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Stereospecific Synthesis of 23-Hydroxyundecylprodiginines and Analogues and Conversion to Antimalarial Premarineosins via a Rieske Oxygenase Catalyzed Bicyclization

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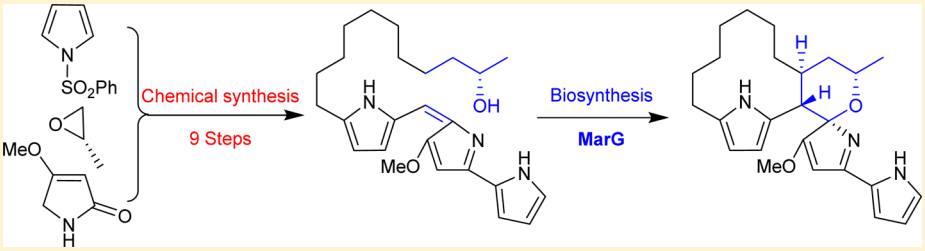
Supporting Information



ABSTRACT: Facile and highly efficient synthetic routes for the synthesis of (*S*)- and (*R*)-23-hydroxyundecylprodiginines ((23*S*)-**2**, and (23*R*)-**2**), 23-ketoundecylprodiginine (**3**), and deuterium-labeled 23-hydroxyundecylprodiginine ([23-*d*]-**2**) have been developed. We demonstrated a novel Rieske oxygenase MarG catalyzed stereoselective bicyclization of (23*S*)-**2** to premarineosin A (**4**), a key step in the tailoring process of the biosynthesis of marineosins, using a *marG* heterologous expression system. The synthesis of various A−C-ring functionalized prodiginines **32**−**41** was achieved to investigate the substrate promiscuity of MarG. The two analogues **32** and **33** exhibit antimalarial and cytotoxic activities stronger than those of the marineosin intermediate **2**, against *Plasmodium falciparum* strains (CQ^S-D6, CQ^R-Dd2, and 7G8) and hepatocellular HepG2 cancer cell line, respectively. Feeding of **34**−**36** to *Streptomyces venezuelae* expressing *marG* led to production of novel premarineosins, paving a way for the production of marineosin analogues via a combinatorial synthetic/biosynthetic approach. This study presents the first example of oxidative bicyclization mediated by a Rieske oxygenase.

INTRODUCTION

Prodiginines (**1**−**3**, Figure 1) are a family of red-pigmented natural products characterized by a 4-methoxy bipyrrole moiety linked to a variety of alkyl-substituted pyrroles.[1] These compounds have been extensively studied for their intriguing biological activities (antimicrobial,[2] immunosuppressive,[3] antitumor,[2a,b,4] anticancer,[5] and antimalarial[6,7]) and modes of action (transmembrane anion transport[5c−e,8] and DNA intercalation[9]). Some of these compounds have shown clinical potential, and the synthetic prodiginine analogue obatoclax-3 (GX15-070) has completed phase II clinical trials for the treatment of small cell lung cancer and is engaged in multiple clinical trials for the treatment of other cancer conditions.[10,11]

As a part of our ongoing interest in developing new antiparasitic agents, we recently reported the antimalarial activity of natural and synthetic prodiginines.[7] This work showed that the terminal nonalkylated pyrrole (ring A) and 3,5-dialkyl substitutions on the other terminal pyrrole (ring C) of natural prodiginines are crucial for potent antimalarial activity. In addition, we had demonstrated in mice that prodiginines can be administered orally with marked parasite clearance, including cures in some cases, without evident weight loss and toxicity. Recently we also have developed new methods for the synthesis of various 2,2′-bipyrrole-5-carboxaldehydes[12] and have subsequently generated a library of middle ring (ring B) functionalized prodiginines and tambjamines for their antimalarial activity and structure−activity relationship (SAR) studies.

In 2008, Fencial and co-workers isolated marineosin A (**6**) and B (**7**) (Figure 1), a new class of modified prodiginines with an unusual spiro-tetrahydropyran-aminal and pyrrole-macrocyclic rings from the marine *Streptomyces* sp. CNQ-617.[13] Marineosins were shown to have strong and selective anticancer activity, as well as antimalarial activity.[13,14] The intriguing structure and biological activities of marineosins has spurred efforts toward total synthesis attempts. In recent years, several research groups have attempted to accomplish the total synthesis but were successful only in the synthesis of key fragments.[15] To date, the total synthesis of marineosins is still incomplete.

Recently we have demonstrated the final steps of the marineosin biosynthetic pathway through identification and characterization of the corresponding *mar* gene cluster from

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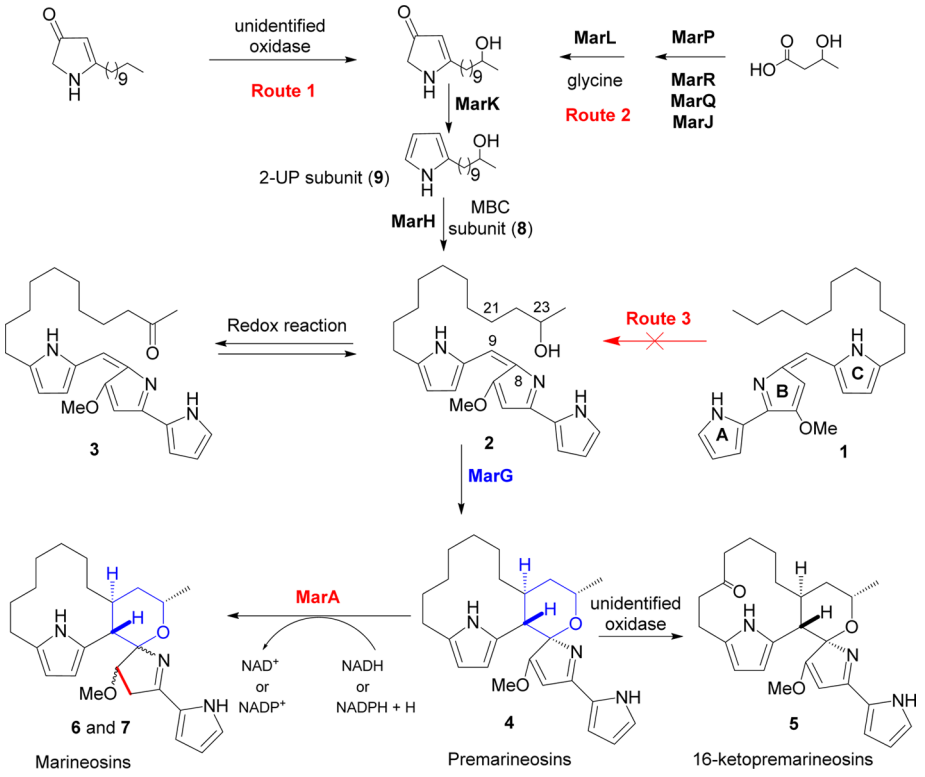

Figure 1. Marineosin biosynthetic pathway.

marine *Streptomyces* sp. CNQ-617 (Figure 1).[14] Expression of the entire gene cluster in a *S. venezuelae* host led to production of marineosins, whereas gene replacement of *marG*, which encodes a Rieske nonheme iron-dependent oxygenase, led to accumulation of 23-hydroxyundecylprodiginine (**2**) and 23-ketoundecylprodiginine (**3**). Replacement of *marA*, encoding a putative dehydrogenase, led to accumulation of premarineosin A (**4**) and 16-ketopremarineosin A (**5**) (Figure 1). These observations did not support either previous marineosin biosynthetic hypotheses, in which the pathway either passes through an enone analogue of undecylprodiginine[13] or involves a hydroxylation of undecylprodiginine by a RedG homologue (MarG).[15a] Rather, they suggested a pathway which culminates in a MarG-catalyzed cyclization of either **2** or **3** to form premarineosin A, which is subsequently reduced by MarA to generate marineosins (Figure 1).

