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A Novel Fungal Flavin-Dependent Halogenase for Natural Product Biosynthesis

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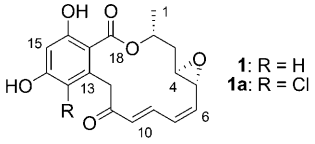
Halogenated molecules represent an important class of natural products, many of which are pharmaceutically relevant, such as chloramphenicol (antibacterial), vancomycin (antibacterial), and rebeccamycin (anticancer). Flavin-dependent halogenases have been identified as a major player in the introduction of halogen into activated organic molecules in natural product biosynthesis.[1] However, the flavin-dependent halogenases identified so far are mainly prokaryotic tryptophan halogenases with strict substrate specificity.[2–5] Most of these enzymes are involved in early biosynthetic steps of natural products to modify precursors such as tryptophan, which has limited their potential as biocatalysts to prepare various halogenated molecules. Considering the biological importance of halogens in natural products,[6] a potent halogenase able to tailor diverse complex structures will be useful for enzymatic synthesis of novel halogenated compounds.

Monocillin I (**1**) and radicicol (**1a**) are potent heat shock protein 90 (Hsp90) inhibitors isolated from various fungi,[7–9] of

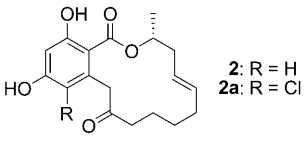


which **1a** is a chlorine-containing resorcylic acid lactone (RAL). The radicicol biosynthetic gene cluster from two different radicicol-producing fungi, *Pochonia chlamydosporia*[10] and *Chaetomium chiversii*,[11] have been recently reported, both containing a putative halogenase (*rdc2* or *radH*) that might be involved in the chlorination of the RAL structure. However, none of these enzymes have been biochemically characterized and their enzymatic properties remain unknown. Herein, we report the identification and reconstitution of a fungal halogenase Rdc2 from *P. chlamydosporia*, and its use for structural modification of diverse bioactive natural products as a promising halogenating biocatalyst.

To biochemically characterize this halogenase, we first need to get the functional enzyme. As predicted by the previous literature,[10] five introns are present in *rdc2*. We extracted the mRNA from *P. chlamydosporia*, which was subsequently used for the synthesis of cDNA. The intron-free *rdc2* gene was cloned by using the cDNA as the template and subsequently ligated into pET28a vector between NdeI and HindIII sites to yield the plasmid pJZ54. The sequencing result of the plasmid confirmed that the cloned *rdc2* is intron-free, and revealed that it is actually longer than the predicted cDNA in GenBank (EU520419). The plasmid was then transformed into *E. coli* BL21-CodonPlus (DE3)-RIL strain (hereafter referred to as RIL) and the protein expression was induced by 200 μM IPTG at 28 °C for 16 h. The N-terminal His-tagged enzyme was purified into homogeneity (Figure S1) by Ni-NTA column at a yield of 6.6 mg L^{-1}.

BLAST analysis has shown that Rdc2 is a putative flavin-dependent halogenase. The function of flavin-dependent halogenases requires a partner flavin reductase to generate $FADH_2$ from FAD and NADH. Because such a reductase was absent in the radicicol biosynthetic gene cluster, we chose Fre, a known flavin reductase from *E. coli*,[12] as the coupling enzyme to test the function of Rdc2. Similarly, the *fre* gene was cloned from the genomic DNA of *E. coli* BL21(DE3), inserted into pET28a, and expressed in RIL. The N-terminal His-tagged enzyme was purified by Ni-NTA column at a yield of 10.3 mg L^{-1} (Figure S2).

With these two soluble enzymes in hand, we first tested the catalytic ability of Rdc2 to transform **1** into **1a**. However, many peaks appeared in the HPLC chromatogram of the reaction mixture, likely due to the instability of the double bonds and epoxide in both **1** and **1a**,[13] in the presence of hypochlorous acid generated during the halogenation reaction.[2] We then sought to use a more stable biosynthetic intermediate or byproduct in radicicol biosynthesis as a substrate to assay the Rdc2-catalyzed halogenation. Thus, we tested monocillin IV (**2**) as the alternative substrate. HPLC analysis of the reaction mixture (Figure 1A) indicated that the substrate was converted into a product **2a**, which has a longer retention time (22.2 min) than **2**. ESI-MS of **2a** showed the $[M+H]^+$ quasimolecular peaks at *m/z* 353 and 355, respectively, with a ratio of 3:1 (Figure S3), which is a characteristic isotope pattern of



monochlorinated compounds. In addition, we also observed a bathochromic shift of the UV absorptions caused by the chlo rination by comparing the UV spectrum of **2a** to that of the substrate (Figure S4). To obtain sufficient amount of **2a** for NMR spectroscopic analysis, we used an in vivo biotransforma-

