A Chemoenzymatic, Enantioconvergent, Asymmetric Total Synthesis of (*R*)-Fridamycin E

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Keywords: Asymmetric synthesis / Biotransformations / Enzyme catalysis / Natural products / Total synthesis

A chemoenzymatic, asymmetric total synthesis of the antibiotic (*R*)-fridamycin E has been accomplished following a biocatalytic deracemization procotol. The key step comprises the construction of the chiral side-chain from a functionalized *rac*-2,2-disubstituted oxirane via a kinetic resolution/stereoinversion sequence without formation of the undesiredstereoisomer.

Introduction

The clinical utility of anthracyclines and their analogs in chemotherapy has initiated many efforts directed towards their synthesis.[1,2] Typically, anthracyclines consist of an anthraquinone nucleus bearing one or more sugar moieties attached through *O*-glycosidic bonds. However, an intriguing subclass of structurally related compounds exists in which the carbohydrate is linked by a *C*-glycosidic bond, prominent representatives of which are the vineomycin[3] and fridamycin families.[4] Among the latter, fridamycin E (**1**) can be regarded as the aglycon core of the more complex *C*-glycosidic fridamycins A, B, and D. Interestingly, the structure of **1** seems to represent the bioactive principle of the fridamycin family as it shows the highest activity against Gram-positive bacteria.

Evaluation of the total syntheses of fridamycin E reported to date reveals that the construction of the (*R*)-configured side-chain is the main challenge. The unnatural (*S*)-enantiomer of **1** has been obtained in low yield by a Marschalk reaction of mono-protected 1,5-dihydroxy-9,10-anthraquinone (anthrarufin, **2**) with a chiral building block derived from (*S*)-lactic acid.[4] Subsequently, nucleophilic addition of the anion of a suitably substituted anthracene derivative to an α-chiral aldehyde was reported to give the coupling product in excellent yields; however, elaboration of the chiral β-hydroxycarboxylic acid side-chain required a further nine steps to complete the synthesis of (*R*)-fridamycin E.[5] A classic approach involving the Ti^{IV}-mediated aldol addition of α-lithiated (–)-menthyl acetate as chiral auxiliary onto an acetonyl-substituted anthrarufin ether suffered similar drawbacks, as the diastereomers thus obtained could only be separated by HPLC.[6] Attempts to introduce chirality into the side-chain by asymmetric dihydroxylation of an alkene were impeded by low asymmetric induction (67% *ee*).[7]

Encouraged by our success in the preparation of 2,2-disubstituted oxiranes in high yield and *ee* by chemoenzymatic deracemization employing bacterial epoxide hydrolases,[8] we envisaged the application of this methodology to the asymmetric total synthesis of natural (*R*)-fridamycin E. In contrast to the acid-catalyzed hydrolysis of epoxides, biohydrolysis of 2,2-disubstituted oxiranes occurs by an S_N2-type mechanism[9] (initiated by an Asp residue within the active site[10]) thus effecting strict retention of configuration.

Retrosynthetic analysis suggested the coupling of an *o*-metalated 1,6-dihydroxyanthracene derivative with a chiral epoxide, which would be generated by chemoenzymatic deracemization (Scheme 1).[11,12] The key features of this process are the avoidance of an unwanted stereoisomer by complete conversion of a racemate into a single stereoisomeric product, and modulation of the enantioselectivity of the biocatalytic kinetic resolution step by appropriate choice of an unsaturated (*E*- or *Z*)-alkene or alkyne moiety.[13] Finally, oxidative cleavage of the latter would furnish the desired carboxylic acid moiety.

Results and Discussion

For the construction of the chiral building block required for the side-chain, a series of racemic 2,2-disubstituted oxiranes *rac*-**4a**–**c**[14] containing a C–C multiple bond attached through a methylene unit were screened with a range of *Actinomyces* spp. known to possess a rich secondary metabolism, and epoxide hydrolase activity in particular (Scheme 2).[15] The enantioselectivities for the kinetic resolution (expressed as the corresponding *E* values[16]) are