In the well-established prodiginine biosynthetic pathway, it has been shown that RedH condenses an 2-undecylpyrrole and a 4-methoxy-2,2′-bipyrrolyl-5-carboxaldehyde (MBC, **8**) to generate undecylprodiginine (**1**), which is then cyclized by the Rieske oxygenase RedG to form streptorubin B (Figure 2).[16,17] An elegant study on the biosynthesis of metacycloprodiginine by the same group demonstrated an analogous oxidative cyclization catalyzed by another RedG homologue, McpG.[17] These two Rieske oxygenases, RedG and McpG, catalyze the regio- and stereoselective cyclization of the same substrate, undecylprodiginine (**1**), to form 10- and 12-membered macrocyclic rings, respectively.[17] More interestingly, the absolute stereochemistry of streptorubin B and metacycloprodiginine is varying at C-20 and C-22, respectively, showing that the oxidative cyclization is stereospecific (Figure 2).[17,18]

The product of the *marH* gene shares 80% similarity with RedH, while the novel Rieske oxygenase MarG shares 80% similarity with RedG and 70% similarity with McpG (Figure 2). It seems plausible that the putative marineosin biosynthetic intermediate (*S*)-23-hydroxyundecylprodiginine ((23*S*)-**2**) might be produced via a MarH condensation of **8** and (10′*S*)-hydroxyundecylpyrrole ((10′*S*)-**9**). Subsequently, the unique bicyclization, including C−C bond formation, and the intramolecular hydroalkoxylation are catalyzed by MarG to generate premarineosins (Figure 2).

The proposed final steps of marineosin biosynthesis provide an inspiration for a new approach for the elusive synthesis of marineosins. To that end, the proposed marineosin biosynthetic pathway intermediates **8** and (10′*S*)-**9** would be synthesized and then condensed to form (23*S*)-**2**. MarG would then provide enzymatic access to premarineosin A (**4**), and thus marineosins (**6** and **7**), after a final chemical reduction step. In this work we report the successful synthesis of (*S*)- and (*R*)-23-hydroxyundecylprodiginines ((23*S*)-**2** and (23*R*)-**2**), 23-ketoundecylprodiginine (**3**), and deuterium-labeled 23-hydroxyundecylprodiginine ([23-*d*]-**2**). In vivo feeding experiments using a *marG* heterologous expression system unequivocally demonstrated the role played by MarG to catalyze stereoselective bicyclization of (23*S*)-**2** to premarineosin A (**4**). In addition, we also report the elucidation of the substrate promiscuity of MarG using a series of chemically synthesized new analogues of **2**. Finally, we report on the potent antimalarial activity of two derivatives of **2**, 23-alkoxyundecylprodiginines **32** and **33**.

RESULTS AND DISCUSSION

Synthesis of (*S*)- and (*R*)-23-Hydroxyundecylprodiginines (23*S*)-2 and (23*R*)-2. Our initial efforts focused on the synthesis of the proposed biosynthetic intermediates **8**, (10′*S*)-**9**, and (10′*R*)-**9** from the key precursors **10**−**13** (Scheme 1). By use of literature methodologies, 4-methoxy-2,2′-bipyrrole-5-

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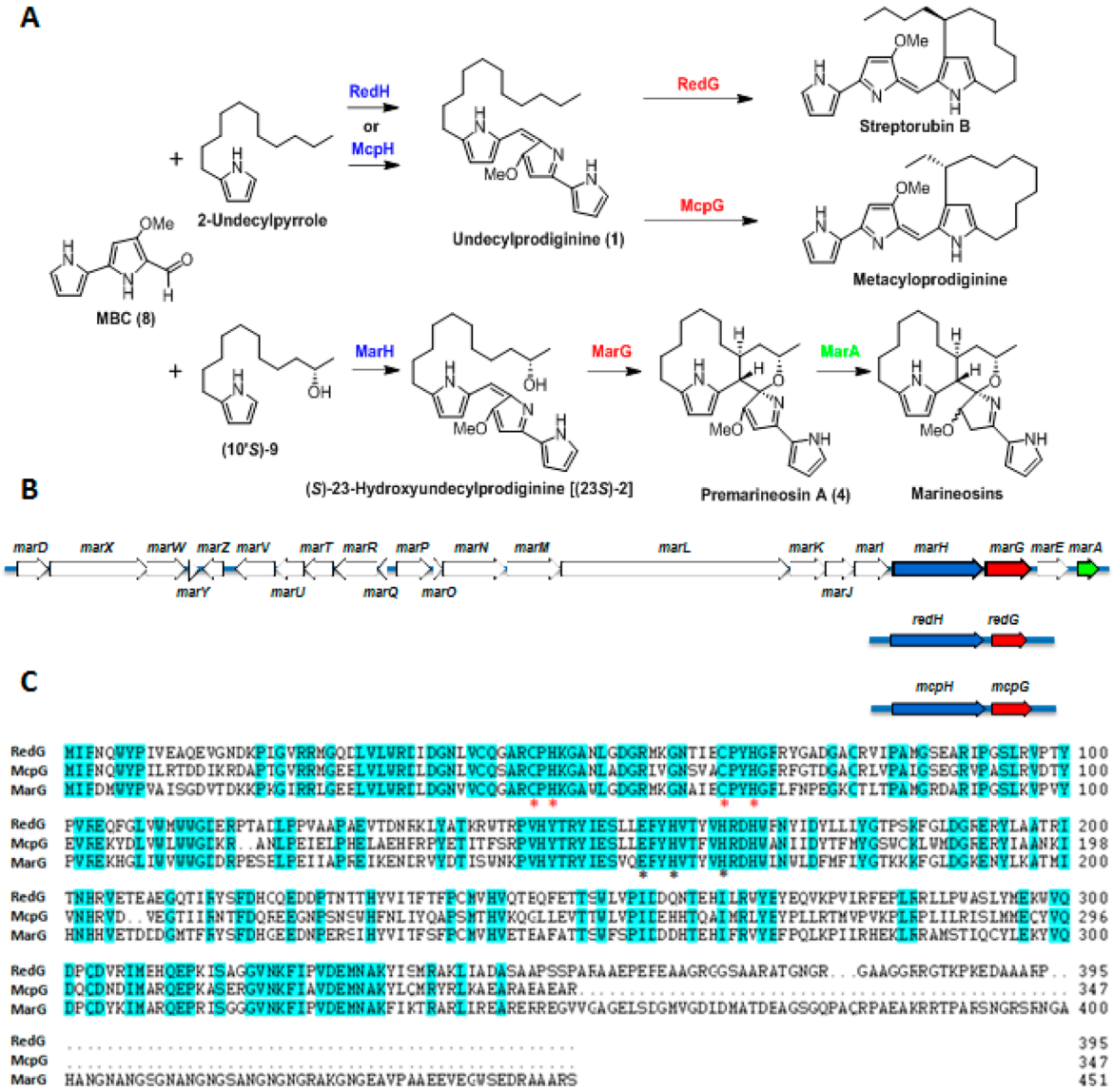

Figure 2. Roles of Rieske oxygenases RedG, McpG, and MarG, along with other tailoring enzymes, in the biosynthesis of the prodiginine natural products streptorubin B, metacycloprodiginine and marineosin: (A) late stages of the biosynthetic pathways leading to streptorubin B, metacycloprodiginine, and marineosins; (B) biosynthetic gene cluster of marineosins and the putative tailoring genes, with the Rieske oxygenase genes *redG*, *mcpG*, and *marG* highlighted in red, genes involved in undecylprodiginine analogue formation highlighted in blue, and the gene encoding premarineosin reductase highlighted in green; (C) multiple amino acid alignment of RedG, McpG, and MarG, with conserved sequences within CXH, CXXH motifs that ligate to the [2Fe-2S] cluster (Rieske center) marked with red asterisks and conserved sequences in the EXXHX$_4$H motif that binds to ferrous ion (nonheme iron center) marked with black asterisks.

carboxaldehyde (MBC, **8**) was prepared from the commercially available 4-methoxy-3-pyrrolin-2-one (**10**) in two steps.[19]

The synthesis of (10′*S*)-hydroxyundecylpyrrole ((10′*S*)-**9**) and (10′*R*)-hydroxyundecylpyrrole ((10′*R*)-**9**) is outlined in Schemes 2 and 3. 1-(Phenylsulfonyl)-pyrrole (**14**) was treated with dichloromethyl methyl ether to give 1-(phenylsulfonyl)-2-pyrrolecarboxaldehyde (**15**) in 82% yield,[20] which was further treated with 3-butenylmagnesium bromide in THF at 0 °C to give compound **16** in 85% yield. To avoid a two-step sequence (oxidation of alcohol group, and deoxygenation, and deprotection by NaBH$_4$/reflux)[15b,c] for the conversion of compound **16** to 2-(4-pentenyl)pyrrole (**11**), we successfully developed a one-pot cascade reaction by using LiAlH$_4$ in THF at 0 °C to reflux conditions (see the Experimental Section), and this proceeded with excellent yields (Scheme 2). Conversely, (*S*)-propylene oxide (**17**) was treated with 4-pentenylmagnesium bromide (**18**) in the presence of CuI to give the (*S*)-2-hydroxy-7-octene (**12**) in 92% yield.[21] Subsequently, a standard cross-metathesis reaction between **11** and **12** by Grubbs II catalyst and further hydrogenation of the olefin bond of **19** with Pd/C (10%) under H$_2$ gas at room temperature led to the desired compound (10′*S*)-**9** (Scheme 2). We employed a similar reaction sequence to furnish the anti isomer (10′*R*)-**9** from (*R*)-propylene oxide (**20**) via the appropriate intermediates (*R*)-2-hydroxy-7-octene (**13**), **11**, and **21** as outlined in Scheme 3.