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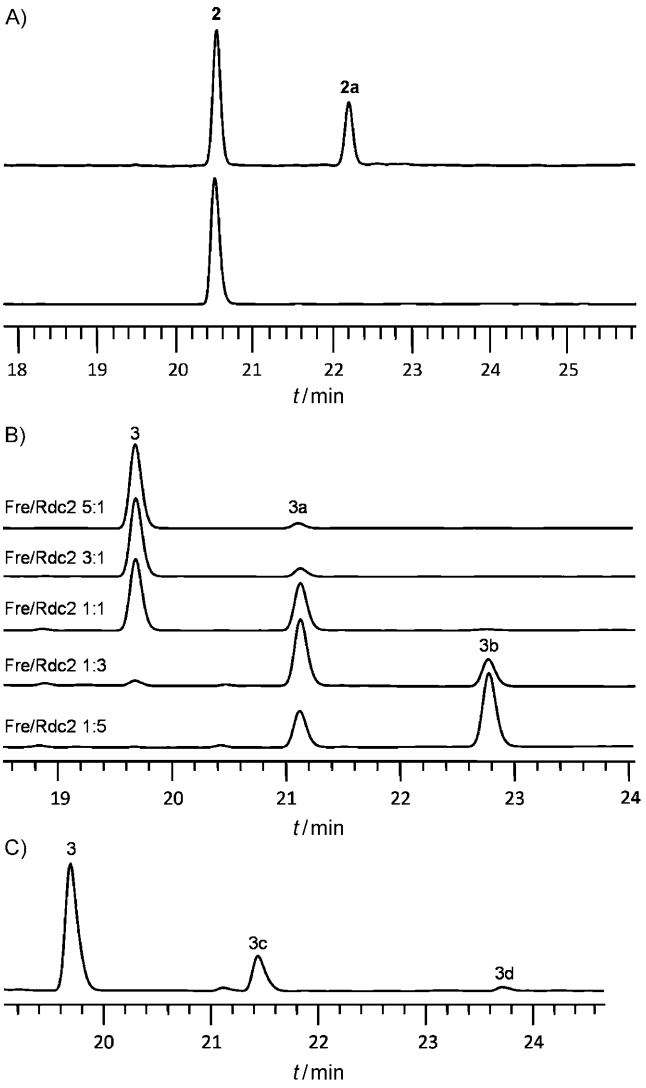


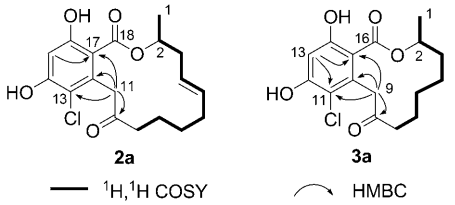
Figure 1. A) HPLC analysis of chlorination of **2** catalyzed by Rdc2 (top: reaction mixture; bottom: substrate control); B) HPLC analysis of chlorination of **3** with different Fre/Rdc2 ratios; C) Bromination of **3** by Rdc2. All samples were analyzed at 310 nm.

tion method to prepare the chlorinated product. We fed 8.2 mg of **2** into 1 L of induced *E. coli* RIL/pJZ54, from which a total of 1.6 mg of **2a** was purified. The ^{1}H NMR spectrum of **2a** indicated that only one aromatic proton signal (singlet at $\delta =$ 6.46, Table 1) is present in **2a**, suggesting that either H13 or H15 was substituted by chlorine. The HMBC correlations of H11 ($\delta = 4.36$ and 4.09) to C13 and C10 (Scheme 1) confirmed that **2a** is 13-chloromonocillin IV. Thus, we were able to identify Rdc2 as a flavin-dependent halogenase and reconstituted this fungal halogenase in *E. coli*. RALs are known to be synthesized by cooperative action of two iterative polyketide synthases (PKSs).[14] Our result indicated that Rdc2 is the dedicated post-PKS tailoring halogenase in radicicol biosynthesis, which is responsible for the introduction of the chlorine atom after the formation of the RAL structure by the PKSs.