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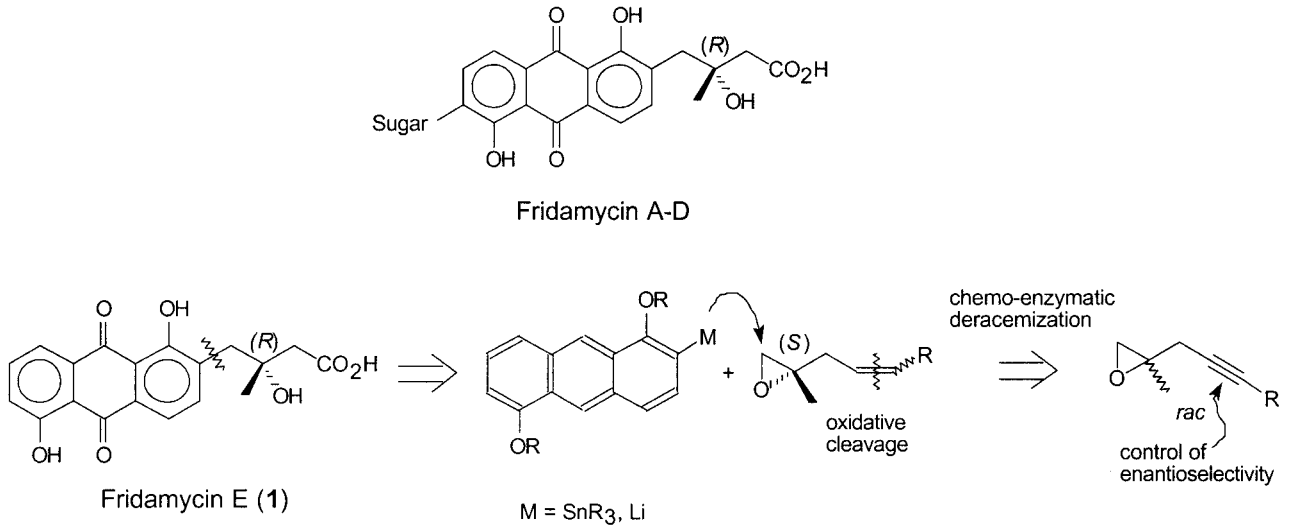
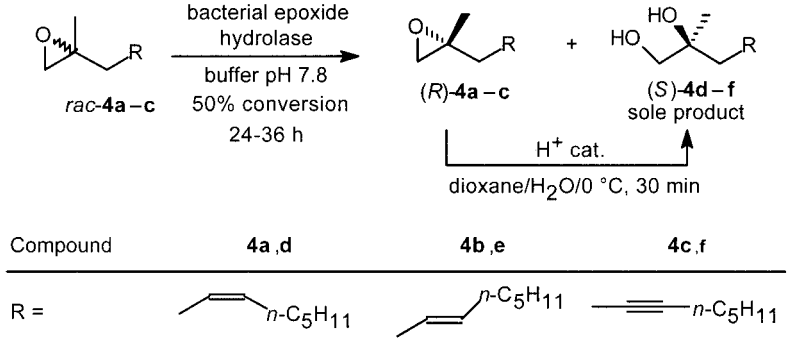


Scheme 1. Fridamycin family and retrosynthetic analysis of fridamycin E.