With the proposed biosynthetic intermediates (10′*S*)-**9** and (10′*R*)-**9** in hand, we then sought and successfully synthesized the desired (*S*)-23-hydroxyundecylprodiginine ((23*S*)-**2**) and (*R*)-23-hydroxyundecylprodiginine ((23*R*)-**2**) using an acid-mediated (methanolic HCl) condensation with 4-methoxy-2,2′-bipyrrole-5-carboxaldehyde (**8**) in good yields as outlined in Scheme 4.[7] Compounds (23*S*)-**2** and (23*R*)-**2** were fully

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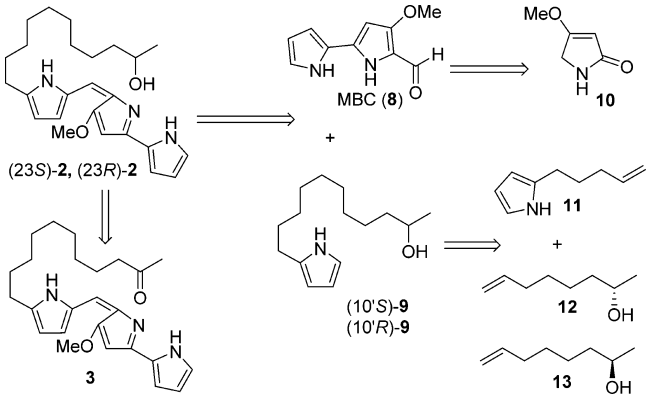
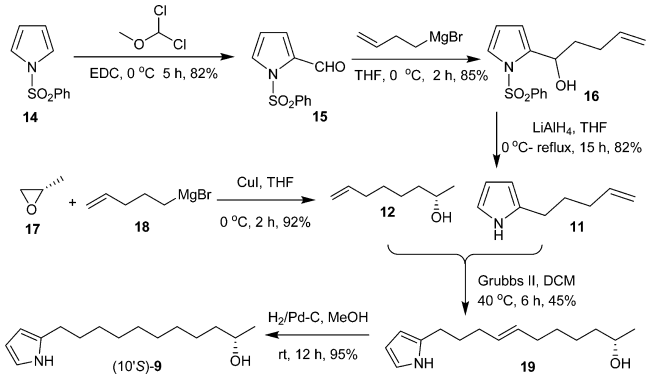
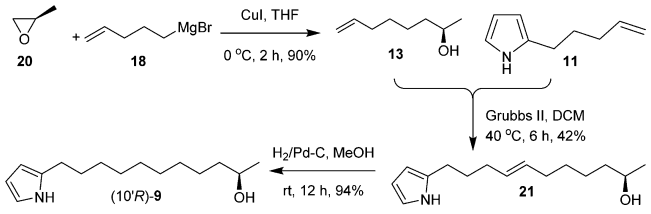
Scheme 1. Retrosynthesis of (*S*)- and (*R*)-23-(Hydroxy/keto)undecylprodiginines (23*S*)-2, (23*R*)-2, and 3



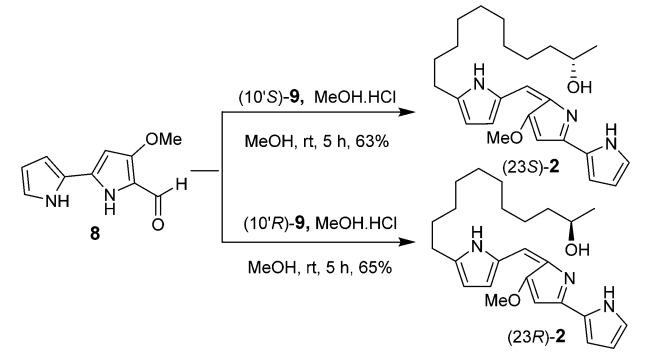
Scheme 2. Synthesis of (10′*S*)-Hydroxyundecylpyrrole ((10′*S*)-9)



Scheme 3. Synthesis of (10′*R*)-Hydroxyundecylpyrrole ((10′*R*)-9)



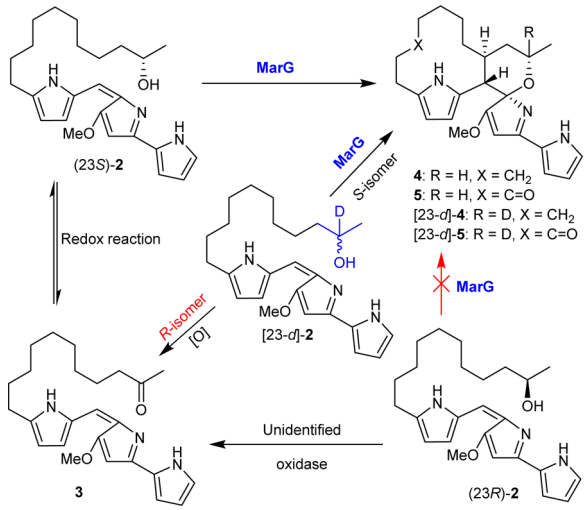
Scheme 4. Synthesis of (*S*)- and (*R*)-23-Hydroxyundecylprodiginines ((23*S*)-2 and (23*R*)-2)



characterized by NMR and MS analysis and compared with the TLC and HPLC profiles of natural product **2** (Figure S1, Supporting Information).

Stereochemical Analysis and Conversion of (23*S*)-2 and (23*R*)-2 to Premarineosin A (4) by a MarG-Catalyzed Bicyclization. Sequence analysis suggests that MarG is a Rieske nonheme iron-dependent oxygenase, containing the universally conserved N-terminal CXH and CXXH motifs that ligate to the [2Fe-2S] cluster in the Rieske center and conserved EXXHX$_4$H motif that binds to ferrous ion in the nonheme iron center. It was initially proposed that MarG is involved in the hydroxylation and subsequent spiroaminal ring formation of marineosin from an undecylprodiginine intermediate.[15a] Our previous work suggests that MarG is not responsible for hydroxylation but catalyzes spiroaminal formation from an isomer of **2** (Figure 1).[14] Taking into consideration the difficulties associated with in vitro protein reconstitution of this class of enzymes, we sought the overexpression of MarG in *S. venezuelae* ATCC 15439 (*S. venezuelae* MarG, see the Experimental Section and Tables S1 and S2 (Supporting Information)) in order to study its role in the biosynthesis of marineosin in vivo. Feeding of synthesized (23*S*)-**2** to *S. venezuelae* MarG resulted in the production of both premarineosin A (**4**) and 16-ketopremarineosin A (**5**, a presumptive shunt product) (Scheme 5). The production of **4**

Scheme 5. Conversion of (23*S*)-2, (23*R*)-2, 3, and [23-*d*]-2 to Premarineosins by MarG



and **5** was confirmed by LC-MS analysis using side by side comparison with standard premarineosins (Figure 3 and Figure S2 (Supporting Information)). Surprisingly, feeding of the opposite isomer, (23*R*)-**2**, to *S. venezuelae* MarG also provided both premarineosin A (**4**) and 16-ketopremarineosin A (**5**), as shown in Scheme 5 (Figure 3 and Figure S2). Unfortunately, the conversion of prodiginines to premarineosins was very poor, and the resulting products were not isolated. However, they were confirmed by LC-MS analysis using side by side comparison with standard premarineosins. The conversion of both isomers (23*S*)-**2** and (23*R*)-**2** into premarinesosin A, which has the *S* stereocenter at C-23, by the *S. venezuelae* MarG strain presented an enigma. The diastereomer of **4**, premarineosin B,[14] was not observed in detectable levels. As premarineosins A and B differ only in the stereochemistry at the spiroaminal carbon (C-8), it is most likely that premarineosin B arises from an inversion of the aminal nitrogen of **4**. The absence of detectable levels of premarineosin B indicates that premarineosin A (**4**) is more thermodynami-