To test the potential of Rdc2 as an effective halogenating biocatalyst, we next examined whether it can function on

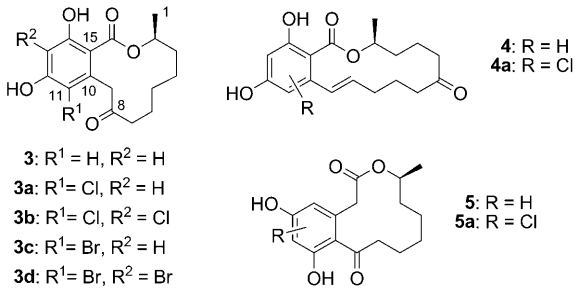
Table 1. ^{1}H NMR spectroscopy data for **2a**, **3a**, and **3b** (in CD_3OD, 300 MHz)

Position	^{1}H, δ (multiplicities, J [Hz])		
	2a	**3a**	**3b**
1	1.36 (d, 6.5 Hz, 3H)	1.31 (d, 6.5 Hz, 3H)	1.34 (d, 6.2 Hz, 3H)
2	5.31 (m, 1H)	5.09 (m, 1H)	5.16 (m, 1H)
3	2.50 (m, 1H)	1.70 (m, 2H)	1.72 (m, 2H)
	2.30 (m, 1H)		
4	5.50 (m, 1H)	1.52 (m, 1H)	1.51 (m, 1H)
		1.97 (m, 1H)	1.97 (m, 1H)
5	5.48 (m, 1H)	1.49 (m, 2H)	1.49 (m, 2H)
6	2.08 (m, 2H)	1.30 (m, 2H)	1.30 (m, 2H)
7	1.62 (m, 2H)	2.64 (m, 1H)	2.66 (m, 1H)
8	1.54 (m, 2H)	2.49 (m, 1H)	2.52 (m, 1H)
9	2.55 (m, 2H)	4.64 (d, 18.5 Hz, 1H)	4.65 (d, 18.9 Hz, 1H)
		4.50 (d, 18.5 Hz, 1H)	4.53 (d, 18.9 Hz, 1H,)
11	4.36 (d, 17.5 Hz, 1H)		
	4.09 (d, 17.5 Hz, 1H)		
13		6.40 (s, 1H)	
15	6.46 (s, 1H)		

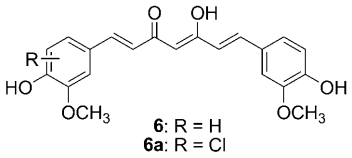


Scheme 1. Selected ^{1}H,^{1}H COSY and HMBC correlations for **2a** and **3a**.

other similar natural lactones, including dihydroresorcylide (**3**), zearalenone (**4**), and curvularin (**5**). The results (Figures S5–S16) showed that Rdc2 can chlorinate all these substrates to the corresponding chlorinated derivatives **3a**–**5a**, confirmed by the characteristic $[M+H]^+$ ion peaks. It should be noted that both **3** and **5** are a pair of regioisomers with the only structural difference in the position of the lactone and ketone groups. Apparently, Rdc2 can accept a variety of macrolactones as the substrates to generate chlorinated derivatives.



Encouraged by these results, we next tested curcumin (**6**), a well-known bioactive natural product that is synthesized by plant PKSs.[15] This compound exhibits many biological properties such as antioxidant and anti-inflammatory activities. LC-MS



6: R = H
6a: R = Cl

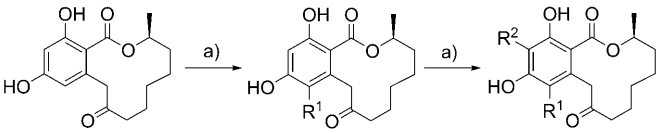
analysis confirmed that a chlorinated product had been formed (Figures S17–S19) at a relatively lower yield compared to those macrolactones. The lower conversion rate might be due to inefficient substrate–enzyme binding. Because **6** has a distinct linear structure and totally different biosynthetic origin, chlorination of this compound further confirmed that Rdc2 has broad substrate specificity.

