounds such as urdamycin B (**5**), which contains

Scheme 1



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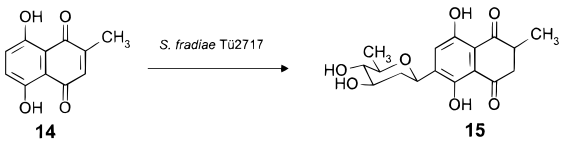
(23) Other observed upfield shifts: C-1 ($\Delta\delta$ 0.02 ppm); upfield shifts at C-7 and C-8 were not observable, due to the broadness of the signals or O-exchange with water, respectively.

Scheme 2



14 **15**

a C-9-C-glycosidically bound olivose but bears no angular OH groups. Thus, UrdGT2 seems to be less substrate specific than the other C-glycosyltransferases involved in angucycline group biosyntheses. This view is further supported by feeding of methylnaphthazarine **14** to *S. fradiae* Tü2717, resulting in the C-glycosylated compound **15** (Scheme 2).[24]

It is also remarkable for this formerly undetected shunt pathway that urdamycins I and J possess a saturated 5,6-bond, a structural variant unknown from the *S. fradiae* Tü2717 wild-type products. This must arise from an enoylreductase-type enzyme normally not active in the urdamycin biosynthetic pathway, which may only act on substrates not containing the C-olivosyl moiety. In contrast to the relatively early C-glycosyl transfer, the O-glycosyl-transfer steps occur as the last three biosynthetic steps in the sequence leading to **1**.[6] The GT responsible for the L-rhodinosyl transfer to the 12b-position seems to show a relaxed substrate specificity, since it can act on a molecule without the C-olivose moiety, as the structure of urdamycin K (**3**) reveals. Interestingly, **3** is a diastereomer of sakyomicin C, with all stereo centers inverted except C-3.[25,26]

Our ongoing work is focused on the cloning of the remaining glycosyltransferase genes and the assignment of these genes to the three O-glycosyl-transfer steps as well as on the investigation of the oxidoreductases of the urdamycin biosynthetic pathway. The work described here allowed the unambiguous assignment of the function of the gene product of *urdGT2*, which catalyzes the C-glycosyl-transfer step leading to the important angucycline antibiotic aquayamycin (**2**).

The results also demonstrate again that it is, indeed, possible to find novel "unnatural" natural products through targeted gene deletion. The latter is obviously leveraged if post-PKS enzymes are present, which are more flexible in their substrate specificity. Such flexible enzymes have been discovered more recently[27−29] and are useful in the extension of combinatorial biosynthetic approaches on post-PKS enzymes. Here, evidence is given that, in particular, oxidoreductases and glycosyltransferases of *S. fradiae* may be good candidates for this purpose.

Besides the elucidation of biosynthetic problems and creation of new molecules, the purpose of such research is always to discover new biologically active drugs. Here we were aiming for antitumor agents. Initial in vitro assays by the National Cancer Institute showed that one of the new urdamycins, namely urdamycin J (**12**), is, indeed, a quite active anticancer drug, while urdamycin I (**11**) showed only weak activity (Table 2).[30] Since both structures differ only in the 2,3-double bond, this double bond seems to be an important structural feature for the antitumor activity. For urdamycin K (**3**), no data are available yet, but anticancer activity is also likely because of the compound's close structural similarity to the sakyomicins. The fact that urdamycin J (**12**) has a much better anticancer activity that the parent compound urdamycin A (**1**) proves arguments to be wrong stating that the observed biological activity is a result of evolutionary pressure and therefore the natural products cannot be optimized anymore with regard to their biological activity. This argument may be true for some antibiotics, but it is definitely untrue for anticancer agents. Therefore, combinatorial biosynthetic approaches are especially fruitful when they aim on the creation of novel anticancer drugs.