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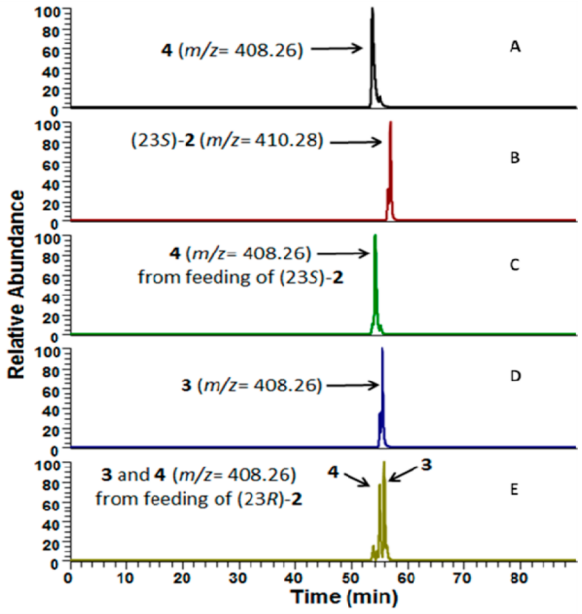
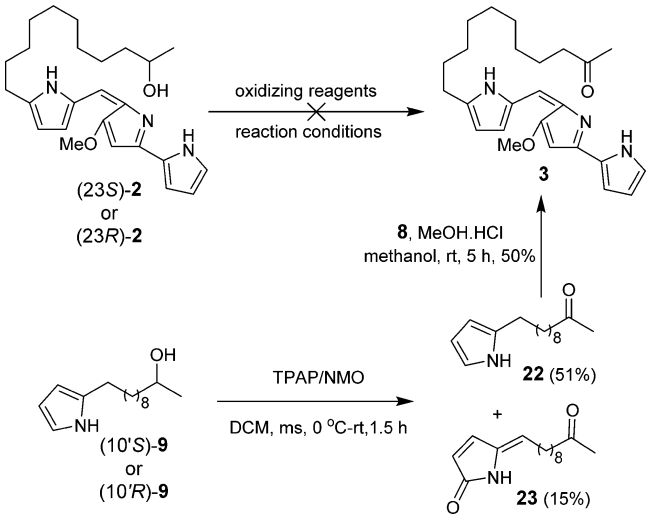


Figure 3. LC-MS (EIC) profiles of feeding (23*S*)-**2** and (23*R*)-**2** to *S. venezuelae* MarG: (A) EIC for *m*/*z* 408.26−408.27, corresponding to $[M + H]^+$ for **4**, from an LC-MS analysis of standard **4**; (B) EIC for *m*/*z* 410.28−410.29, corresponding to $[M + H]^+$ for (23*S*)-**2**, from an LC-MS analysis of synthesized (23*S*)-**2**; (C) EIC for *m*/*z* 408.26−408.27, corresponding to $[M + H]^+$ for **4**, from an LC-MS analysis of extracts of *S. venezuelae* MarG fed with (23*S*)-**2**; (D) EIC for *m*/*z* 408.26−408.27, corresponding to $[M + H]^+$ for **3**, from an LC-MS analysis of synthesized **3**; (E) EIC for *m*/*z* 408.26−408.27, corresponding to $[M + H]^+$ for **4** and $[M + H]^+$ for **3**, from an LC-MS analysis of extracts of *S. venezuelae* MarG fed with (23*R*)-**2**.

cally stable under pathway conditions. It is noteworthy that feeding (23*R*)-**2** to *S. venezuelae* MarG resulted in the production of 23-ketoundecylprodiginine (**3**) as detected by LC-MS analysis (Figure 3). Intriguingly, **3** was not observed when the experiment was carried out with (23*S*)-**2** (Figure 3). The production of **3** suggests that (23*R*)-**2** is initially converted into **3**, and the latter may also be participating in the MarG-catalyzed cyclization to provide premarineosins. To test this hypothesis and determine the actual intermediate for MarG, we sought the synthesis of **3**.

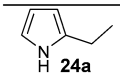
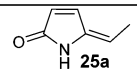
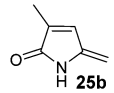
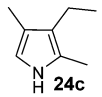
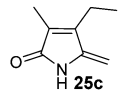
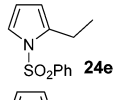
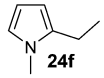
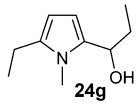
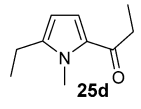
Synthesis of 23-Ketoundecylprodiginine (3) and Subsequent Conversion to Premarineosins. We anticipated that the prodiginine **3** could be obtained directly from (23*S*)-**2** and/or (23*R*)-**2** by an oxidation of the hydroxyl group at C-23, as shown in Scheme 6. Unfortunately, we were unable to convert compound (23*S*)-**2** and/or (23*R*)-**2** to the desired product **3** under a variety of oxidation reagents/conditions, which resulted in either formation of several products or extensive decomposition. We therefore took an alternative route that exclusively leads to the final product **3** (Scheme 6). To achieve this, we took either the (10′*S*)-**9** and/or (10′*R*)-**9** as a starting material and subjected them to several oxidizing agents. A Ley−Griffith oxidation provided the desired 10′-ketoundecylpyrrole (**22**) in 51% isolated yield (Scheme 6). It is noteworthy that the Ley−Griffith oxidation of (10′*S*)-**9** and/or (10′*R*)-**9** also provided the 1,5-dihydropyrrol-2-one **23** as a side product in considerable amount (15% isolated yield). The individual compound **23** was fully characterized by extensive NMR and MS analysis (Supporting Information). This kind of controlled oxidation of N-protected pyrroles has been

Scheme 6. Synthesis of 23-Ketoundecylprodiginine (3)



previously reported by using Dess−Martin periodinane,[22] but to our knowledge, Ley−Griffith oxidation conditions (TPAP/NMO) have never been exploited to unprotected pyrroles. To demonstrate the generality of this controlled oxidation, the reaction was carried out under the same reaction conditions on 2-ethylpyrrole (**24a**), 2,4-dimethylpyrrole (**24b**), and 3-ethyl-2,4-dimethylpyrrole (**24c**) and successfully led to the desired 5-alkylene-1,5-dihydropyrrol-2-ones **25a−c** (Table 1). Conversely, the same reaction conditions with N-protected 2-ethylpyrroles **24d−f** failed to provide the corresponding oxidative products in detectable levels. The *N*-methylpyrrole **24g** was completely converted into the corresponding oxidized product **25d** in excellent yield (Table 1).

Table 1. Synthesis of 5-Alkylene-1,5-dihydropyrrol-2-ones (25a−c) and 25d

Reactant	Product	Yield
 **24a**	 **25a**	32%
 **24b**	 **25b**	35%
 **24c**	 **25c**	39%
 **24d**	nr[a]	-
 **24e**	nr	-
 **24f**	nr	-
 **24g**	 **25d**	89%

[a]nr = no reaction.

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These 5-alkylene-1,5-dihydropyrrol-2-ones **23** and **25a−c** are highly functionalized and therefore are excellent building blocks for the synthesis of natural and synthetic products of biological importance: for example, pulchellalactam is a potent CD45 protein tyrosine phosphatase (PTP) inhibitor, which was isolated from the marine fungus *Corollospora pulchella*.[23] Finally, the acid-catalyzed condensation of the key intermediate **22** with **8** provided the desired 23-ketoundecylprodiginine (**3**) in 50% isolated yield (Scheme 6), and it was fully characterized by NMR and MS analysis and compared with the TLC and HPLC profiles of natural product **3** (Figure S1, Supporting Information).