To investigate the catalytic properties of Rdc2, we chose **3** as the representative substrate. First, we prepared **3a** through in vivo biotransformation as we did for **2a**. A total of 9.8 mg of **3a** was prepared in pure form. ESI-MS showed two quasimolecular ion peaks $[M+H]^+$ at *m/z* 327 and 329 (3:1), indicating that it is a monochlorinated derivative of **3**. Similar to **2a**, only one aromatic proton signal (singlet at $\delta = 6.40$, Table 1) was observed in the ^{1}H NMR spectrum of **3a**. Further 1D and 2D NMR spectroscopic analyses (Scheme 1) confirmed that H11 in the substrate has been substituted by chlorine. Thus, the structure of **3a** can be established as 11-chlorodihydroresorcylide.

It has been shown that the ratio of the molar concentrations of a halogenase and its partner flavin reductase will influence the catalytic efficiency. Previous research reported that a ratio of 3:1 of RebF(reductase)/RebH(halogenase) is optimal for the halogenation.[16] To achieve the best catalytic efficiency of Rdc2, we set up a series of reactions with five different Fre/Rdc2 ratios. As shown in Figure 1B, a ratio of 5:1 can completely chlorinate **3** in 2 h, indicating that reduction of FAD to $FADH_2$ by Fre is more efficient than the Rdc2-catalyzed halogenation in the system. More surprisingly, the second product **3b** appeared when relatively high concentrations of Rdc2 were present. ESI-MS of **3b** showed the quasimolecular ion $[M+H]^+$ peaks at *m/z* 361, 363, and 365, respectively, with an approximate ratio of 10:6:1 (Figure S6), suggesting that a dichlorinated derivative was synthesized. We then tried to obtain enough of **3b** for NMR spectroscopic analysis. However, no product was detected in the biotransformation broth of induced *E. coli* RIL/pJZ54 when **3a** was fed as the substrate, likely due to the relatively low concentration of Rdc2 in the cells. Alternatively, we prepared 0.6 mg of **3b** from **3a** by scaling up the in vitro reaction. The ^{1}H NMR spectroscopic analysis (Table 1) has revealed the absence of any aromatic protons, confirming that H13 of **3a** has also been substituted by chlo rine.

We next tested **2** as the substrate by using the same in vitro reaction system for **3**. Similarly, dichlorinated monocillin IV was detected by LC-MS analysis (data not shown). The discovery of these dichlorinated products was unexpected, because dichlorinated monocillin I has never been found in nature. Although the role of Rdc2 was originally believed to be a monohalogenase corresponding to the fact of one chlorine atom in **1a**, synthesis of **3b** has clearly revealed its ability to catalyze dichlorination. PltA, a flavin-dependent dichlorinase involved in pyoluteorin biosynthesis, was previously identified, which catalyzes 4,5-dichlorination after proline is tethered by thioester linkage to the carrier protein and desaturated to the pyrrolyl-S-PltL.[17] In contrast, it is apparent that Rdc2 can take free natural molecules for dichlorination. This makes Rdc2 a unique modifying enzyme to prepare various chlorinated structures. This result also indicated that whereas the functions of biosynthetic enzymes are often assigned based on the structure of intermediates and final products, some enzymes might have additional catalytic properties that could not be interpreted from the original metabolites. We reasoned that the missing of the dichlorinated products in the original host might be attributed to the low physiological concentration of Rdc2 or possibly the strong toxicity of dihalogenated metabolites.

As shown in Scheme 2, it has been observed that **3** was first halogenated at C11, followed by C13 halogenation. To understand the reaction sequence, we next measured the kinetic parameters of the two chlorination steps of **3** (Scheme 2). To test C11 chlorination, **3** was used as the substrate and the corresponding k_{cat}/K_m ratio is 2.93 min^{-1} mM^{-1} (Table 2). Similarly, the k_{cat}/K_m value of C13 chlorination was determined as 0.11 min^{-1} mM^{-1} (Table 2) with **3a** as the substrate. Apparently, the C11 halogenation step is much more efficient and thus favored by Rdc2.