Table 2. Examples[a] of the Results of in Vitro Antitumor Tests with 60 Human Antitumor Cell Lines (GI_{50} in mol^{-1})[30]

	11	**12**
leukemia HL-60 (TB)	8.19×10^{-6}	3.45×10^{-8}
non-small cell lung cancer NCI-H522	1.42×10^{-5}	1.10×10^{-7}
colon cancer HCT-116	1.61×10^{-5}	2.22×10^{-7}
melanoma UACC-257	1.79×10^{-5}	3.69×10^{-7}
ovarian cancer OVCAR-3	1.72×10^{-5}	3.11×10^{-7}
breast cancer MCF7	1.07×10^{-5}	1.34×10^{-7}
prostate cancer DU-145	1.61×10^{-5}	1.23×10^{-6}
renal cancer CAKI-1	2.57×10^{-5}	6.80×10^{-7}
CNS cancer U251	—[b]	1.67×10^{-6}

[a] Only one example of each type of human cancer cell lines is shown. [b] No inhibition ($>5.00 \times 10^{-5}$ M)

Experimental Section

Analytical Methods. NMR spectra were recorded on a Varian VXR 300, a Varian VXR-S 400, or a Bruker DMX 500 spectrometer; chemical shifts are recorded in ppm relative the solvent resonance. UV spectra were recorded on a Perkin-Elmer Lambda 4A UV/vis spectrophotometer; acidic and alkaline UV spectra were obtained after addition of 1 drop of 0.1 N HCl and NaOH, respectively. CD spectra were recorded at 27 °C on an Aviv CD spectrometer model 60DS.

Generation of the Chromosomal *urdGT2* Mutant BF-1-1. For the generation of a chromosomal *urdGT2* mutant of *S. fradiae* by homologous recombination, the plasmid pSP-urdGT2d was constructed. A 1.88 kb *Sal*I DNA fragment containing parts of a putative NDP-hexose 3,5-epimerase gene (*urdZ1*), the entire glycosyltransferase genes *urdGT2*, and parts of a NDP-hexose synthetase gene (*urdG*) was restricted with *Sty*I to generate a 327-bp deletion (207 bp + 120 bp) within *urdGT2*. A 1.55-kb *Xba*I-*Kpn*I fragment carrying the deletion was subcloned into the corresponding sites of pSP1, generating pSP-urdGT2d. pSP-urdGT2d was used to transform protoplasts of *S. fradiae* Tü 2717. Selection of primary transformants was performed on erythromycin-containing plates. For characterization of transformants by Southern hybridization, an internal 1.7-kb *Sma*I fragment of the *ermE* gene, a 207-bp *Sty*I fragment containing parts of *urdGT2*, and the 1.88-kb *Sal*I fragment were used as probes. After screening for erythromycin sensitivity, a double-crossover mutant was generated in which the mutated allele had replaced the wild-type copy.

Fermentation of Mutant BF-1-1 and Isolation of the New Urdamycins. *S. fradiae* mutant BF-1-1 was cultured in 250-mL Erlenmeyer flasks with three baffles, each containing 100 mL of medium, consisting of 2% soybean meal and 2% glucose. The pH was adjusted to 7.2 before autoclaving. Cultures were grown at 30 °C and 220 rpm for 72 h, after which the culture filtrate and the cell mycelium were separated by filtration. The mycelium was extracted with acetone, and the filtrate was extracted with ethyl acetate. To isolate urdamycins I and J, the combined extracts were concentrated to dryness, dissolved in methanol, and purified by HPLC (Kromasil 100 C18 column, 250

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× 10 mm, flow rate 6 mL/min, gradient H_2O/acetonitrile/MeOH = 70:25:5 → 60:34:6, within 13 min). The products were finally purified by column chromatography on Sephadex LH-20 (CH_2Cl_2). To isolate urdamycin K, the combined extracts were concentrated and purified by column chromatography (1, silica gel, CH_2Cl_2/MeOH 15:1; 2, silica gel, CH_2Cl_2/MeOH 30:1; 3, Sephadex LH-20, MeOH).

