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# Stereochemistry and Mechanism of Undecylprodigiosin Oxidative Carbocyclization to Streptorubin B by the Rieske Oxygenase RedG

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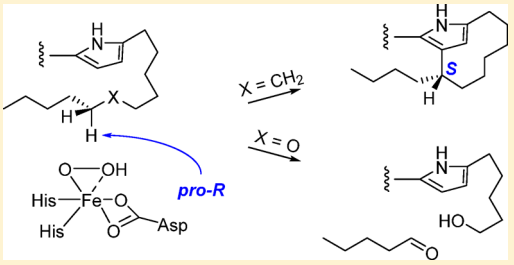
Supporting Information

**ABSTRACT:** The prodiginines are a group of specialized metabolites that share a 4-methoxypyrrolyldipyrromethene core structure. Streptorubin B is a structurally remarkable member of the prodiginine group produced by *Streptomyces coelicolor* A3(2) and other actinobacteria. It is biosynthesized from undecylprodigiosin by an oxidative carbocyclization catalyzed by the Rieske oxygenase-like enzyme RedG. Undecylprodigiosin derives from the RedH-catalyzed condensation of 2-undecylpyrrole and 4-methoxy-2,2′-bipyrrole-5-carboxaldehyde (MBC). To probe the mechanism of the RedG-catalyzed reaction, we synthesized 2-(5-pentoxypentyl)-pyrrole, an analogue of 2-undecylpyrrole with an oxygen atom next to the site of C−C bond formation, and fed it, along with synthetic MBC, to *Streptomyces albus* expressing *redH* and *redG*. This resulted in the production of the 6′-oxa analogue of undecylprodigiosin. In addition, a small amount of a derivative of this analogue lacking the *n*-pentyl group was produced, consistent with a RedG catalytic mechanism involving hydrogen abstraction from the alkyl chain of undecylprodigiosin prior to pyrrole functionalization. To investigate the stereochemistry of the RedG-catalyzed oxidative carbocyclization, [7′-$^2$H](7′*R*)-2-undecylpyrrole and [7′-$^2$H](7′*S*)-2-undecylpyrrole were synthesized and fed separately, along with MBC, to *S. albus* expressing *redH* and *redG*. Analysis of the extent of deuterium incorporation into the streptorubin B produced in these experiments showed that the *pro-R* hydrogen atom is abstracted from C-7′ of undecylprodigiosin and that the reaction proceeds with inversion of configuration at C-7′. This contrasts sharply with oxidative heterocyclization reactions catalyzed by other nonheme iron-dependent oxygenase-like enzymes, such as isopenicillin N synthase and clavaminate synthase, which proceed with retention of configuration at the carbon center undergoing functionalization.




## INTRODUCTION

The prodiginines are a group of specialized metabolites with a common 4-methoxypyrrolyldipyrromethene core produced by a variety of bacteria, including *Streptomyces* and related actinobacteria (Figure 1).[1] Members of the prodiginine family possess a range of interesting biological activities and have recently been shown to be orally effective antimalarials in a murine infection model.[1,2]

*Streptomyces coelicolor* A3(2) produces undecylprodigiosin **1** and its carbocyclic derivative streptorubin B **2** (Figure 1).[3,4] Transannular interactions in the 10-membered carbocycle of streptorubin B **2** restrict rotation around the C2−C1′ and C4−C7′ bonds, resulting in the formation of atropisomers.[5] Nuclear Overhauser effect spectroscopy (NOESY) NMR experiments showed that the hydrochloride salt of **2** exists as predominantly the *anti* atropisomer in solution.[5,6] The absolute configuration of this atropisomer was assigned as 7′*S* by $^1$H and $^2$H NMR spectroscopic analyses of stereoselectively deuterium labeled streptorubin B.HCl produced via a mutasynthesis approach.[5] This stereochemical assignment was confirmed by enantioselective total synthesis and X-ray crystallographic analysis.[6] Chiral high pressure liquid chromatography (HPLC) comparisons of **2** isolated from *S. coelicolor* with chemically synthesized (7′*S*)-, (7′*R*)-, and (7′*RS*)-streptorubin B showed that the natural material consists of the *anti*-(7′*S*), *syn*-(7′*S*), and *anti*-(7′*R*) isomers in an approximately 88:7:5 ratio.[5,6]

The biosynthesis of undecylprodigiosin **1** and streptorubin B **2** in *S. coelicolor* has been extensively studied.[7] Hybrid nonribosmomal peptide synthetase/polyketide synthase/α-oxoamine synthase assembly lines construct 4-methoxy-2,2′-bipyrrole-5-carboxaldehyde (MBC) **3** and 2-undecylpyrrole **4** from L-proline, malonyl-CoA, L-serine, and *S*-adenosyl-L-methionine, and one unit of acetyl-CoA, six units of malonyl-CoA, and a unit of glycine, respectively (Figure 1) (CoA = coenzyme A, Ad = adenosine).[4,8] MBC **3** and 2-undecylpyrrole **4** are condensed to form undecylprodigiosin **1** by RedH (Figure 1).[9] The broad substrate tolerance of RedH has been exploited to produce undecylprodigiosin analogues via a mutasynthesis approach.[9] Streptorubin B **2** is formed from undecylprodigiosin **1** via an unusual oxidative carbocyclisation reaction catalyzed by RedG,[10,11] a Rieske nonheme iron-dependent oxygenase-like enzyme (Figure 1).[12] Remarkably, in *Streptomyces longisporusruber* DSM 40667 McpG, which is 75% similar in sequence to RedG, catalyzes the regio- and stereodivergent carbocylisation of undecylprodigiosin **1** to form metacycloprodigiosin **5** (Figure 1).[10]