3 **3a**: R^1 = Cl **3b**: R^1 = Cl, R^2 = Cl
3c: R^1 = Br **3d**: R^1 = Br, R^2 = Br

Scheme 2. Sequential halogenations of **3** by Rdc2. a) Rdc2, Fre, NADH, FAD, O_2, X^-

Table 2. Steady-state kinetic parameters of Rdc2.[a]

Kinetic parameters	C11 halogenation	C13 halogenation
K_m [μM]	281 ± 20	846 ± 26
k_{cat} [min^{-1}]	0.824 ± 0.040	0.096 ± 0.004
k_{cat}/K_m [min^{-1} mM^{-1}]	2.93	0.11

[a] Data were from three independent experiments.

To further explore the potential of Rdc2, we next examined whether Rdc2 can accept other halogen donors such as bromide and iodide. The results showed that Rdc2 can incorporate bromine into **3** to yield the corresponding mono- (**3c**) and dibrominated (**3d**) products (Figure 1C and Scheme 2) in a pattern similar to the chlorinations. No iodinated products were observed, which was not surprising considering the big size of iodine atom.

In summary, we have identified a fungal RAL dihalogenase from the radicicol biosynthetic gene cluster in *P. chlamydo-*

sporia. Unlike those previously characterized bacterial halogenases such as PrnA,[2] RebH,[16] KtzR, and KtzQ[3] that are specifically involved in the early steps in natural product biosynthesis, Rdc2 is a late tailoring enzyme that can halogenate various natural products. Recently, a eukaryotic flavin-dependent halogenase ChlA was reported to be involved in the biosynthesis of DIF-1, a polyketide-derived morphogen.[18] Our results not only further gave another example of eukaryotic halogenases involved in polyketide biosynthesis, but also for the first time provided a promising biocatalyst for in vivo or in vitro preparation of both mono- and dihalogenated derivatives from diverse bioactive molecules.

Experimental Section

General methods and materials: Products were analyzed and isolated on an Agilent 1200 high-performance liquid chromatography (HPLC) instrument. Mass spectra of the compounds were collected by the same HPLC coupled with an Agilent 6130 Single Quad mass spectrometer. Nuclear magnetic resonance (NMR) spectra were recorded in CD_3OD on a JEOL instrument (at 300 MHz for ^1H NMR and 75 MHz for ^{13}C NMR). The chemical shift (δ) values were referenced to the solvent signals for CD_3OD ($\delta_H = 3.31$) and ($\delta_C = 49.15$).

E. coli XL1-Blue and RIL were purchased from Stratagene for routine cloning and protein expression, respectively. T4 DNA ligase, 1 kb Plus DNA ladder, SuperScript III First-Strand cDNA Kit and Platinum Pfx DNA polymerase were from Invitrogen. Restriction enzymes and protein ladder were purchased from New England Biolabs, and pET28a vector was from Novagen. *P. chlamydosporia* ATCC 16683 was obtained from American Type Culture Collection (ATCC). *Acremonium zeae* NRRL 45893 and *Penicillium baradicum* NRRL 3754 were obtained from Agricultural Research Service (ARS) of the United States Department of Agriculture. Flavin adenine dinucleotide disodium salt hydrate (FAD), β-nicotinamide adenine dinucleotide, reduced dipotassium salt (NADH), zearalenone (**4**), and curcumin (**6**) were purchased from Sigma–Aldrich. Compound **6** was further purified on HPLC to remove demethoxycurcumin and bisdemethoxycurcumin before it was used as a substrate. Monocillin I (**1**), radicicol (**1a**) and monocillin IV (**2**) were isolated from the potato dextrose agar (PDA) culture of *P. chlamydosporia* ATCC 16683. Dihydroresorcylide (**3**) and curvularin (**5**) were isolated from *A. zeae* NRRL 45893 and *P. baradicum* NRRL 3754, respectively.