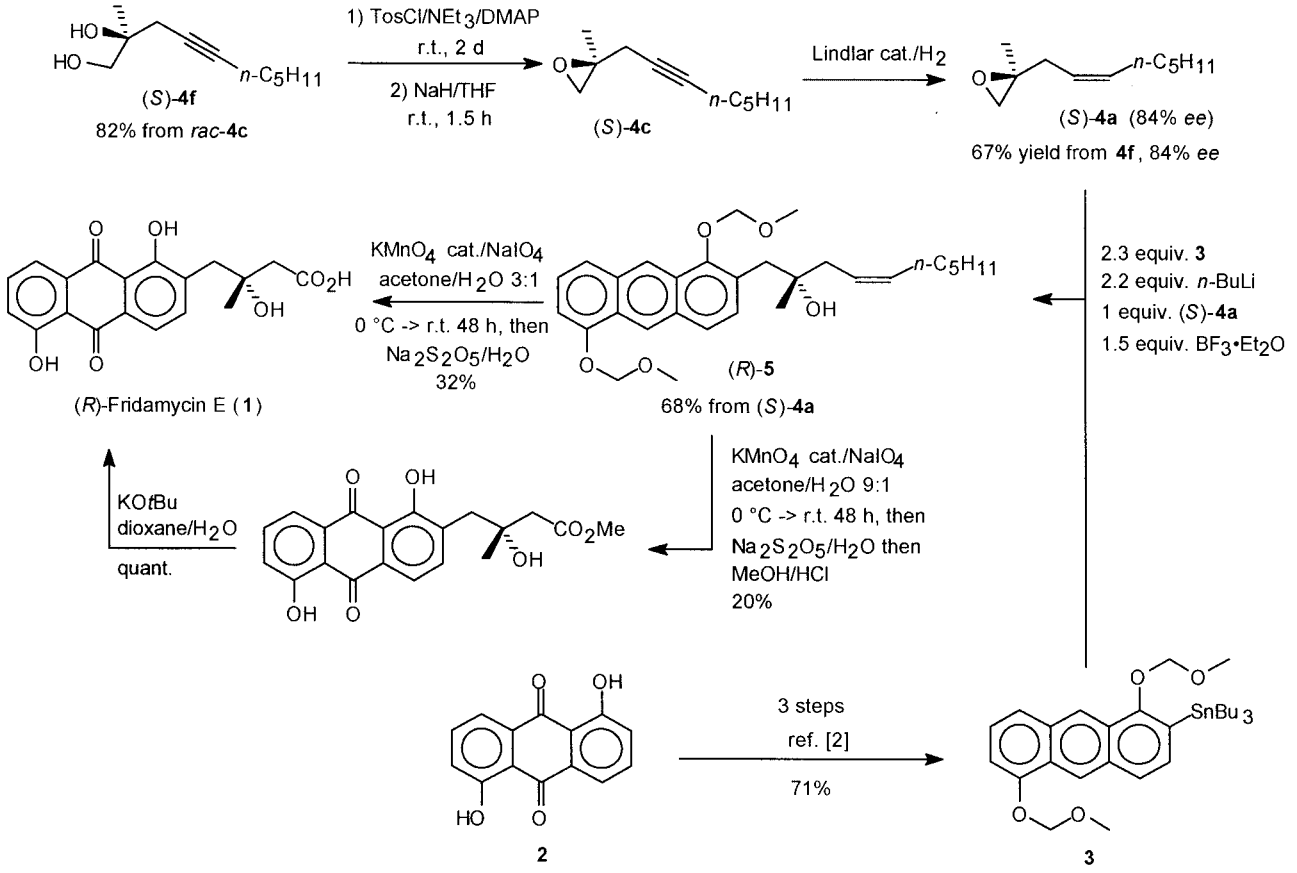
strongly dependent on the nature of the C–C multiple bond: whereas oxirane **4a**, containing a "bent" (*Z*)-alkene unit was generally resolved with low selectivities (*E* = 30, *Mycobacterium paraffinicum* NCIMB 10420), the "stretched" (*E*)-analog **4b** gave better results (*E* = 49, *Rhodococcus* sp. NCIMB 11216). In line with these observations, substrate **4c**, which bears the least flexible and straightest side-chain, gave the best results and could be resolved by *Methylobacterium* sp. FCC 031 on a preparative scale with an *E* value of 66. After the biohydrolysis was terminated at 50% conversion, the mixture of formed diol (*S*)-**4f** and remaining unreacted oxirane (*R*)-**4c** was extracted and treated with an H_2O/dioxane mixture under acid catalysis to effect hydrolysis of **4c** with inversion of configuration to yield (*S*)-**4f** in 84% *ee* and 82% overall yield from the racemate.

With (*S*)-**4f** in hand, the total synthesis could be completed as follows (Scheme 3). Ring-closure of diol **4f** (TosCl/NEt_3, NaH) gave oxirane (*S*)-**4c**, which was hydrogenated under Lindlar conditions to furnish (*S*)-**4a** in a one-pot procedure in good yield. The coupling of building block (*S*)-**4a** with the *o*-metalated anthracene unit **3**, which was obtained in three steps from commercially available anthrarufin (**2**) according to a literature procedure,[2] proved to be unexpectedly difficult: both stannane **3** and the corresponding Li



Biocatalyst	Enantioselectivity (*E* value)		
	4a	**4b**	**4c**
Rhodococcus sp. NCIMB 11216	14	**49**	12
Rhodococcus ruber DSM 43338	10	32	32
Mycobacterium paraffinicum NCIMB 10420	**30**	2.4	17
Rhodococcus sp. DSM 44539	25	10	4.4
Methylobacterium sp. FCC 031	25	slow reaction	**66 !**

Scheme 2. Chemoenzymatic deracemization of side-chain building block.