Urdamycin I (11). Colorless amorphous powder, soluble in chloroform, acetone, and methanol. ^{1}H NMR and ^{13}C NMR: see Table 1. FAB MS: *m*/*z* 376, M^-. HR MS: found 399.1062 ($M + Na^+$), calcd for $C_{19}H_{20}O_8Na$ 399.1056. UV: λ_{max} (ϵ) (MeOH and MeOH/HCl) 202 (16 000), 232 (24 000), 348 (7000); (MeOH/NaOH) 215 (10 000), 267 (7400), 343 (3900), 364 (3500); CD (MeOH) λ_{extr} (Θ) 270 (−21 700), 274 (−20 600), 287 (−35 300), 291 (−24 700), 296 (−25 600), 342 (4800), 369 (7300).

Urdamycin J (12). Colorless amorphous powder, soluble in chloroform, acetone, and methanol. ^{1}H NMR and ^{13}C NMR: see Table 1. FAB MS: *m*/*z* 358, M^-. HR MS: found 381.0955 ($M + Na^+$), calcd for $C_{19}H_{18}O_7Na$ 381.0950. UV: λ_{max} (ϵ) (MeOH and MeOH/HCl) 202 (7000), 234 (8000), 340 (1000); (MeOH/NaOH) 203 (2400), 212 (4300), 262 (1700), 343 (7000), 365 (7000); CD (MeOH) λ_{extr} (Θ) 280 (−36 200), 297 (−4000), 313 (−4200), 344 (11 100), 367 (8800).

Urdamycin K (3). Yellow amorphous powder, soluble in chloroform, acetone, or methanol. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.58 (d, 3H, J = 6 Hz; 6′-H_3), 1.23 (s, 3H; 13-H_3), 1.4−1.6 (m, 2H; 3′-H_2), 1.72 (dddd, 1H, J = 12, 5, 5, 2 Hz; 2′-H_e), 1.89 (dd, 1H, J = 15, 2 Hz; 4-H_e), 1.89 (dddd, 1H, J = 12, 9, 9, 5 Hz; 2′-H_a), 2.18 (d, 1H, J = 15 Hz; 4-H_a), 2.52 (d, 1H, J = 13 Hz; 2-H_a), 2.79 (dd, 1H, J = 13, 2 Hz; 2-H_e), 3.48 (d, 1H, J = 2 Hz; 4′-H), 3.65 (dq, 1H, J = 6, 2 Hz; 5′-H); 4.02 (s, 1H (exchangeable with D_2O; 4a-OH); 5.38 (d, 1H, J = 1 Hz; 1′-H), 6.40 (d, 1H, J = 10 Hz; 5-H), 6.86 (d, 1H, J = 10 Hz; 6-H), 7.30 (dd, 1H, J = 8, 3 Hz; 9-H), 7.63 (m, 2H; 10-H, 11-H), 11.92 (s, 1H, exchangeable with D_2O; 8-OH). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 16.4 (C-6′), 23.0 (C-3′), 25.3 (C-2′), 29.9 (3-CH_3), 43.2 (C-4), 53.9 (C-2), 67.0 (C-4′), 67.0 (C-5′), 75.3 (C-3), 80.3 (C-4a), 81.8 (C-12b), 94.7 (C-1′), 114.4 (C-7a), 116.8 (C-6), 120.2 (C-11), 124.9 (C-9), 131.5 (C-11a), 137.2 (C-6a), 137.3 (C-10), 138.6 (C-12a), 144.9 (C-5), 162.8 (C-8), 182.4 (C-12), 187.7 (C-7), 201.6 (C-1). ES MS: *m*/*z* 470. FAB MS: *m*/*z* 493 ($M + Na^+$). UV: λ_{max} (ϵ) (MeOH) 212 (20 100), 308 (4600), 349 (2600), 418 (3100); (MeOH/HCl) 213 (20 600), 356 (2200), 417 (3500); (MeOH/NaOH) 221 (14 800), 279 (4800), 315 (4100), 362 (1900).