Expression and purification of Rdc2: The radicicol-producer strain *P. chlamydosporia* ATCC 16683 was grown in potato dextrose broth (50 mL; PDB) at 28 °C for 4 d. The culture was filtered and the mycelium was ground in liquid N_2. The RNA was extracted from the ground mycelium by using a RNeasy Plant Mini Kit from Qiagen. The resulting RNA was used as the template to synthesize the cDNA through a SuperScript® III First-Strand cDNA Kit from Invitrogen. The cDNA was subsequently used as the PCR template to clone the intron-free *rdc2* gene. Primers included rdc2–5′-NdeI (AA*C ATA TG*T CGG TAC CCA AGT CTT G) and rdc2–3′-HindIII (AA*A AGC TT*T CAA ACT TTG TTG AGG CCA A). The introduced restriction sites are shown in italics. The PCR product was digested with NdeI and HindIII and inserted into pET28a to yield pJZ54. The plasmid was transformed into *E. coli* RIL strain for protein expression. For 500 mL of liquid culture, the cells were grown at 37 °C in Luria–Bertani (LB) medium supplemented with 35 μg mL^{-1} kanamycin and 25 μg mL^{-1} chloramphenicol to an OD_{600} of 0.4–0.6, and then induced by 200 μM isopropyl-1-thio-β-D-galactoside (IPTG) for 16 h at 28 °C. The cells were harvested by centrifugation at 3,500 rpm for 10 min, resuspended in cold lysis buffer [30 mL; 20 mM Tris–HCl (pH 7.9), 0.5 M NaCl, 10 mM imidazole, 4 °C] and lysed by using sonication on ice. Cellular debris was removed by centrifugation at 20,000 rpm for 45 min at 4 °C. Ni-NTA agarose resin (Qiagen) was added to the supernatant (4 mL L^{-1} of culture) and the mixture was shaken at 4 °C for 3 h to ensure the His_6-tagged protein was well absorbed. The protein resin mixture was loaded into a gravity flow column and proteins were purified with an increasing concentration of imidazole in buffer A (50 mM Tris–HCl, pH 7.9, 2 mM EDTA, 2 mM DTT). Purified Rdc2 was concentrated and exchanged into buffer A with Centriprep filter devices (Amicon Inc.). The concentration of purified protein was determined by a Coomassie Protein Assay Kit from Fisher Scientific. The purity of Rdc2 (58 kDa) was checked on SDS-PAGE (Figure S1).

Expression and purification of Fre: The *fre* gene was directly cloned from the genomic DNA of *E. coli* BL21(DE3) by using a pair of primers including fre-5′-NdeI (AA*C ATA TG*A CAA CCT TAA GCT GTA A) and fre-3′-EcoRI (AA*G AAT TC*T CAG ATA AAT GCA AAC GCA T). The introduced restriction sites are shown in italics. The gene was inserted into pET28a between NdeI and EcoRI sites to yield plasmid pJZ62. The gene was confirmed by sequencing. The expression plasmid pJZ62 was transformed into *E. coli* RIL strain for protein expression. The expression and purification procedure is same as that for Rdc2 except that the expression temperature is 16 °C. The purity of Fre (26 kDa) was checked on SDS-PAGE (Figure S2).

Halogenation assays: A typical halogenation assay mixture (100 μL) consisted of 100 μM FAD, 10 mM NADH, 10 mM NaCl, 0.1 mM substrate, 16 μM Fre, and 16 μM Rdc2 in 100 mM phosphate buffer (pH 7.0). The reaction mixtures were incubated at 28 °C for 2 h and then quenched with MeOH (200 μL). Substrate controls included all components except Rdc2. The mixtures were briefly vortexed and then centrifuged at 15000 rpm for 5 min to remove the precipitated proteins before the samples were injected into LC-MS for analysis. The samples were analyzed on an Agilent Single Quad LC-MS by using a Zorbax SB-C18 reversed-phase analytical column (5 μm, 150 mm × 4.6 mm) at 310 nm, with a flow rate of 1 mL min^{-1}. A gradient of MeCN/H_2O system (10–90%) containing 0.1% trifluoroacetic acid (TFA) was programmed over 30 min.

To test other halogen donors, the purified Rdc2 and Fre were exchanged into phosphate buffer after purification to maximally remove Cl^-. NaCl in the reaction mixtures was also replaced by NaBr or NaI as the halogen donor. Small amount of monochlorinated product **3a** might still be observed in these reactions such as the bromination reaction shown in Figure 1C due to the residual Cl^- in the system.