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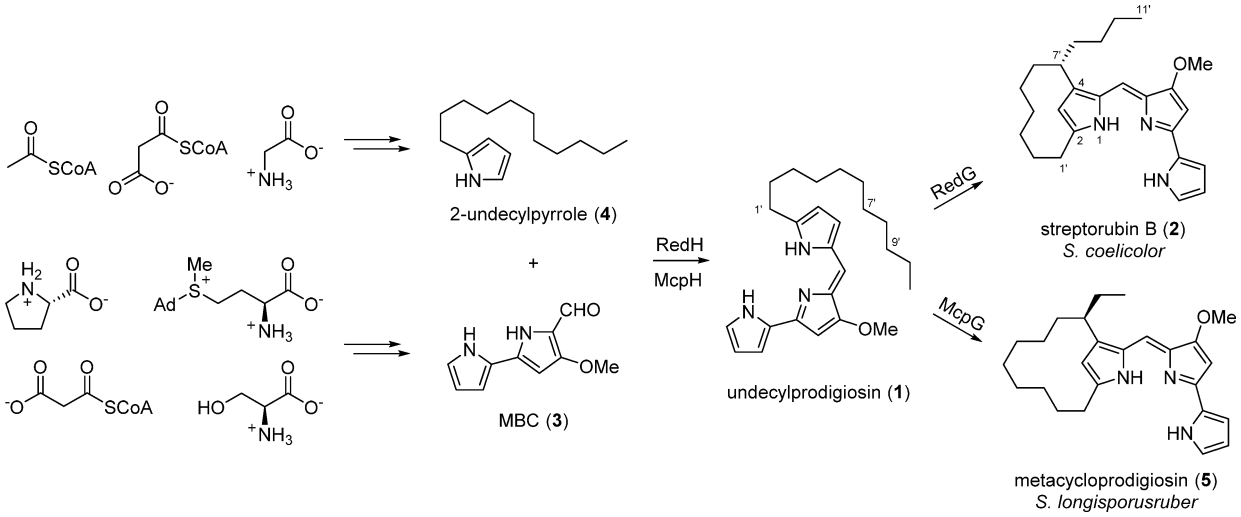


**Figure 1.** Pathway for the biosynthesis of undecylprodigiosin **1** and streptorubin B **2** in *S. coelicolor* A3(2). RedH catalyzes the condensation of 2-undecylpyrrole **4** and 4-methoxy-2,2′-bipyrrole-5-carboxaldehyde **3** to form **1**, which undergoes RedG-catalyzed oxidative carbocyclization to form **2**. In *Streptomyces longisporusruber* DSM40667, McpG catalyzes the oxidative carbocyclisation of **1** to form metacycloprodigiosin **5**.

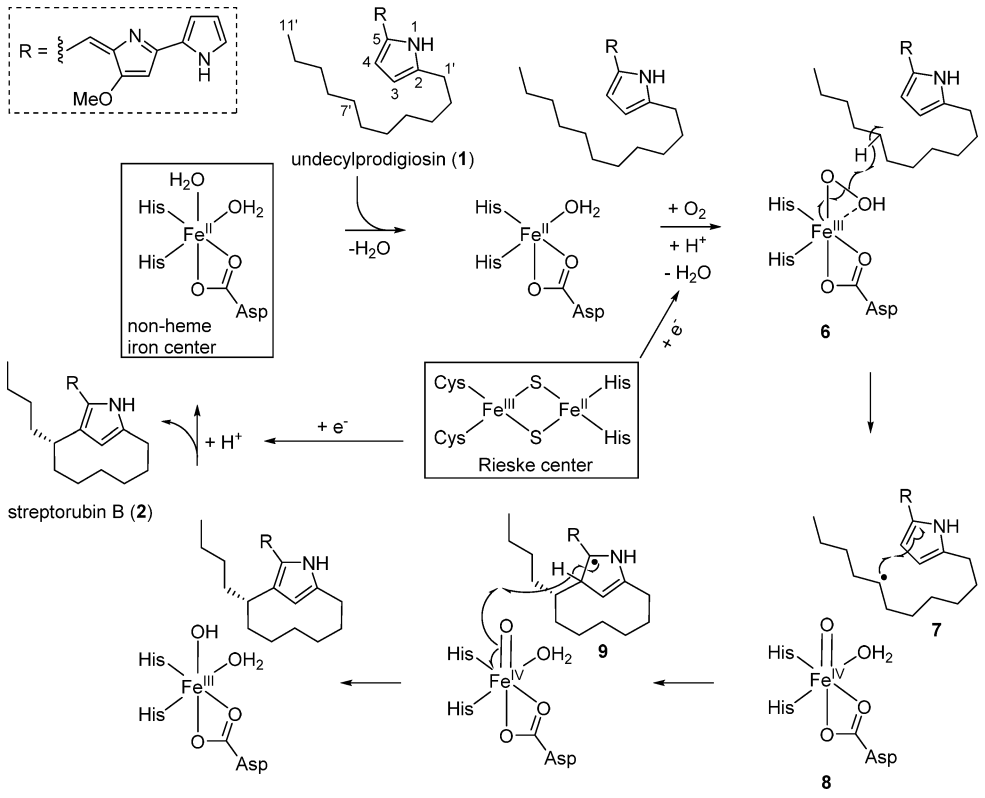


**Figure 2.** Proposed mechanism for the RedG-catalyzed conversion of undecylprodigiosin **1** to streptorubin **2**. Alternative Fe(V)O(OH) and C-7′/C-5 cation intermediates are also possible.

Based on the well-studied catalytic mechanism of naphthalene dioxygenase, we recently proposed a mechanism for the RedG-catalyzed oxidative carbocyclization reaction involving abstraction of a hydrogen atom from C-7′ of enzyme bound undecylprodigiosin **1** by the active site Fe(III)OOH complex **6** to form a carbon-centered radical **7** and an Fe(IV)O($OH_2$) complex **8** (Figure 2).[12] Addition of the radical to C-4 of the proximal pyrrole leads to formation of the 10-membered carbocycle **9** bearing a radical at C-5, which can be stabilized by delocalization into the adjacent $\pi$-system. Hydrogen abstraction from C-4 of **9** by the Fe(IV)O($OH_2$) complex **8** yields streptorubin B **2**. It was noted that an Fe(V)O(OH) complex, a C-7′ carbocation and a 10-membered carbocycle bearing a cation at C-5 are plausible alternative intermediates to **6**, **7** and **9**, respectively.[12] One of the more remarkable facets of this mechanistic proposal is that the hydrocarbon chain of undecylprodigiosin **1** is oxidized by the Fe(III)OOH complex in preference to the considerably more electron rich 4-methoxypyrrolyldipyrromethene $\pi$-system.