Feeding of 23-ketoundecylprodiginine (**3**) to the *S. venezuelae* MarG expression strain clearly provided both premarineosin A (**4**) and 16-ketopremarineosin A (**5**), as shown in Scheme 5 (Figure 4 and Figures S2 and S3

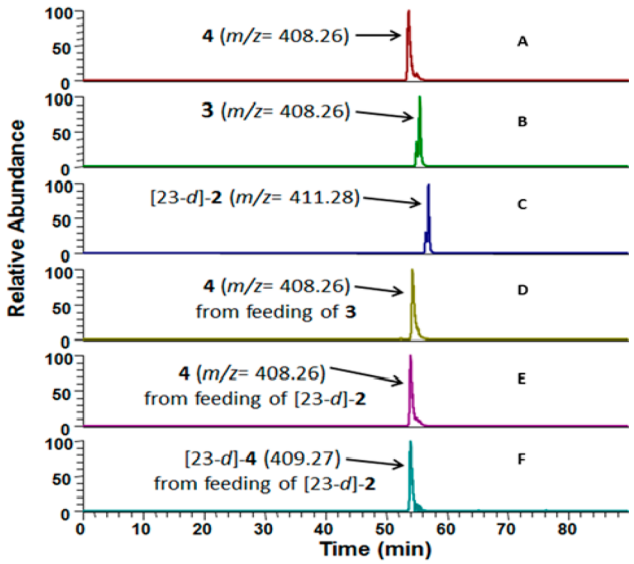

Figure 4. LC-MS (EIC) profiles of **4** and [23-*d*]-**4** from feeding of **3** and [23-*d*]-**2** to *S. venezuelae* MarG: (A) EIC for *m*/*z* 408.26−408.27, corresponding to $[M + H]^+$ for **4**, from an LC-MS analysis of standard **4**; (B) EIC for *m*/*z* 408.26−408.27, corresponding to $[M + H]^+$ for **3**, from an LC-MS analysis of synthesized **3**; (C) EIC for *m*/*z* 411.28−411.29, corresponding to $[M + H]^+$ for [23-*d*]-**2**, from an LC-MS analysis of synthesized [23-*d*]-**2**; (D) EIC for *m*/*z* 408.26−408.27, corresponding to $[M + H]^+$ for **4**, from an LC-MS analysis of extracts of *S. venezuelae* MarG fed with **3**; (E) EIC for *m*/*z* 408.26−408.27, corresponding to $[M + H]^+$ for **4**, from an LC-MS analysis of extracts of *S. venezuelae* MarG fed with [23-*d*]-**2**; (F) EIC for *m*/*z* 409.27−409.28, corresponding to $[M + H]^+$ for [23-*d*]-**4**, from LC-MS analysis of extracts of *S. venezuelae* MarG fed with [23-*d*]-**2**.

(Supporting Information)). The conversion of hydroxy- and ketoprodiginines (23*S*)-**2**, (23*R*)-**2**, and **3** to premarinesosin A by MarG expression strain did not resolve the question of whether the keto or hydroxyl derivative is the correct intermediate. We hypothesized that hydroxy- and ketoprodiginines are most likely interconverting via a redox reaction in the MarG expression host, *S. venezuelae* (Figure 1 and Scheme 5). Therefore, we sought the synthesis of [23-*d*]-23-hydroxyundecylprodiginine ([23-*d*]-**2**) to further study the intermediates involved in marineosin biosynthesis.

Synthesis of [23-*d*]-23-Hydroxyundecylprodiginine ([23-*d*]-2) and Subsequent Conversion into Deuterium-Labeled Premarineosin A ([23-*d*]-4) via MarG-Catalyzed Cyclization. Adopting an approach developed by Li and Herzon in our syntheses,[24] 10-undecynoic acid (**26**) was treated with Au(IPr)Cl (0.02 equiv), AgO_2CCF_3 (0.02 equiv), and Shvo's catalyst (0.02 equiv) in a mixture of water and isopropyl alcohol (14% v/v), to give the desired 10-hydroxyundecanoic acid (**27**) in 82% yield (Scheme 7). 10-Oxoundecanoic acid (**28**) was synthesized by oxidation of the hydroxyl group of **27** using Dess−Martin periodinane conditions, which upon treatment with sodium borodeuteride ($NaBD_4$) gave the [10-*d*]-10-hydroxyundecanoic acid ([10-*d*]-**27**). Attempts to convert the acid group of [10-*d*]-**27** to acid chloride using either oxalyl chloride $(COCl)_2$ or thionyl chloride ($SOCl_2$) in dichloromethane/toluene, with a subsequent Friedel−Crafts acylation on pyrrole in the presence of Zn/toluene[25] led to the formation of several products. Therefore, the hydroxyl group at C-10 needed to be protected. The efficiency of this reaction sequence (formation of acid chloride and Friedel−Crafts acylation of pyrrole) toward the 2-acylpyrrole [10′-*d*]-**30** was improved by the protection of the hydroxyl group (C10−OH) of [10-*d*]-**27** with Ac_2O/pyridine. The reaction of [10-*d*]-10-acetoxyundecanoic acid ([10-*d*]-**29**) with $SOCl_2$/DMF (catalytic)/toluene with a subsequent acylation of pyrrole in the presence of Zn/toluene provided the desired 2-acylpyrrole [10′-*d*]-**30** as a major product along with the 3-acylpyrrole [10′-*d*]-**31** as a minor product. This ratio of products is in contrast with the standard regioselective acylation method, in which 2-acylpyrrole is the single product from pyrrole and acid chloride.[25] The two isomers [10′-*d*]-**30** (57%) and [10′-*d*]-**31** (8%) were isolated by silica gel column chromatography and characterized by NMR and MS analysis. Then the 2-acylpyrrole [10′-*d*]-**30** was smoothly converted to [10′-*d*]-**9** in 67% isolated yield by using an excess (12 equiv) of $NaBH_4$ in isopropyl alcohol (IPA) under reflux (Scheme 7).[7,26] The acid-catalyzed condensation of [10′-*d*]-**9** with **8** provided the desired racemic [23-*d*]-23-hydroxyundecylprodiginine ([23-*d*]-**2**) in 70% isolated yield (Scheme 7).

Feeding of ∼90% deuterium-labeled racemic compound [23-*d*]-**2** to the *S. venezuelae* MarG expression host led to production of [23-*d*]-**4** and **4** in ∼1.2:1 ratio, along with the shunt products [23-*d*]-**5** and **5**, as shown in Scheme 5 (Figure 4 and Figures S2−S4 (Supporting Information)). Production of deuterium-labeled premarineosin A ([23-*d*]-**4**) is consistent with a pathway that directly proceeds from the isomer [23*S*-23-*d*]-**2** without oxidation to **3** (Scheme 5). The detection of ∼45% unlabeled product premarineosin A (**4**) via MS analysis (Figure S4) from ∼90% labeled [23-*d*]-**2** is consistent with a pathway in which the hydroxyl group at C-23 of [23*R*-23-*d*]-**2** is oxidized to **3** (as observed with (23*R*)-**2** feeding experiment), the deuterium label is lost, and then the compound is reduced to the unlabeled (23*S*)-**2**, which is then converted by MarG to premarineosin A (**4**) (Scheme 5). Our findings are thus all consistent with a role of MarG in catalyzing formation of premarineosins exclusively from (*S*)-23-hydroxyundecylprodiginine ((23*S*)-**2**).

Synthesis of Hydroxyundecylprodiginine Analogues To Probe MarG Substrate Promiscuity. Having determined the intermediates involved in the biosynthesis of marineosin and the role of MarG, we wanted to study the potential of MarG-generated marineosin analogues. Using the general approach for synthesis of (23*S*)-**2**, (23*R*)-**2**, **3**, and [23-*d*]-**2** and various 2,2′-bipyrrole-5-carboxaldehydes (**52a−d**),[7,12] we synthesized various A- and B-ring functionalized (hydroxy/alkoxy)undecylprodiginines **2** and **32−41**, as shown in Schemes