In vivo biosynthesis of 13-chloromonocillin IV (2a) and 11-chlorodihydroresorcylide (3a) in *E. coli*: *E. coli* RIL/pJZ54 was grown in 1 L of LB medium and induced as described above. Three hours after IPTG induction, **2** (8.2 mg, 820 μL of a 10 mg mL^{-1} solution in MeOH) was added, and the culture was maintained at 28 °C for an additional 36 h. The biotransformation broth was then centrifuged to harvest the supernatant and pellet. The resulting pellet and supernatant were extracted (3×) with the same volume of MeOH and EtOAc, respectively. The extracts were combined and evaporated under reduced pressure. The residue was dissolved in MeOH and purified by HPLC by using the same conditions described above. A total of 1.6 mg of **2a** was isolated in pure form. ^1H NMR data are listed in Table 1. The signals were assigned based on the 1D and 2D NMR spectra; ^{13}C NMR (CD_3OD, 75 MHz): $\delta = 209.9$

(C10), 171.2 (C18), 162.7 (C16), 159.3 (C14), 137.2 (C12), 135.8 (C5), 126.6 (C4), 115.6 (C13), 108.9 (C17), 104.0 (C15), 74.3 (C2), 47.3 (C11), 42.2 (C9), 39.1 (C3), 33.4 (C6), 27.0 (C7), 23.5 (C8), 19.5 ppm (C1).

The same procedure was used for the preparation of **3a**. A total of 34 mg of **3** was fed into 4 L of induced culture of *E. coli* RIL/pJZ54, from which pure product (9.8 mg) was isolated by HPLC. ^{1}H NMR spectroscopy data were listed in Table 1. The signals were assigned based on the 1D and 2D NMR spectra; ^{13}C NMR (CD_3OD, 75 MHz): $\delta = 210.8$ (C8), 171.9 (C16), 163.7 (C14), 160.7 (C12), 136.9 (C10), 117.1 (C11), 108.2 (C15), 104.1 (C13), 75.5 (C2), 47.5 (C9), 43.2 (C7), 33.0 (C3), 28.6 (C5), 22.8 (C6), 22.5 (C4), 19.4 ppm (C1).

In vitro enzymatic preparation of 11,13-dichloro-dihydroresorcylide (3b): Although in vivo biosynthesis of **3b** from **3a** in *E. coli* was attempted, no product was detected. We then chose to scale up the in vitro reaction to prepare enough **3b**. A 40 mL reaction was set up that contained 100 μM FAD, 10 mM NADH, 10 mM NaCl, 0.1 mM substrate, 16 μM Fre, and 16 μM Rdc2 in 100 mM phosphate buffer (pH 7.0). The reaction mixture was incubated at 28 °C for 16 h, before it was quenched with MeOH (80 mL). After brief vortexing, the mixture was centrifuged at 15000 rpm for 10 min. The supernatant was evaporated and redissolved in MeOH. The product was then purified on the Agilent 1200 HPLC by using the same conditions as described for the analysis of small-scale reactions. A total of 0.6 mg of **3b** was isolated in pure form. ^{1}H NMR data of **3b** were listed in Table 1. The proton signals were assigned by a comparison with **3a** and the ^{1}H,^{1}H COSY analysis.

Kinetic analysis of two chlorination steps of dihydroresorcylide (3) by Rdc2: Dihydroresorcylide (**3**) was chosen as the substrate to study the kinetics of Rdc2. To measure the k_{cat} and K_m for the C11 chlorination, we set up a series of 100 μL reaction systems containing 100 μM FAD, 10 mM NADH, 10 mM NaCl, 16 μM Fre, and 16 μM Rdc2 in 100 mM phosphate buffer (pH 7.0) with varying amount of **3** (0.17 to 1.71 mM) in each tube. All the reaction components and Fre were mixed thoroughly and maintained at 28 °C for 2 min. Rec2 was then added to initiate the reaction. After 30 min, the reactions were quenched with MeOH (200 μL), briefly vortexed, and centrifuged at 15,000 rpm for 5 min. Product formation was quantified on the HPLC at 315 nm based on the area of the peaks and standard curve of **3a**. Reactions were run in triplicate and the steady-state parameters k_{cat} and K_m were determined by nonlinear fitting of Michaelis–Menten equation.

For the second halogenations step, C13 chlorination, we used the same method to determine k_{cat} and K_m except that 11-chlorodihydroresorcylide (**3a**) was used as the substrate (0.03–0.18 mM).

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Keywords: biosynthesis · flavin · halogens · natural products · radicicol · substrate specificity

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