Here we report the synthesis of 2-(5-pentoxypentyl)-pyrrole **10**, a 2-undecylpyrrole analogue designed to probe the RedG catalytic mechanism. The products resulting from feeding of


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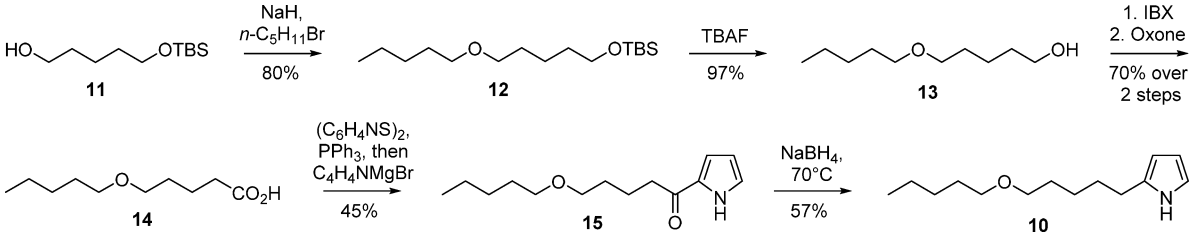
**Scheme 1. Synthesis of 2-(5-Pentoxypentyl)-pyrrole 10**



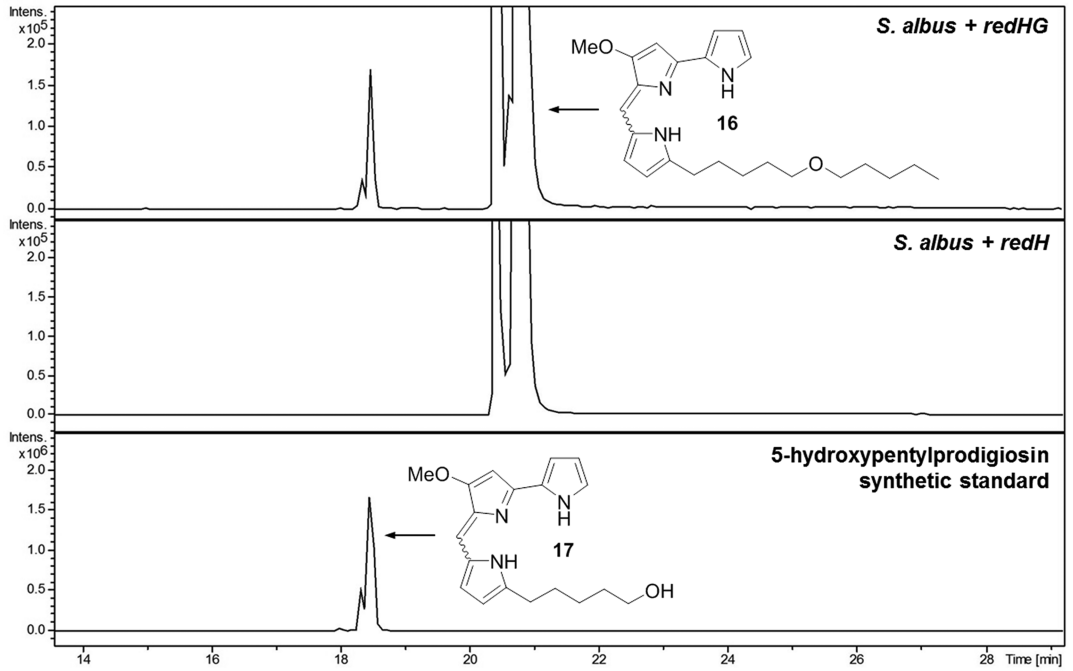




**Figure 3.** Extracted ion chromatograms (EICs) at $m/z$ = 396.26; 326.18 from LC-MS analyses of culture extracts of *S. albus* expressing *redHG* fed with MBC **3** and 2-(5-pentoxypentyl)-pyrrole **10** (top panel), and *S. albus* expressing *redH* fed with MBC **3** and 2-(5-pentoxypentyl)-pyrrole **10** (middle panel). The corresponding EIC from LC-MS analysis of a synthetic standard of 5-hydroxypentylprodigiosin **17** is shown in the bottom panel. Peak doubling is due to geometrical isomerism, resulting from rotation around the bond between C-1″ and C-5 of ring C.

this 2-undecylpyrrole analogue, together with synthetic MBC **3**, to *Streptomyces albus* J1704 expressing *redH* and *redG* demonstrate that hydrocarbon oxidation precedes functionalization of the 4-methoxypyrrolyldipyrromethene moiety. We also report the synthesis of both enantiomers of [7′-$^2$H]-2-undecylpyrrole. Feeding of these stereoselectively deuterium labeled 2-undecylpyrroles, along with synthetic MBC **3**, to *S. albus* expressing *redH* and *redG* elucidated the stereochemical course of the undecylprodigiosin to streptorubin B conversion, allowing the remarkable chemoselectivity of RedG to be rationalized.

## EXPERIMENTAL PROCEDURES

See the Supporting Information.

## RESULTS AND DISCUSSION

**Synthesis of 2-(5-Pentoxypentyl)-pyrrole 10.** We reasoned that an analogue of undecylprodigiosin **1** with an oxygen atom in place of the C-6′ methylene group would allow us to probe the timing of hydrocarbon oxidation relative to pyrrole functionalization in the RedG-catalyzed carbocyclization reaction, by stabilizing a radical/cationic intermediate at C-7′, which may lead to the formation of shunt products. Thus, we synthesized 2-(5-pentoxypentyl)-pyrrole **10** from *t*-butyldimethylsilyl (TBS)-protected 1,5-pentandiol **11**[13] in six steps (Scheme 1). Deprotonation of **11** with sodium hydride and reaction of the resulting alkoxide with 1-bromopentane afforded the corresponding ether **12**. Deprotection of **12** with tetra-*n*-butyl ammonium fluoride (TBAF) gave alcohol **13**, which was oxidized to carboxylic acid **14**, via the aldehyde, by sequential treatment with iodoxybenzoic acid (IBX) and oxone.[14,15] Coupling of **14** with pyrryl magnesium bromide, followed by reduction of the resulting 2-acylpyrrole **15**, were both accomplished according to established procedures,[4] affording 2-(5-pentoxypentyl)-pyrrole **10**.