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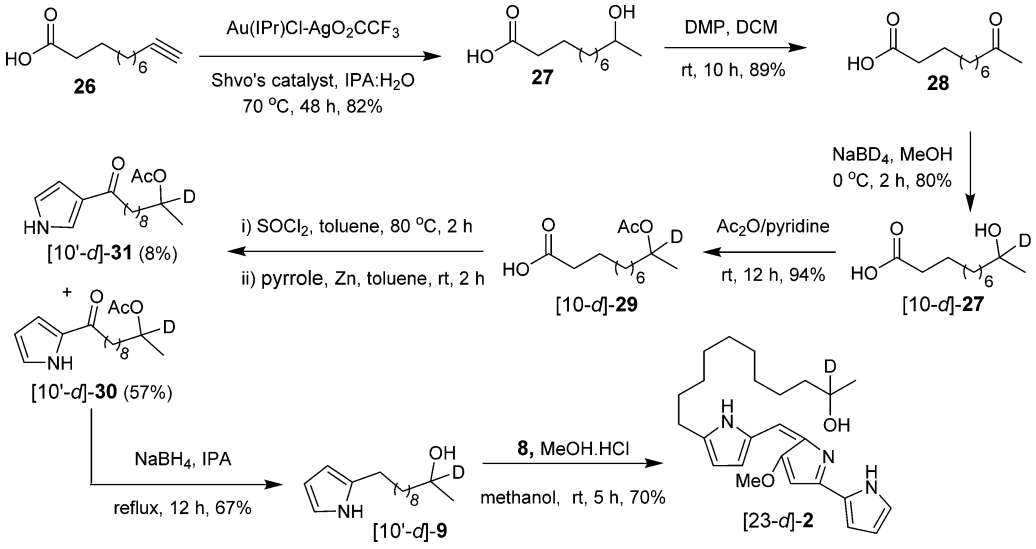
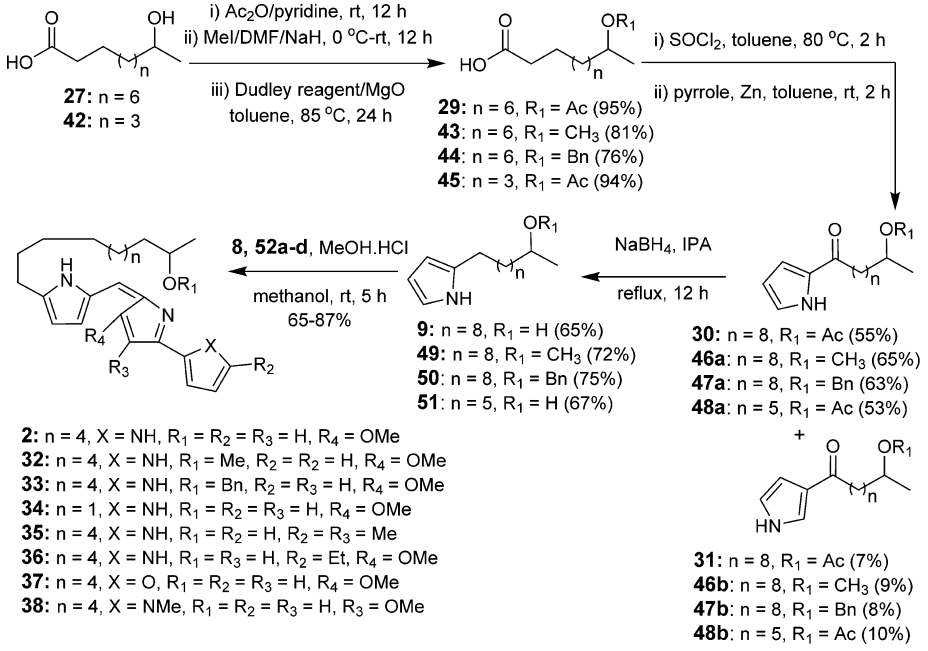
Scheme 7. Synthesis of Racemic [23-*d*]-23-Hydroxyundecylprodiginine ([23-*d*]-2)



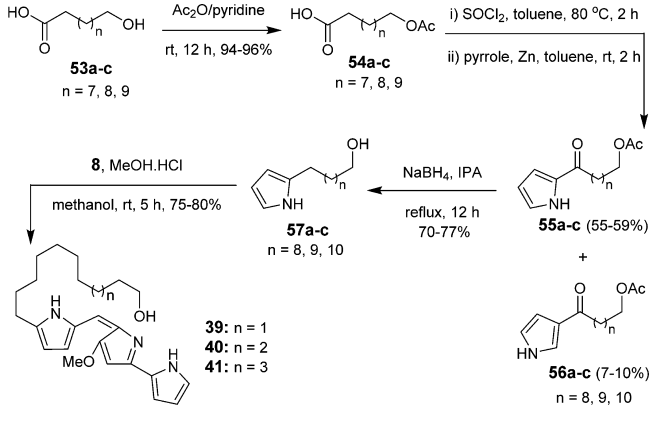
Scheme 8. Synthesis of A- and B-Ring Functionalized (Hydroxy/alkoxy)undecylprodiginines 2 and 32−38



8 and 9. These compounds and undecylprodiginine (**1**) were fed to the MarG expression strain.

Neither premarineosin A (**4**) nor C9−C21 cyclized prodiginine was observed at a detectable level after feeding undecylprodiginine (**1**), which has no hydroxyl group at C-23 of the alkyl chain (Figure 1). This observation clearly indicates that the hydroxyl group of **2** is required for the MarG-catalyzed process and must be introduced early in the pathway. One option is the hydroxylation of the 2-UP subunit by an unidentified oxidase (route 1, Figure 1) or recruitment of 2-hydroxybutyric acid starter unit by MarP (route 2, Figure 1). The gene product(s) required for introduction of the hydroxyl group remains unknown. We also did not observe any C9−C21 cyclized prodiginine related products after feeding of 23-alkoxyundecylprodiginines **32** and **33** (Scheme 8), further demonstrating that the hydroxyl group at C-23 is required for the formation of the spiro-tetrahydropyran-aminal ring and that

Scheme 9. Synthesis of Hydroxyundecylprodiginines 39−41 Containing a Terminal Hydroxyl Group at the Alkyl Chain



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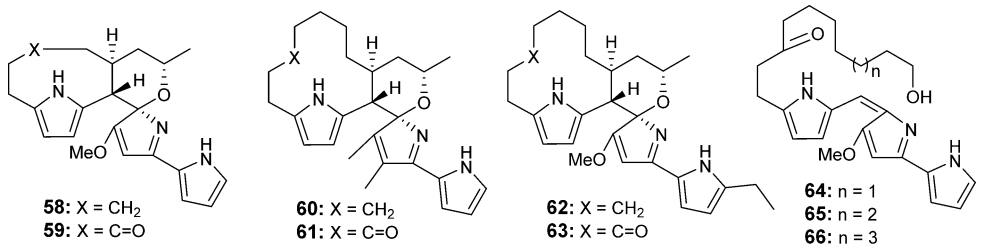

Figure 5. Proposed structures of expected products from feeding of **34**−**36** and **39**−**41** to *S. venezuelae* MarG.

hydroxyundecylprodiginines are the preferred substrates for MarG. Prodiginines **34**−**36** with shorter alkyl chain length (octyl) on ring C and *C*-alkyl substitutions on rings A and B, containing the key secondary alcohol substituent, were synthesized (Scheme 8) and fed to *S. venezuelae* MarG mutant. LC-MS analyses clearly demonstrated the production of both the corresponding premarineosin A and 16-ketopremarineosin analogues **58**−**63**. The compounds were not isolated and characterized due to poor conversion of hydroxyundecylprodiginines to premarineosins; however, their corresponding chemical structures were proposed on the basis of the above feeding results of *S. venezuelae* MarG mutant, MS/MS, and LC-MS analyses (Figure 5 and Figures S5−S8 (Supporting Information)). Conversely, cyclic products of prodiginines with *N*-methylpyrrole and furan in the place of terminal pyrrole (ring A) (**37** and **38**, Scheme 8), were not detected via LC-MS after feeding their respective substrates to the *S. venezuelae* MarG expression strain. Interestingly, prodiginines **39**−**41** (Scheme 9), with a terminal hydroxyl group at the alkyl chain (primary alcohols), were converted to their corresponding 16-ketoprodiginines **64**−**66** (Figure 5), but their corresponding cyclized products were not detected via LC-MS. These products **64**−**66** also were not isolated and characterized, but their chemical structures were proposed on the basis of the above feeding results of *S. venezuelae* MarG mutant and MS analysis (Figure S9 (Supporting Information)). The above data demonstrate that MarG catalyzed bicyclization requires the presence of a free secondary hydroxyl group on an alkyl chain on ring C but can tolerate *C*-alkyl substitutions on rings A and B (notably replacement of the methoxy group). Substitution of terminal pyrrole (ring A) with other heterocycles does not lead to cyclized products.