Scheme 3. Asymmetric total synthesis of (*R*)-fridamcin E.

analog − obtained by transmetalation of **3** with *n*BuLi − were unreactive in the absence of a Lewis acid. After some experimentation, it was found that addition of a slight molar excess of $BF_3{\cdot}Et_2O$ successfully activated the oxirane to yield coupling product (*R*)-**5** in 68% yield from (*S*)-**4f**. Several attempts to promote the coupling, such as variation of solvent and temperature, addition of HMPA and copper(I) salts, failed. Finally, a one-pot, multistep oxidation of (*R*)-**5** using $KMnO_4$ cat./$NaIO_4$ involving initial deprotection of both MOM-aryl ethers, subsequent oxidation of the anthracene core to form the anthraquinone moiety, and oxidative cleavage of the alkene unit to furnish the carboxylic acid, gave (*R*)-fridamycin E in 32% yield. In order to allow spectroscopic characterization of the target compound, (*R*)-fridamycin E methyl ester (**6**) was obtained by subsequent esterification. Hydrolysis of the latter gave (*R*)-**1** in quantitative yield. Comparison of the optical rotation of this material $[\alpha]_D^{20}$ = +8.2 (*c* = 0.7, $CHCl_3$) with literature data ($[\alpha]_D^{27}$ = +8.9)[5] revealed its absolute configuration to be identical with that of the natural material. Its physical and spectroscopic data were in good agreement with literature values.[4–6]

In conclusion, we have reported the asymmetric total synthesis of the antibacterial agent (*R*)-fridamycin E following a chemoenzymatic approach. The key feature of this synthesis lies in the construction of the chiral side-chain, which is based on a biocatalytic kinetic resolution/deracemization sequence, which provides the desired chiral building block in 84% *ee* and 82% overall yield from the racemate by avoiding the formation of the unwanted stereoisomer.

Experimental Section

General: NMR spectra were recorded in $CDCl_3$ on a Bruker AMX360 spectrometer at 360 MHz (^1H) and 90 MHz (^{13}C) or on a Bruker DMX Avance 500 at 500 MHz (^1H) and 125 MHz (^{13}C). Chemical shifts are reported relative to TMS (δ = 0.00 ppm) with $CHCl_3$ as internal standard [δ = 7.23 (^1H) and 76.90 ppm (^{13}C)]. TLC was performed on silica gel Merck 60_{F254}, and compounds were visualized by spraying with Mo reagent [$(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$ (100 g L^{-1}), $Ce(SO_4)_2{\cdot}4H_2O$ (4 g L^{-1}) in H_2SO_4 (10%)]. Compounds were purified by flash chromatography on silica gel (Merck 60, 230–400 mesh). GC analyses were carried out on a Varian 3800 gas chromatograph equipped with a FID and an HP1301 capillary column (30 m × 0.25 mm × 0.25 μm film, N_2). Enantiomeric purities were analyzed with a CP-Chirasil DEXCB column (25 m × 0.32 mm × 0.25 μm film, H_2). Optical rotation values were measured on a Perkin–Elmer polarimeter 341 at 589 nm (Na line) in a 1 dm cuvette and are given in units of 10 deg cm^2 g^{-1}. Solvents were dried and freshly distilled as usual. Substrates *rac*-**4a**–**c**[9] and stannane **3**[2] were synthesized as reported previously. Lyophilized bacteria were grown as reported before.[13,17] FCC stands for our in-house strain collection. Enantioselectivities from the screening listed in the table in Scheme 2 were determined as

described before;[11] for the determination of selectivities from scale-up reactions see below.