**Feeding of 2-(5-Pentoxypentyl)-pyrrole 10 and MBC 3 to *S. albus* Expressing redHG.** We previously reported that feeding of MBC **3** and 2-undecylpyrrole **4** to a strain of *S. albus* expressing *redH* under the control of the constitutive *ermE** promoter produces undecylprodigiosin **1**.[11] We also reported that a similar experiment using a strain of *S. albus* expressing *redHG* results in the production of streptorubin B **2**, in addition to undecylprodigiosin **1**.[11]

Feeding of 2-(5-pentoxypentyl)-pyrrole **10** and synthetic MBC **3**[8] to *S. albus* expressing *redH* resulted in the formation of a red pigment that gave rise to ions with $m/z$ = 396.26 in liquid chromatography-mass spectrometry (LC-MS) analyses, corresponding to the $[M + H]^+$ ion for the 6′-oxa undecylprodi-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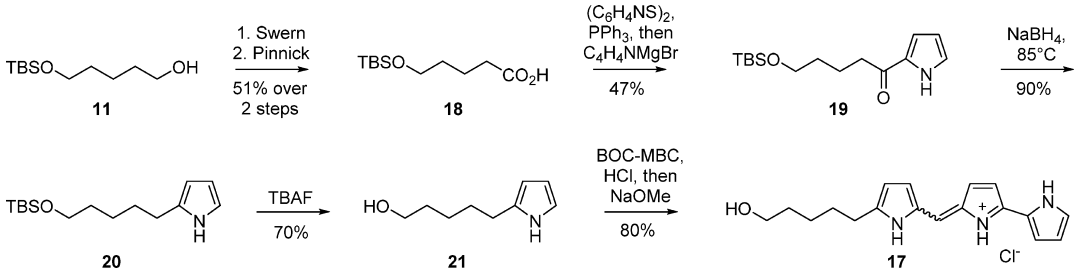
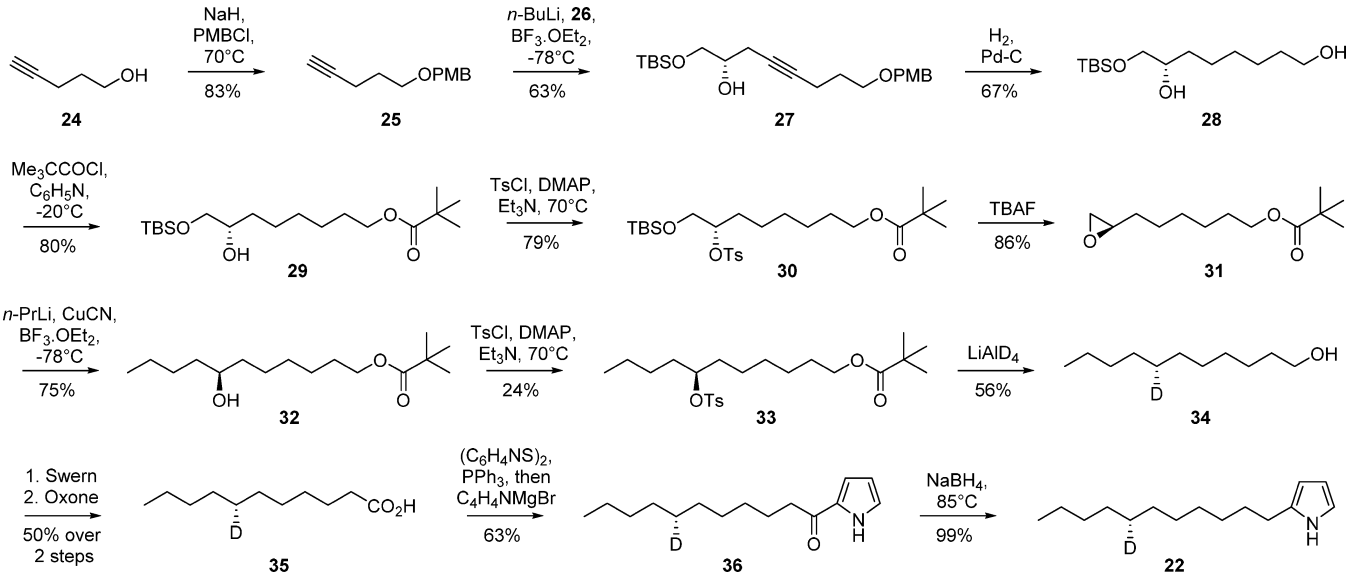
**Scheme 2. Synthesis of 5-Hydroxypentylprodigiosin 17**




**Scheme 3. Synthesis of [7′-$^2$H](7′*R*)-2-Undecylpyrrole 22**[a]




[a]Compound **26** is the TBS ether of (*R*)-glycidol, which was prepared according to a literature procedure.[18] [7′-$^2$H](7′*S*)-2-undecylpyrrole **23** was synthesized via the same route using the TBS ether of (*S*)-glycidol.

giosin analogue **16** (Figure 3). No production of **16** was observed when **10** and **3** were fed to wild type *S. albus*. When 2-(5-pentoxypentyl)-pyrrole **10** and synthetic MBC **3** were fed to *S. albus* expressing *redHG*, the 6′-oxa undecylprodigiosin analogue **16** was also produced (Figure 3), but none of the corresponding streptorubin B analogue could be detected. Instead ultrahigh resolution LC-MS analyses showed that a small amount of a compound with a molecular formula corresponding to 5-hydroxypentylprodigiosin **17** (calculated for $C_{19}H_{24}N_3O_2^+$, 326.1863; found, 326.1858), a derivative of **16** lacking the *n*-pentyl group, was produced (Figure 3). Compound **17** was not produced when **10** and **3** were fed to *S. albus* expressing *redH* alone (Figure 3).