Antimalarial Activity and Cytotoxicity of Prodiginines. All synthesized natural prodiginines **1**−**3**, and their analogues **32**−**41**, were evaluated for their antimalarial activity against the chloroquine-sensitive (CQ^S) D6 and the chloroquine-resistant (CQ^R) Dd2 and 7G8 strains of *Plasmodium falciparum* with chloroquine (CQ) as a reference drug,[27] and the results are shown in Table 2. In parallel, the cytotoxicity of all tested compounds was also tested against hepatocellular HepG2 cancer cell line using mefloquine (MQ) as a control drug.[27d] The synthesized prodiginines **2**, (23*R*)-**2**, (23*S*)-**2**, and **3**, containing an extra hydroxyl and/or keto group at C-23 of the alkyl chain, have decreased antimalarial activity and selectivity in comparison to undecylprodiginine (**1**), which has no substitutions on the alkyl chain (Table 2 and Figure 1). It is noteworthy that the isomer (23*S*)-**2** is only slightly more potent than the isomer (23*R*)-**2** and racemic compound **2** (Table 2), which demonstrates that the stereochemistry at C-23 does not alter the antimalarial activity. More significantly, 23-alkoxy analogues **32** and **33** have much improved antimalarial activity and selectivity in comparison to **2** and **3**. Indeed, **32** and **33** have antimalarial activity comparable to that of **1**, with slightly reduced toxicity (as measured by activity against HepG2 cancer cells), and thus offer increased selectivity. In contrast, retention of the free hydroxyl group on the alkyl chain, with a reduction in the alkyl chain length, led to a 3-fold decrease in the antimalarial potency (**34** versus **2**). Similarly, a loss of antimalarial activity was observed with replacement of the OMe group of ring B by methyl groups (**35** versus **2**), demonstrating the importance of the methoxy group on ring B for potency. Surprisingly, prodiginine **36**, which contains an extra alkyl residue (ethyl) on ring A, showed a substantially higher potency with an IC_{50} of <24 nM against all strains of *P. falciparum* (**36** versus **2**), and it had reduced selectivity. Replacement of the terminal nonalkylated pyrrole ring (ring A) of the core moiety by *N*-methylpyrrole and furan moieties (compounds **37** and **38**) resulted in a large decrease in antimalarial activity (**37** and **38** versus **2**). These results, indicating that the pyrrole NH of the prodiginines is required for potent antimalarial activity, support our previous findings.[7] Our previous studies also demonstrated that undecylprodiginine, with a terminal amine group at the alkyl chain, showed poor antimalarial activity (IC_{50} = 1700 nM).[7] However, prodiginines **39**−**41**, with a terminal hydroxyl group at the alkyl chain (primary alcohols), exhibited better activity (IC_{50} < 110 nM). Notably, in these cases, longer alkyl chains led to an

Table 2. Antimalarial Activity and Cytotoxicity of Prodiginines

	antimalarial activity (IC_{50} in nM)[a]				
compd	D6	Dd2	7G8	cytotoxicity (IC_{50} in nM)[a] HepG2	SI[b] (D6)
1	7.2	7.5	7.0	1713	238
2	110	98	118	7358	67
(23*R*)-**2**	92	51	114	7961	86
(23*S*)-**2**	66	46	100	9152	139
3	191	121	199	14667	77
32	7.1	10.2	11.3	2682	378
33	5.7	3.7	2.3	2770	486
34	383	293	368	12050	31
35	1084	1188	627	9385	9
36	17	15	24	1757	103
37	>2500	>2500	>2500	>100000	>40
38	2328	>2500	1001	>100000	>43
39	108	80	168	11416	106
40	59	36	93	11363	193
41	38	23	58	6184	163
CQ	10	102	63	ND[c]	ND
MQ	ND	ND	ND	10970	ND

[a]IC_{50} values are represented as averages of triplicate measurements (SD ± 10%). [b]SI (selectivity index) = IC_{50} (cytotoxicity)/IC_{50} (D6). [c]ND: not determined.

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increase in the antimalarial activity (IC_{50} of **39** (C10) > **40** (C11) > **41** (C12)). These results thus demonstrate that the presence of hydroxyl and/or keto substituents on the alkyl chain, replacement of methoxy group of ring B by alkyl substituents, and replacement of the terminal nonalkylated pyrrole ring (ring A) by *N*-methylpyrrole and/or other heterocycles have an adverse effect on the antimalarial activities. Conversely, the hydroxyl group of **2** masked with methyl and benzyl groups, as in **32** and **33**, respectively, dramatically increased the potency and selectivity (Table 2).

CONCLUSIONS

In summary, we have accomplished the first synthesis of (*S*)-23-hydroxyundecylprodiginine ((23*S*)-**2**), (*R*)-23-hydroxyundecylprodiginine ((23*R*)-**2**)), 23-ketoundecylprodiginine (**3**), [23-*d*]-23-hydroxyundecylprodiginine ([23-*d*]-**2**), and their synthetic analogues **32**−**41** from commercially available starting materials in straightforward approaches. These feasible synthetic routes can be carried out on a large scale and are suited for the generation of a library of novel prodiginines for their advanced biological activities and SAR studies. Formation of the critical unusual spiro-tetrahydropyran-aminal ring of marineosins and conversion of (23*S*)-**2** to premarineosin A (**4**) were accomplished by MarG expressed in *S. venezuelae*. Conversion of [23-*d*]-**2** to the deuterium-labeled premarineosin A ([23-*d*]-**4**) unambiguously demonstrates that the pathway directly proceeds stereospecifically from (23*S*)-**2**. The synthesis of 23-alkoxyundecylprodiginines (**32** and **33**), 20-hydroxyoctylprodiginine (**34**), and A- and B-ring functionalized prodiginines (**35**−**41**) was used to investigate the substrate promiscuity of MarG. As such, this one-step enzymatic (C−C bond formation and intramolecular hydroalkoxylation) work represents a complementary approach to the widespread synthetic approaches directed toward this class of complex natural products. Investigations to improve the conversion of prodiginines to premarineosins by MarG and subsequent isolation and chemical reduction step to the final marineosins and their analogues are underway in our laboratory. Significantly, alkoxy analogues **32** and **33** exhibited potent antimalarial activity with better selectivity than natural prodiginines **1**−**3**. In the course of this work, we also have discovered a simple and convenient method for the synthesis of 5-alkylene-1,5-dihydropyrrol-2-ones from *C*-alkyl-substituted pyrroles by using TPAP/NMO. These pyrrol-2-ones are excellent building blocks for the synthesis of natural and synthetic products of biological importance, and further investigations to improve the yields and expand the substrate scope of the oxidation with this reagent as well as mechanistic studies are underway in our laboratory.

EXPERIMENTAL SECTION

General Considerations. NMR spectra were recorded on a spectrometer at 400 MHz (^{1}H) and 100 MHz (^{13}C). Experiments were recorded in $CDCl_3$ and acetone-d_6 at 25 °C. Chemical shifts are given in parts per million (ppm) downfield from internal standard Me_4Si (TMS). HRMS (ESI) were recorded on a high-resolution (30000) LTQ-Orbitrap mass spectrometer. Unless otherwise stated, all reagents and solvents were purchased from commercial suppliers and used without further purification. Reactions which required the use of anhydrous, inert atmosphere techniques were carried out under an atmosphere of argon/nitrogen. Chromatography was executed using silica gel (230−400 mesh) and/or neutral alumina as the stationary phase and mixtures of ethyl acetate and hexane as eluents. Analytical HPLC analyses were performed using a C18 column (4.6 × 150 mm) with a linear elution gradient ranging from $CH_3OH/CH_3CN/H_2O$ (40%/10%/50%) to CH_3OH (100%) acidified with 0.1% trifluoroacetic acid at a flow rate of 0.3 mL/min.

Synthesis of 1-(1-(Phenylsulfonyl)-pyrrol-2-yl)pent-4-en-1-ol (16). To a stirred solution of **15** (4.0 g, 17.02 mmol) in 200 mL of THF was added 3-butenylmagnesium bromide solution (0.5 M in THF) (68 mL, 34.04 mmol) at 0 °C, and the reaction mixture was stirred at 0 °C for 2 h. Then the reaction was quenched by addition of 2 N HCl, with the temperature maintained at <10 °C, and extracted with diethyl ether (3 × 100 mL). The combined organic extracts were washed with water and brine and dried over anhydrous Na_2SO_4. The solvent was evaporated under reduced pressure, and the crude product was chromatographed on silica gel to afford the title compound **16** (4.21 g, 85%) as a syrup: R_f = 0.45 (20% EtOAc/hexanes); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.78 (d, J = 8.1 Hz, 2H), 7.59 (m, 1H), 7.50 (m, 2H), 7.31 (dd, J = 1.7, 3.3 Hz, 1H), 6.29 (m, 2H), 5.72 (m, 1H), 4.93 (m, 2H), 4.85 (m, 1H), 2.92 (br s, 1H), 2.12 (m, 2H), 1.90 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 139.1, 138.1, 137.8, 134.0, 129.5 (2C), 126.6 (2C), 123.5, 115.1, 112.5, 111.8, 64.5, 34.2, 30.1; HRMS (ESI) calcd for $C_{15}H_{17}NaNO_3S$ $(M + Na)^+$ 314.0821, found 314.0815; IR (KBr) ν_{max} 3365, 2956, 2846, 1678, 1425 cm^{-1}.