Chemoenzymatic Deracemization of 2-Methyl-2-(2-octinyl)oxirane (*rac*-4c): Lyophilized cells of *Methylobacterium* sp. FCC 031 (600 mg) were rehydrated in Tris-buffer (30 mL, 0.05 M, pH 8.0) for 1 h in a shaking flask on a rotary shaker at 30 °C. Substrate *rac*-**4c** (290 mg) was then added and the mixture was shaken at 30 °C and 130 rpm. The conversion was monitored by GC analysis of 50 μL samples (after micro-workup by extraction with 3 × 100 μL EtOAc, centrifugation and phase separation) and reached 50% after 18 h. The mixture was extracted four times with EtOAc (200 mL total, phase separation was facilitated by centrifugation). The combined organic layers were dried with Na_2SO_4, filtered, and carefully concentrated under reduced pressure to avoid losses of the volatile epoxide. The resulting mixture of epoxide (*R*)-**4c** and diol (*S*)-**4f** thus obtained was subsequently subjected to acid-promoted epoxide opening under inversion of configuration without further purification. Thus, the mixture (370 mg) was dissolved in 20 mL of dioxane, the solution was cooled to 0 °C, and 93% aqueous sulfuric acid (0.2 mL) was added dropwise. The reaction mixture was allowed to reach room temperature and was stirred for 20 min, after which TLC control indicated complete hydrolysis of the epoxide. The reaction was quenched by neutralization with saturated aqueous $NaHCO_3$ (20 mL). EtOAc was then added and the resulting biphasic mixture was stirred vigorously for an additional 30 min. The organic layer was separated, the aqueous layer was extracted with EtOAc (2 × 15 mL), and the combined organic phases were dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Column chromatography of the crude product on silica gel (petroleum ether/EtOAc, 2:1) afforded 2-methyldec-4-yne-1,2-diol [(*S*)-**4f**; 240 mg, 82% yield from *rac*-**4c**]. R_f (petroleum ether/EtOAc, 2:1) = 0.2. $[\alpha]_D^{20}$ = –4.6 (*c* = 1.3, EtOH, 82% *ee*). GC data: HP1301 capillary column, 14.5 psi N_2, 150 °C (8 min), 15 °C min^{-1} to 200 °C; t_R. 9.1 min. ^{1}H NMR ($CDCl_3$, 360 MHz): δ = 0.90 (t, *J* = 7 Hz, 3 H, C*H*$_3$-CH$_2$), 1.25 (s, 3 H, C*H*$_3$-C$_q$), 1.25–1.57 [m, 6 H, (C*H*$_2$)$_3$-CH$_3$], 2.06–2.52 (m, 6 H, C*H*$_2$-C≡C-C*H*$_2$, 2 × O*H*), 2.48, 3.58 (dd, *J* = 11 and 5 Hz, 1 H each, C*H*$_2$-OH) ppm. ^{13}C NMR ($CDCl_3$, 90 MHz): δ = 14.0 (*C*H$_3$-CH$_2$); 18.7, 22.2, 23.6, 28.7, 29.5, 31.1 (5 × *C*H$_2$, *C*H$_3$-C$_q$); 69.1 (*C*H$_2$-OH); 72.1 (C$_q$); 75.5, 84.0 (*C*≡*C*) ppm.

Derivatization of (*S*)-4f to its Monomethyl Ether to Give 1-Methoxy-2-methyldec-4-yne-2-ol for *ee* Determination: Diol (*S*)-**4f** (8 mg, 0.04 mmol) was dissolved in DMSO (1 mL) at room temperature, and KOH (20 mg, 0.35 mmol) was added. To the resulting mixture, 10 μL (0.16 mmol) of MeI was added and stirring was continued for 30 min. The reaction was quenched by addition of distilled water (3 mL) and the resulting solution was extracted with petroleum ether (3 × 10 mL). The organic phases were combined, dried with Na_2SO_4, filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography to afford approximately 1 mg (12%) of the monomethyl ether 1-methoxy-2-methyldec-4-yne-2-ol. GC analysis of the latter revealed an *ee* of 82%. Conditions: CP-Chirasil DEXCB column, 1 bar H_2, 100 °C isothermal, retention times: 19.5 min (*R*), 21.0 min (*S*).

(*S*)-2-Methyl-2-(2-octenyl)-oxirane (4a): Triethylamine (0.49 mL, 3.51 mmol), DMAP (72 mg, 0.59 mmol), and 4-toluenesulfonyl chloride (444 mg, 2.34 mmol) were sequentially addedto a solution of (*S*)-**4f** (214 mg, 1.17 mmol) in 10 mL of dichloromethane. The mixture was stirred at room temperature for 48 h, when TLC indicated that all starting material had been consumed. The reaction was quenched by addition of saturated aqueous NH_4Cl solution (20 mL). The phases were separated, the aqueous layer was extracted with dichloromethane (3 × 20 mL), and the combined organic layers were washed with saturated NH_4Cl solution (10 mL), saturated $NaHCO_3$ solution (2 × 10 mL), distilled water (10 mL), dried with Na_2SO_4, filtered, and concentrated under reduced pressure. The resulting yellow oil was dissolved in 5 mL of anhydrous THF, the solution was cooled to 0 °C, and NaH (85 mg, 2.1 mmol, 60% in mineral oil) was added. The mixture was allowed to reach room temperature and was stirred for 1.5 h. It was then poured into a mixture of ice and NH_4Cl. Distilled water (5 mL) was added, the phases were separated, and the aqueous layer was extracted with dichloromethane (2 × 15 mL). The combined organic layers were dried with Na_2SO_4, filtered, and carefully concentrated under reduced pressure to afford 250 mg of crude epoxide (*S*)-**4c**, which was directly used in the following Lindlar hydrogenation without further purification due to its high volatility.