To confirm the production of 5-hydroxypentylprodigiosin **17** when 2-(5-pentoxypentyl)-pyrrole **10** and MBC **3** are fed to *S. albus* expressing *redHG*, an authentic synthetic standard was prepared in six steps from TBS-protected 1,5-pentandiol **11**[13] (Scheme 2). Swern oxidation of **11** yielded the corresponding aldehyde,[16] which was further oxidized to the acid **18** using Pinnick's reagents.[17] Coupling of acid **18** with pyrryl magnesium bromide and reduction of the resulting 2-acylpyrrole **19** to the corresponding alkylpyrrole **20**,[4] was followed by deprotection with TBAF to give 2-(5-hydroxypentyl)-pyrrole **21**. Acid-promoted condensation of **21** with BOC-protected MBC[6] afforded 5-hydroxypentylprodigiosin **17** as its HCl salt. LC-MS/MS analyses confirmed that the dealkylated undecylprodigiosin analogue produced when 2-(5-pentoxypentyl)-pyrrole **10** and MBC **3** are fed to *S. albus* expressing *redHG* has the same retention time and fragmentation pattern as the authentic standard of **17** (Figure 3 and Supporting Information).

**Synthesis of 2-Undecylpyrrole Stereoselectively Deuterium Labeled at C-7′.** To probe the stereoselectivity of hydrogen abstraction from C-7′ and the stereochemical course of the subsequent carbocyclization reaction, we synthesized [7′-$^2$H](7′*R*)-2-undecylpyrrole **22** and [7′-$^2$H](7′*S*)-2-undecylpyrrole **23** from commercially available *R*- and *S*-glycidol, respectively, (Scheme 3). 4-Pentyn-1-ol **24** was protected as its 4-methoxybenzyl ether **25**. TBS-protected *R*-glycidol **26**[18] was reacted in the presence of $BF_3{\cdot}OEt_2$ with the acetylide derived from **25** by deprotonation with *n*-BuLi to yield homopropargyllic alcohol **27**. Reduction with $H_2$ over 10% Pd−C converted **27** to the saturated diol **28**. Selective protection of the primary alcohol in **28** with pivaloyl choride in pyridine gave ester **29**, which was converted to the corresponding tosylate **30** by treatment with 4-toluenesulfonyl chloride (TsCl), 4-dimethylaminopyridine (DMAP) and $Et_3N$. Reaction of **30** with TBAF yielded epoxide **31**, which was converted to the alcohol **32** using *n*-PrLi,[19] CuCN, and $BF_3{\cdot}OEt_2$.[20] The enantiopurity of **32** was assessed by converting a small portion to the corresponding Mosher's esters,[21] which were analyzed by $^1$H NMR spectroscopy (see the Supporting Information). While a precise enantiometric excess for the esters could not be determined due to signal overlap, the $^1$H NMR data confirmed



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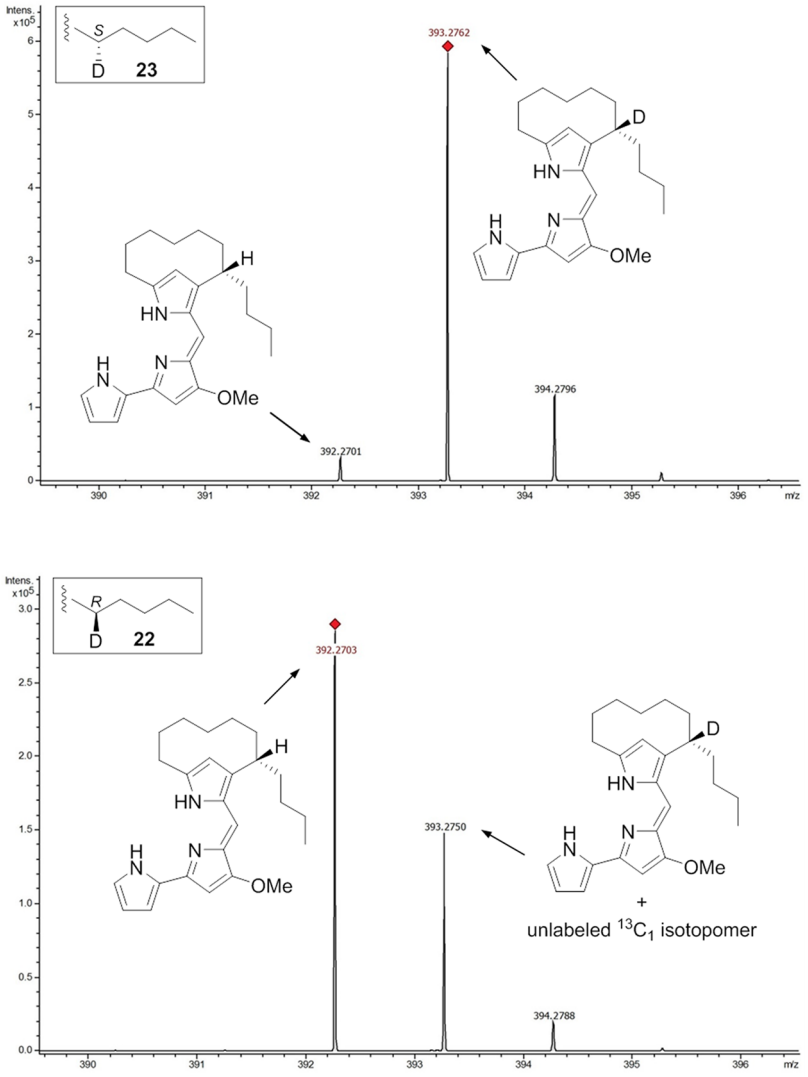


**Figure 4.** Mass spectra of streptorubin B **2** from LC-MS analyses of mycelial extracts of *S. albus* expressing *redHG* fed with MBC **3** and [7′-$^2$H](7′*S*)-2-undecylpyrrole **23** (top spectrum) or [7′-$^2$H](7′*R*)-2-undecylpyrrole **22** (bottom spectrum). When **23** is fed, 92% of the label is incorporated into streptorubin B, whereas when **22** is fed, 77% of the label is calculated to be lost (after subtraction from the $m/z$ = 393.2750 signal in the bottom panel of the component due to unlabeled $^{13}C_1$ streptorubin B isotopomer).

that **32** is predominantly the 7*S* enantiomer. Alcohol **32** was converted to the tosylate **33**, which was reacted with $LiAlD_4$ to afford (7*R*)-7-deuterioundecan-1-ol **34**.[22] Swern oxidation of **34** gave the corresponding aldehyde,[16] which was further oxidized to the acid **35** using oxone.[15] Coupling of acid **35** with pyrryl magnesium bromide and reduction of the resulting 2-acylpyrrole **36** yielded [7′-$^2$H](7′*R*)-2-undecylpyrrole **22**.[4] Using TBS-protected *S*-glycidol in place of **26**, [7′-$^2$H](7′*S*)-2-undecylpyrrole **23** was synthesized via an analogous sequence of transformations.