2,3-Dihydro-5,8-dihydroxy-6-(4,5-dihydroxy-6-methyltetrahydropyran-2-yl)-2-methyl-1,4-naphthoquinone (15). 2-Methylnaphthazarine (5,8-dihydroxy-2-methylnaphthoquinone)[31] (**14**, 25 mg, dissolved in 2 mL of DMSO) was added to a 1-L culture of the urdamycin producer *S. fradiae* Tü2717 in four portions, 24, 36, 48, and 60 h after inoculation. Harvesting after 74 h, centrifugation (500 rpm, 20 min), extraction of the culture filtrate with ethyl acetate, and chromatography on silica gel (column 1, CH_2Cl_2/MeOH 10:1; column 2, CH_2Cl_2/MeOH 15:1) and Sephadex LH-20 (CH_2Cl_2) yielded 3 mg of **15** as a red crystalline powder (soluble in chloroform or methanol). R_f = 0.47 (CH_2Cl_2/MeOH 10:1). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.35 (d, 3H, J = 7 Hz; 2-CH_3), 1.41 (d, 3H, J = 6 Hz; 5′-CH_3), 1.38 (partially obscured; 2′-H_a), 2.18 (s, 1H, exchangeable with D_2O; 3′- or 4′-OH), 2.28 (s, 1H, exchangeable with D_2O; 3′- or 4′-OH), 2.51 (ddd, 1H, J = 12, 5, 2 Hz; 2′-H_e), 2.84 (dd, 1H, J = 16.5, 10 Hz; 3-H_α), 3.05 (dd, 1H, J = 16.5, 15.5 Hz; 3-H_β), 3.16 (obscured; 2-H), 3.20 (t, 1H, J = 8.5 Hz; 4′-H), 3.48 (dq, 1H, J = 8.5, 6 Hz, 5′-H), 3.82 (ddd, 1H, J = 11.5, 8.5, 5 Hz; 3′-H), 4.85 (dd, 1H, J = 11, 2 Hz; 1′-H), 7.50 (s, 1H; 7-H), 11.9 (s, exchangeable with D_2O, 8-OH), 12.3 (s, exchangeable with D_2O, 5-OH). ^{13}C NMR (125.7 MHz, $CDCl_3$): δ 15.9 (C-6′ or 2-*C*H_3), 18.0 (2-*C*H_3 or C-6′), 39.2 (C-3), 41.2 (C-2), 44.1 (C-2′), 71.2 (C-1′), 73.0 (C-5′), 75.8 (C-3′), 77.9 (C-4′), 113.5 (C-10 or C-9), 113.6 (C-9 or C-10), 124.6 (C-7), 142.3 (C-6), 151.8 (C-5 or C-8), 155.1 (C-8 or C-5), 201.0 (C-4 or C-1), 204.7 (C-1 or C-4). EI MS: *m*/*z* (relative intensity) 336 (80, M^+), 232 (100). HR MS: calcd for $C_{17}H_{20}O_7$ 336.1209, found 336.1206. UV: λ_{max} (ϵ) (MeOH and MeOH/HCl) 216 (19 500), 231 (13 200), 262 (7600), 394 (5600), 509 (1400); (MeOH/NaOH) 219 (36 500), 304 (6000), 579 (8300), 610 (8000). CD (MeOH) λ_{extr} (Θ) 217 (11 500), 232 (8300), 263 (5500), 386 (2400).

Feeding Experiment with [1^{13}C,$^{18}O_2$]Acetate. This experiment was performed in analogy to a feeding experiment described earlier.[21]

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Supporting Information Available: ^{1}H, ^{13}C, and 2D-NOESY NMR spectra (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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