Quinoline (211 mg, 1.64 mmol) and Lindlar catalyst (100 mg, Pd on $CaCO_3$ 5%, poisoned with lead) were added to a solution of crude (*S*)-**4c** (250 mg) in ethanol (20 mL) at room temperature. The reaction flask was evacuated, purged with H_2 three times, and the mixture was stirred for 45 min under hydrogen atmosphere (balloon). In order to avoid over-reduction, the reaction was stopped when GC monitoring indicated almost complete conversion of starting material. The mixture was then filtered through Celite 545 and the solvent was carefully evaporated. Column chromatography of the crude product on silica gel (petroleum ether/ethyl acetate, 20:1) afforded 131 mg of (*S*)-**4a** [67% from (*S*)-**4f**]. R_f (petroleum ether/EtOAc, 2:1) = 0.8. $[\alpha]_D^{20}$ = +7.3 (*c* = 1.3, $CHCl_3$), *ee* 84%. GC data: CP-Chirasil DEXCB column, 1 bar H_2, 80 °C isothermal, t_R.: 10.3 min (*R*), 10.8 min (*S*). ^{1}H NMR ($CDCl_3$, 360 MHz): δ = 0.88 (t, *J* = 6.6 Hz, 3 H, C*H*$_3$-CH$_2$), 1.30–1.42 [m, 7 H, (C*H*$_2$)$_2$-CH$_3$, C*H*$_3$-C$_q$], 2.00–2.08 (q, *J* = 6.8 Hz, 2 H, C*H*$_2$), 2.20–2.45 (m, 4 H, 2 × C-C*H*$_2$), 2.58, 2.64 (d, *J* = 5 Hz, 1 H each, C*H*$_2$-O), 5.3–5.6 (m, 2 H, C*H*=C*H*) ppm. ^{13}C NMR ($CDCl_3$, 90 MHz): δ = 14.1 (*C*H$_3$-CH$_2$); 21.2, 22.6, 27.4, 29.3, 31.5, 34.5 (5 × *C*H$_2$, *C*H$_3$-C$_q$); 53.3 (*C*H$_2$-O); 56.9 (*C*$_q$); 123.7, 133.0 (*C*=*C*) ppm.

Coupling of Epoxide (*S*)-4a to Stannane 3: A solution of stannane **3** (978 mg, 1.67 mmol, prepared as described in ref.[2]) in anhydrous THF (10 mL) was cooled to –80 °C in a flame dried flask under argon atmosphere and *n*-butyllithium (1 mL of a 1.6 M solution in hexane, 1.6 mmol) was added dropwise. The reaction was stirred at between –80 °C and –30 °C for 45 min until complete consumption of the stannane was observed by TLC (ethyl acetate/petroleum ether, 1:5, containing 2% triethylamine). The reaction mixture was cooled to –80 °C and a solution of epoxide (*S*)-**4a** (120 mg, 0.72 mmol) in 1 mL of anhydrous THF was added dropwise, followed by 0.14 mL (1.1 mmol) of $BF_3{\cdot}Et_2O$. The resulting green solution was allowed to warm to –10 °C within 5 h and poured into 20 mL of a saturated aqueous NH_4Cl solution. The phases were separated, the aqueous layer was extracted with dichloromethane (3 × 30 mL), and the organic layers were combined and washed with brine (20 mL), dried with Na_2SO_4, filtered, and concentrated under reduced pressure. The crude product was purified by flash chromatography (petroleum ether/EtOAc, 5:1) to give the coupling product (*R*)-**5** as a yellow oil [228 mg, 68% based on (*S*)-**4a**]. R_f (petroleum ether/EtOAc, 5:1) = 0.25. $[\alpha]_D^{20}$ = –12.1 (*c* = 0.7, $CHCl_3$). ^{1}H NMR (360 MHz, $CDCl_3$): δ = 0.89 (t, *J* = 7.2 Hz, 3 H, C*H*$_3$-CH$_2$), 1.22 (s, 3 H, C*H*$_3$-C$_q$), 1.30–1.40 [m, 6 H, -(C*H*$_2$)$_3$-CH$_3$], 2.09 [q, *J* = 5.8 Hz, 2 H, -C*H*$_2$-(CH$_2$)$_3$-CH$_3$], 2.37 (d, *J* = 5.0 Hz, 2 H, aryl-C*H*$_2$-), 2.85 (s, 1 H, O*H*), 3.07, 3.21 (d, *J* = 14 Hz, 1 H each, aryl-C*H*$_2$), 3.61 (s, 3 H, C*H*$_3$-O), 3.78 (s, 3 H, C*H*$_3$-O), 5.28–5.33 (q, *J* = 3.6 Hz, 2 H, -C*H*=C*H*-), 5.50 (s, 2 H, O-C*H*$_2$-O), 5.62 (t, *J* = 5.0 Hz, 2 H, O-C*H*$_2$-O), 7.05 (d, *J* = 7.2 Hz, 1 H, aryl-*H*), 7.39 (t, *J* = 8.3 Hz, 2 H, aryl-*H*), 7.69 (d, *J* = 8.3 Hz, 1 H), 7.82 (d, *J*