**Feeding of Deuterium-Labeled Pyrroles 22 and 23, and MBC 3, to *S. albus* Expressing redHG.** LC-MS analysis of mycelial extracts of *S. albus* expressing *redHG* that had been fed with [7′-$^2$H](7′*S*)-2-undecylpyrrole **23** and MBC **3** showed that 92% of the resulting streptorubin B **2** is deuterium labeled (Figure 4). In contrast, 77% of the deuterium label is lost in streptorubin B **2** resulting from the feeding of [7′-$^2$H](7′*R*)-2-undecylpyrrole **22** and MBC **3** to *S. albus* expressing *redHG* (Figure 4). These data show that the *pro-R* hydrogen atom is preferentially abstracted from C-7′ in the RedG-catalyzed conversion of undecylprodigiosin **1** to streptorubin B **2**. Comparison of chromatograms resulting from monitoring of absorbance at 533 nm (corresponding to the $\lambda_{max}$ for the protonated 4-methoxypyrrolyldipyrromethene chromophore of **1** and **2**) showed that the amount of streptorubin B **2** produced relative to undecylprodigiosin **1** was much lower in the experiment utilizing [7′-$^2$H](7′*R*)-2-undecylpyrrole **22** than in that using [7′-$^2$H](7′*S*)-2-undecylpyrrole **23** (see the Supporting Information). This is consistent with a primary kinetic isotope effect for the abstraction of the deuterium atom from [7′-$^2$H](7′*R*)-undecylprodigiosin, which may account for the discrepancy in the extent of deuterium retention in streptorubin B **2** when [7′-$^2$H](7′*S*)-2-undecylpyrrole **23** was used compared with the amount of deuterium loss from streptorubin B **2** when [7′-$^2$H](7′*R*)-2-undecylpyrrole **22** was employed.

**Mechanistic Implications.** The observed production of 5-hydroxypentylprodigiosin **17** when 2-(5-pentoxypentyl)-pyrrole **10** and MBC **3** are fed to *S. albus* expressing *redHG* is consistent with a RedG catalytic mechanism involving oxidation of C-7′ prior to pyrrole functionalization. Presumably, **17** arises via abstraction of a hydrogen atom at C-7′ of **10** by the Fe(III)OOH complex **6** to form a radical (or cation) that is


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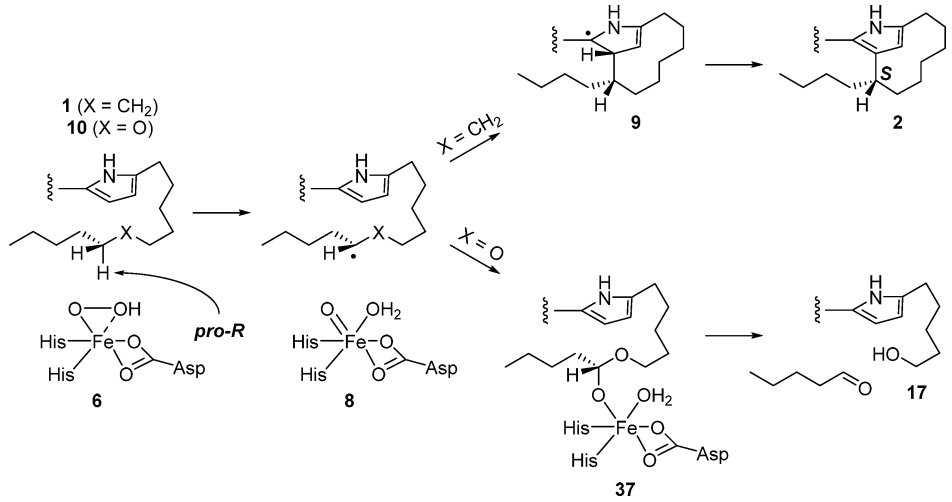


**Figure 5.** Mechanistic interpretation of the results from feeding of 2-(5-pentoxypentyl)-pyrrole **10** and stereoselectively deuterium labeled 2-undecylpyrroles **22** and **23**, along with MBC **3**, to *S. albus* expressing *redHG*. The C-7′ radical formed by abstraction of the *pro-R* hydrogen atom in undecylprodigiosin **1** by the RedG ferric hydroperoxide complex **6** undergoes inversion prior to adding to C-4 of the proximal pyrrole, resulting in *S*-configured streptorubin B **2**. The reduced reactivity of the radical formed via abstraction of a hydrogen atom from C-7′ of 2-(5-pentoxypentyl)-pyrrole **10** by ferric hydroperoxide complex **6** prevents it from adding to C-4 of the proximal pyrrole. Instead, the radical "rebounds" onto the ferryl complex **8** to form the hemiketal complex **38**, which undergoes protonation-triggered collapse to liberate pentanal and form 5-hydroxypentylprodigiosin **17**.

unable to add to C-4 of the proximal pyrrole, either because the reactivity of the radical (cation) toward the pyrrole is reduced by the neighboring oxygen lone pair, or because of repulsion between the lone pairs of the C-6′ oxygen atom and the pyrrole $\pi$-face (or both). The Fe(IV)O($OH_2$) complex **8** formed by hydrogen abstraction from **10** by **6** reacts with the C-7′ radical instead, forming the hemiketal complex **37**, which upon protonation can collapse with loss of *n*-pentanal to give 5-hydroxypentylprodigiosin **17** (Figure 5). Similar dealkylation reactions are known to be catalyzed by other members of the Rieske nonheme iron-dependent oxygenase family, such as Dicamba monoxygenase, which demethylates Dicamba (3, 6-dichloro-2-methoxybenzoate) via hydroxylation to form a hemiacetal that collapses with elimination of formaldehyde.[23]