= 9.0 Hz, 1 H, aryl-*H*), 8.55 (s, 1 H, aryl-*H*), 8.85 (s, 1 H, aryl-*H*) ppm. ^{13}C NMR (90 MHz, $CDCl_3$): δ = 14.1 (CH_3-CH_2-); 22.6, 26.9, 27.5, 29.3, 31.6 (4× CH_2, CH_3-C_q); 40.4, 42.2 (CH_2-aryl, CH_2-C_q); 56.4 (O-CH_3); 57.9 (O-CH_3); 74.1 (C_q); 94.8, 100.3 (2× O-CH_2-O); 105.9 (aryl); 120.5; 121.2; 122.0; 124.8; 125.0; 125.1; 125.5; 126.2; 127.3; 129.8; 132.1; 132.7; 133.3; 151.5, 152.8 (2× C_{aryl}-O) ppm.

Oxidative cleavage of (*R*)-5 to Form (*R*)-Fridamycin E (1): $NaIO_4$ (134 mg, 0.63 mmol, 8 equiv.) was added to a solution of (*R*)-**5** (37 mg, 0.079 mmol) in acetone/water (9:1, 2 mL) and the reaction mixture was cooled to 0 °C. $KMnO_4$ (2 mg, 0.012 mmol) was then added and the reaction was allowed to reach room temperature. The flask was protected from light and the reaction mixture was stirred for 48 h at room temperature, when complete consumption of the starting material was observed by TLC. The mixture was diluted with 5 mL of distilled water, cooled to 0 °C, and quenched by slow addition of solid $Na_2S_2O_5$ (120 mg, 0.63 mmol, 8 equiv.) to destroy the excess of oxidants. The resulting yellow, acidic (pH 1) solution was stirred for 6 h at room temperature to ensure complete cleavage of the protecting groups. After removal of acetone under reduced pressure, the aqueous solution was extracted with dichloromethane (3× 10 mL) and ethyl acetate (3× 10 mL), and the combined organic phases dried with Na_2SO_4, filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel (CH_2Cl_2/MeOH, 10:1), to afford 9 mg of (*R*)-fridamycin E [**1**; 32% yield from (*R*)-**5**] as an orange-colored solid. R_f (CH_2Cl_2/MeOH, 10:1) = 0.25. $[\alpha]_D^{20}$ = +8.2 (*c* = 0.7, $CHCl_3$) (ref.:[5] +8.9). Mp.: 162–163 °C (ref.:[5] 164–165 °C). ^{1}H NMR (360 MHz, $CDCl_3$): δ = 1.24 (s, 3 H, CH_3-C_q), 2.63 (s, 2 H, CH_2), 3.12 (AB, *J* = 13.3 Hz, 2 H, CH_2), 7.35 (d, *J* = 8.3 Hz, 1 H), 7.67 (d, *J* = 7.9 Hz, 1 H); 7.7 (t, *J* = 8.3 Hz, 1 H), 7.85 (m, 2 H), 12.66 (s, 1 H), 13.39 (s, 1 H) ppm. ^{13}C NMR (90 MHz, $CDCl_3$): δ = 27.2 (CH_3-C_q); 41.3 (CH_2-aryl); 44.7 (CH_2-COOH); 72.2 (C_q); 115.2, 115.5 (2× COH_{aryl}-C_{aryl}-C=O_{aryl}), 119.2, 119.5 (2× CH_{aryl}-C_{aryl}-C=O_{aryl}); 125.2 (CH_{aryl}-C_{aryl}-OH); 131.7, 132.4 (2× CH_{aryl}-C_{aryl}-C=O_{aryl}); 133.8 (CH_{aryl}-CH_2);136.8 (CH_{aryl}-CH_{aryl}-C_{aryl}-OH); 139.6 (CH_{aryl}-C_{aryl}-CH_2); 160.6 (CH_2-CH_{aryl}-C_{aryl}-OH); 162.8(C_{aryl}-OH); 173.9 (*C*OOH);188.2, 188.5 (*C*=O) ppm. Correlation of peaks was performed by HMBC and HSQC.

Oxidative Cleavage of (*R*)-5 and Transformation to (*R*)-Fridamycin E Methyl Ester: $NaIO_4$ (206 mg, 0.97 mmol, 8 equiv.) was added to a solution of (*R*)-**5** (37 mg, 0.12 mmol) in acetone/water (9:1, 2 mL) and the mixture was cooled to 0 °C. $KMnO_4$ (2 mg, 0.012 mmol) was then added and the reaction was allowed to reach room temperature. The flask was protected from light and the mixture was stirred for 48 h at room temperature. The mixture was diluted with 5 mL of distilled water, cooled to 0 °C, and the reaction was quenched by slow addition of solid $Na_2S_2O_5$ (184 mg, 0.97 mmol). The resulting yellow, acidic solution was extracted with dichloromethane (2× 15 mL) and ethyl acetate (2× 10 mL) and the combined organic phases were dried with Na_2SO_4, filtered, and concentrated under reduced pressure. The resulting brown oil was dissolved in anhydrous methanol (2 mL), the solution was cooled to 0 °C, and treated with methanolic HCl, (2 mL, prepared by addition of 0.3 mL of acetyl chloride to 2 mL of methanol). This mixture was stirred at 0 °C for 30 min and at room temperature for 4 h. Solvent evaporation followed by column chromatography of the residue on silica gel (CH_2Cl_2/MeOH, 20:1) produced 9 mg of (*R*)-fridamycin E methyl ester (20%). R_f (CH_2Cl_2/MeOH, 20:1) = 0.2. $[\alpha]_D^{20}$ = –10.8 (*c* = 0.7, $CHCl_3$) {ref.:[5] $[\alpha]_D^{28}$ = –12 (*c* = 0.80, $CHCl_3$)}. ^{1}H NMR ($CDCl_3$, 360 MHz): δ = 1.32 (s, 3 H, CH_3-C_q), 2.59 (s, 2 H, CH_2), 3.09 (q, *J* = 12.0 Hz, 2 H), 3.73 (s, 3 H, O-CH_3), 3.91 (s, 1 H), 7.34 (d, *J* = 8.2 Hz, 1 H), 7.68 (d, *J* = 8.1 Hz, 1 H), 7.72 (d, *J* = 7.8 Hz, 1 H), 7.84 (d, *J* = 8.1 Hz, 1 H), 7.86 (d, *J* = 7.6 Hz, 1 H), 12.69 (s, 1 H), 13.21 (s, 1 H) ppm. ^{13}C NMR ($CDCl_3$, 90 MHz): δ = 27.3 (CH_3-C_q), 40.5 (CH_2-aryl), 44.4 (CH_2-CO_2Me), 51.7 (O-CH_3), 71.8 (C_q), 115.6, 116.1, 118.9, 119.4, 125.0, 131.8, 133.2, 134.6, 136.6, 139.7, 161.4 (C_{aryl}-OH), 162.7 (C_{aryl}-OH), 173.2 (CO_2Me), 187.8 (*C*=O), 188.4 (*C*=O) ppm.

Hydrolysis of (*R*)-Fridamycin E Methyl Ester to (*R*)-Fridamycin E (1): A solution of (*R*)-fridamycin E methyl ester (8 mg, 0.022 mmol) in THF (0.5 mL) was added dropwise to a solution of *t*BuOK (10 mg, 0.09 mmol) in THF/H_2O (9:1, 0.5 mL) at 0 °C. After 2 h of stirring at room temperature, complete conversion of the ester was observed by TLC (CH_2Cl_2/MeOH, 10:1). The reaction mixture was diluted with 3 mL of distilled water and acidified to pH 1 with 0.2 M HCl. The resulting solution was extracted with CH_2Cl_2 (3× 5 mL), the organic layers were dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by filtration through a silica pad (CH_2Cl_2/MeOH, 9:1) to afford 8 mg (100%) of (*R*)-fridamycin E (**1**). Melting point, optical rotation and spectroscopic properties were identical to those of the material produced by oxidative cleavage of (*R*)-**5**.

Acknowledgments

This study was performed within the Spezialforschungsbereich Biokatalyse. Financial support by the Fonds zur Förderung der wissenschaftlichen Forschung (project no. F-104) is gratefully acknowledged. H. Sterk and G. Kollenz (Graz) are cordially thanked for their valuable support and expertise.

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Received: October 11, 2004