Analysis of deuterium incorporation into streptorubin B **2** resulting from feeding of stereoselectively deuterium labeled 2-undecylpyrroles **22** and **23**, together with MBC **3**, to *S. albus* expressing *redHG* shows that RedG abstracts predominantly the *pro-R* hydrogen atom from C-7′ of undecylprodigiosin **1**. Together with the previous observation that streptorubin B **2** produced by *S. coelicolor* has predominantly the 7′*S* configuration,[5] this result shows that the RedG-catalyzed C−C bond forming reaction proceeds with inversion of configuration at C-7′ (Figure 5). In contrast, carbon-heteroatom bond formation in oxidative cyclizations catalyzed by other nonheme iron-dependent enzymes, such as isopenicillin N synthase and clavaminate synthase proceeds with retention of configuration at the carbon atom undergoing functionalization.[24,25] This stereochemical outcome is observed because the heteroatom participating in oxidative cyclization is ligated *cis* to the oxygen binding site of the nonheme iron center in these enzymes.[26,27]

The inversion of configuration at C-7′ in the RedG-catalyzed reaction suggests that the alkyl chain of the substrate may be juxtaposed between its 4-methoxypyrrolyldipyrromethene moiety and the nonheme iron center (Figure 5), preventing the former from coordinating to the latter. Such an arrangement uses the alkyl chain to "insulate" the nonheme iron center from 4-methoxypyrrolyldipyrromethene moiety, which could prevent its electron rich $\pi$-system from being directly oxidized by the Fe(III)OOH complex **6**. However, this arrangement would probably place the Fe(IV)O($OH_2$) complex **8** too far away from the hydrogen atom that needs to be removed from intermediate **9** to produce streptorubin B **2** for this to occur via direct abstraction. This hydrogen atom could be extracted directly if positioned near one of the oxygen atoms of the hydroperoxide ligand in the Fe(III)OOH complex **6**, but this would require a change in the conformation of the alkyl chain after abstraction of the *pro-R* hydrogen atom from C-7′ to permit C−C bond formation. Alternatively, the orientation of intermediate **9** in the active site could change to allow direct hydrogen abstraction by the Fe(IV)O($OH_2$) complex **8**, or indirect hydrogen abstraction from intermediate **9** by complex **8** could occur via an appropriately placed active site water molecule. It is even conceivable that an electron is transferred from intermediate **9** to complex **8**. The resulting C-5 cation intermediate could then be deprotonated by a basic residue within the active site, obviating the need for the nonheme iron center to be located near H-4 of the pyrrole. This C-5 cation intermediate could also be formed via hydride abstraction from C-7′ by the Fe(III)OOH complex **6** to form a transient alkyl cation that reacts with C-4 of the pyrrole. Alternatively, the transient alkyl cation could result from reaction of intermediate **7** with complex **8**, installing a hydroxyl group that undergoes subsequent protonation to promote its departure. Further experiments will be required to discriminate between these various possibilities.

## ■ CONCLUSIONS

The results reported herein not only illuminate the mechanism of the RedG-catalyzed oxidative carbocyclization of undecylprodigiosin **1** to streptorubin B **2**, but also provide a starting point for understanding and investigating the mechanism and stereochemistry of similar oxidative carbocyclization reactions catalyzed by RedG homologues in the biosynthesis of other prodiginine alkaloids. For example, McpG has been shown to


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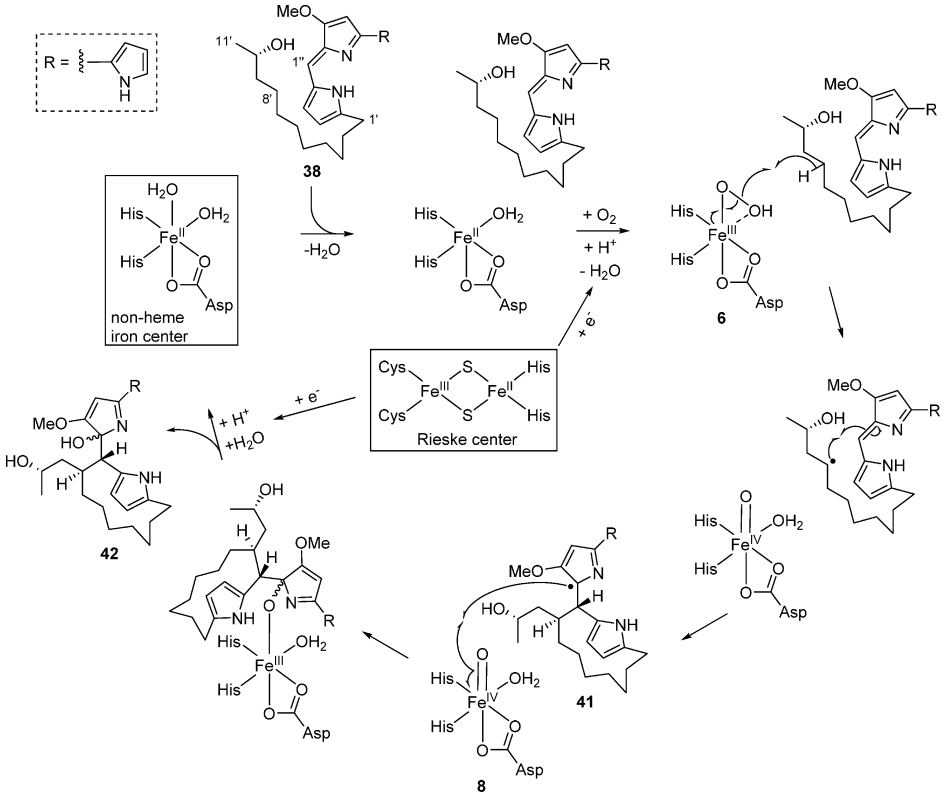


**Figure 6.** Proposed catalytic mechanism for MarG. The key difference from the RedG catalytic mechanism is that the intermediate **41** resulting from addition of the alkyl radical to the 4-methoxypyrrolyldipyrromethene $\pi$-system is hypothesized to react with the Fe(IV)O($OH_2$) complex **8** to produce the hydroxylated product **42**.

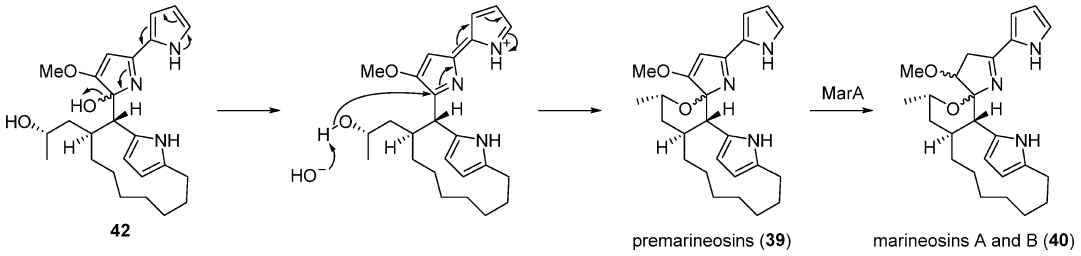


**Figure 7.** Proposed mechanism for formation of the premarineosins **39** from intermediate **42**. The premarineosins are reduced to form the marineosins **40** by MarA.

catalyze the conversion of undecylprodigiosin **1** to metacycloprodigiosin **5** with opposite absolute stereocontrol, resulting in the 9′*R* absolute configuration (Figure 1).[10] It seems reasonable to propose that this also proceeds with inversion of configuration, for the reasons discussed above, but involves abstraction of the *pro-S* hydrogen atom from C-9′. This hypothesis could be tested by synthesizing both enantiomers of 2-undecylpyrrole stereoselectively deuterium-labeled at C-9′ and analyzing whether the deuterium atom is retained when each is fed, along with MBC, to strains of *S. albus* expressing *mcpH* and *mcpG*.

Recently, Reynolds and co-workers reported that the RedG homologue MarG catalyzes the conversion of (10′*S*)-10′-hydroxyundecylprodigiosin **38** to the premarineosins **39** (Figures 6 and 7), which undergo MarA-catalyzed reduction to marineosins A and B **40** (Figure 7).[28,29] We hypothesize that the MarG catalytic cycle involves abstraction of a hydrogen atom from C-8′ of **38** by the Fe(III)OOH complex **6** (or an Fe(V)O(OH) complex) to yield a radical (or cation) that attacks C-1″. The resulting delocalized radical **41** can be quenched by "rebounding" onto the Fe(IV)O($OH_2$) complex **8** (rather than by hydrogen atom abstraction as in the RedG proposed catalytic mechanism) to give a product **42** that has undergone both oxidative carbocyclisation and hydroxylation (Figure 6). Elimination of water from **42**, followed by addition of the C-10′ hydroxyl group to the $\pi$-system would yield the premarineosins **39** (Figure 7). Addition of the C-10′ hydroxyl group to the alternative delocalized cation intermediate would yield the premarineosins **39** directly.

The gene cluster believed to direct the biosynthesis of deschlororoseophilin **43** and prodigiosin R1 **44** in *Streptomyces griseoviridis* contains four RedG homologues (RphG, RphG2, RphG3 and RphG4).[30] One of these (RphG3) is likely nonfunctional because it lacks the conserved Cys and His residues that ligate the [2Fe-2S] cluster in the N-terminal Rieske domain. Another presumably catalyzes C−C bond formation between C-4 and C-9′ in 11′-dimethyl-undecylprodigiosin **45** to form prodigiosin R1 **44** via a very similar process


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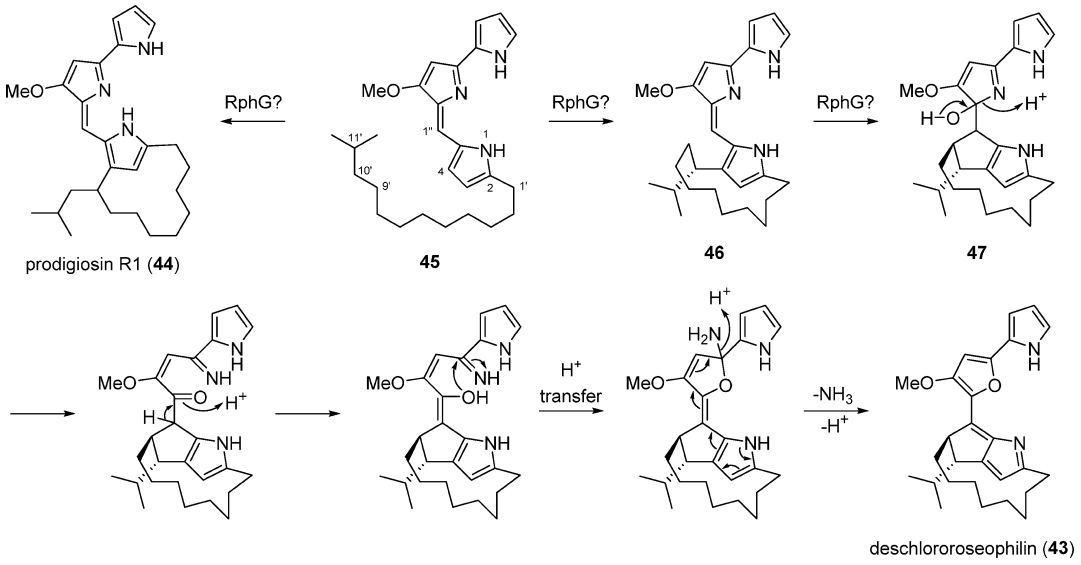


**Figure 8.** Proposed mechanism for the biosynthesis of deschlororoseophilin **43** and prodigiosin R1 **44** in *Streptomyces griseoviridis*.

to the McpG-catalyzed conversion of undecylprodigiosin **1** to metacycloprodigiosin **5** (Figure 8).[10] The remaining two RedG homologues are hypothesized to be responsible for the conversion of **45** to deschlororoseophilin **43** (Figure 8). One is proposed to catalyze C−C bond formation between C-10′ and C-4 in **45** to give **46** (Figure 8). The other is proposed to catalyze the conversion of **46** to **47** via an analogous process to the MarG-catalyzed conversion of (10′*S*)-10′-hydroxyundecylprodigiosin **38** to **42** (Figures 6 and 8). Rearrangement of intermediate **47** via ring opening, tautomerization, ring closure and elimination of ammonia would give deschloroseophilin **43** (Figure 8).

## ■ ASSOCIATED CONTENT

### Supporting Information

Experimental procedures and $^1$H NMR spectra of the Mosher's esters of alcohol **32** and its enantiomer. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/jacs.5b03994.

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### Notes

The authors declare no competing financial interest.

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