Synthesis of 2-(4-Pentenyl)pyrrole (11). To a stirred suspension of $LiAlH_4$ (2.7 g, 72.16 mmol) in dry THF (200 mL) was added dropwise **16** (3.0 g, 10.30 mmol) in THF (50 mL) at 0 °C, the reaction mixture was stirred for 3 h at 0 °C, and it was warmed to room temperature. Then the resulting solution was heated to reflux for 12 h. The reaction was quenched with a saturated solution of sodium sulfate. The insoluble solid was filtrated off and washed with DCM (200 mL). Then the combined organic solution was concentrated under reduced pressure to give the crude product, which was further chromatographed on silica gel, with ethyl acetate/hexanes as eluent, to afford **11** (1.14 g, 82%) as a colorless syrup: R_f = 0.80 (20% EtOAc/hexanes); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.95 (br s, 1H), 6.72 (m, 1H), 6.22 (dd, J = 2.8, 5.8 Hz, 1H), 6.01 (m, 1H), 5.88 (m, 1H), 5.09 (m, 2H), 2.68 (t, J = 7.2 Hz, 2H), 2.18 (m, 2H), 1.80 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 138.5, 132.4, 116.2, 115.0, 108.3, 105.1, 33.3, 28.9, 27.1; HRMS (ESI) calcd for $C_9H_{14}N$ $(M + H)^+$ 136.1121, found 136.1126; IR (KBr) ν_{max} 2931, 2857, 1681, 1439 cm^{-1}.

Representative Procedure for the Synthesis of (*S*)-2-Hydroxy-7-octene (12). To a stirred solution of (*S*)-propylene oxide (**17**; 1.0 g, 17.24 mmol) and copper iodide (327 mg, 1.72 mmol) in 50 mL of THF was added 4-pentenylmagnesium bromide solution (**18**; 0.5 M in THF) (52 mL, 25.86 mmol) at 0 °C, and the reaction mixture was stirred at 0 °C for 2 h. Then the reaction mixture was quenched by addition of 2 N HCl, with the temperature maintained at <10 °C, and extracted with diethyl ether (3 × 100 mL). Then the combined organic extracts were washed with 10% sodium thiosulfate solution, water, and brine and dried over anhydrous Na_2SO_4. The solvent was evaporated under reduced pressure, and the crude product was chromatographed on silica gel to afford the title compound **12** (2.03 g, 92%) as a colorless liquid: R_f = 0.50 (50% EtOAc/hexanes); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.79 (m, 1H), 4.98−4.92 (m, 2H), 3.80 (m, 1H), 2.04 (m, 2H), 1.40 (m, 6H), 1.18 (d, J = 6.2 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 138.9, 114.4, 68.1, 39.2, 33.7, 28.9, 25.2, 23.5; HRMS (ESI) calcd for $C_8H_{16}NaO$ $(M + Na)^+$ 151.1093, found 151.1079; IR (KBr) ν_{max} 3361, 2972, 2930, 2857, 1640 cm^{-1}. Compound **13** has the same spectral data.

Representative Procedure for the Synthesis of (*S*)-11-(Pyrrol-2-yl)undec-7-en-2-ol (19). Grubbs' second-generation catalyst (Grubbs' II; 160 mg, 0.19 mmol) was added to a stirred solution of **11** (500 mg, 3.70 mmol), and **12** (948 mg, 7.40 mmol) in dichloromethane (25 mL) at room temperature. Then the solution was stirred for 6 h at 40 °C. After completion of the reaction, the solvent was removed under reduced pressure and the product was chromatographed on silica gel, with ethyl acetate/hexanes as eluent, to afford the desired product **19** (390 mg, 45%) as a syrup: R_f = 0.45 (30% EtOAc/hexanes); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.15 (br s, 1H), 6.65 (dd, J = 2.5, 6.6 Hz, 1H), 6.13 (dd, J = 2.5, 5.7 Hz, 1H), 5.92 (m, 1H), 5.42 (m, 2H), 3.81 (m, 1H), 2.60 (t, J = 7.6 Hz, 2H), 2.01 (m, 4H), 1.66 (m, 2H), 1.35 (m, 6H), 1.18 (d, J = 6.2 Hz, 3H); ^{13}C

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NMR ($CDCl_3$, 100 MHz) δ 132.5, 130.8, 129.9, 116.0, 108.1, 104.8, 68.1, 39.1, 32.4, 32.0, 29.4 (2C), 27.0, 25.1, 23.4; HRMS (ESI) calcd for $C_{15}H_{25}NaNO$ $(M + Na)^+$ 258.1828, found 258.1819; IR (KBr) ν_{max} 3381, 2928, 2856, 1679, 1437 cm^{-1}. Compound **21** has the same spectral data.

Representative Procedure for the Synthesis of (10′*S*)-Hydroxyundecylpyrrole ((10′S)-9). To a degassed solution of **19** (200 mg, 0.85 mmol) in methanol (10 mL) was added a catalytic amount of 10% Pd/C. The reaction mixture was stirred at room temperature for 12 h under hydrogen gas. After replacement of air by nitrogen, Pd/C was filtered off and methanol was evaporated under reduced pressure. The crude product was chromatographed on silica gel to afford the title compound (10′*S*)-**9** (190 mg, 95%) as a white solid: mp 43−45 °C; R_f = 0.56 (20% EtOAc/hexanes); ^1H NMR ($CDCl_3$, 400 MHz) δ 8.01 (br s, 1H), 6.66 (m, 1H), 6.13 (dd, *J* = 2.8, 5.7 Hz, 1H), 5.91 (m, 1H), 3.80 (m, 1H), 2.59 (t, *J* = 7.4 Hz, 2H), 1.65−1.58 (m, 3H), 1.47−1.29 (m, 13H), 1.18 (d, *J* = 6.2 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 132.9, 116.0, 108.2, 104.8, 68.2, 39.4, 29.7, 29.6 (2C), 29.5, 29.4 (2C), 27.7, 25.8, 23.5; HRMS (ESI) calcd for $C_{15}H_{28}NO$ $(M + H)^+$ 238.2165, found 238.2159; IR (KBr) ν_{max} 3511, 3243, 2923, 2849, 1574, 1470 cm^{-1}. Compound (10′*R*)-**9** has the same spectral data.

Representative Procedure for the Synthesis of (*S*)-23-Hydroxyundecylprodiginines ((23*S*)-2). To a stirred suspension of MBC (**8**; 75 mg, 0.39 mmol) and (10′*S*)-**9** (187 mg, 0.78 mmol) in anhydrous methanol (10 mL) was added methanolic 2 N HCl (catalytic amount) at room temperature. The resulting brightly colored solution was stirred for 5 h at same temperature. The methanol was removed under reduced pressure, and the crude material was dissolved in ethyl acetate (75 mL) and washed with saturated $NaHCO_3$ solution (2 × 25 mL). The organic layer was dried over anhydrous Na_2SO_4, solvent was removed under reduced pressure, and the crude product was chromatographed on neutral alumina as the stationary phase and hexane/ethyl acetate as the mobile phase to afford the desired (23*S*)-**2** (102 mg, 63%): mp 93−95 °C; R_f = 0.30 (70% EtOAc/hexanes); ^1H NMR ($CDCl_3$, 400 MHz) δ 6.86 (s, 1H), 6.67 (dd, *J* = 1.1, 3.6 Hz, 1H), 6.61 (dd, *J* = 1.1, 2.7 Hz, 1H), 6.45 (d, *J* = 3.6 Hz, 1H), 6.11 (dd, *J* = 2.7, 3.6 Hz, 1H), 6.08 (s, 1H), 5.82 (d, *J* = 3.6 Hz, 1H), 3.97 (s, 3H), 3.79 (m, 1H), 2.05 (t, *J* = 7.6 Hz, 2H), 1.46−1.12 (m, 16H), 1.19 (d, *J* = 6.2 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 169.1, 160.2, 144.7, 138.5, 128.4, 128.3, 123.0, 121.1, 116.0, 112.9, 110.0, 108.6, 95.7, 68.2, 58.4, 39.4, 29.7, 29.6 (3C), 29.4, 29.2, 27.3, 25.8, 23.5; HRMS (ESI) calcd for $C_{25}H_{36}N_3O_2$ $(M + H)^+$ 410.2802, found 410.2791; IR (KBr) ν_{max} 3368, 3227, 2925, 2851, 1606, 1574, 1192 cm^{-1}. Compound (23*R*)-**2** has the same spectral data.

Synthesis of 10′-Ketoundecylpyrrole (22). Tetrapropylammonium perruthenate (TPAP; 44 mg, 0.12 mmol) was added to a stirred solution of (10′*S*)-**9** (200 mg, 0.84 mmol), NMO (197 mg, 1.68 mmol), and 4 Å molecular sieves (1.0 g) in dichloromethane (10 mL) at 0 °C. The solution was warmed to room temperature and stirred for 1.5 h. The reaction mixture was filtered through Celite and washed with dichloromethane (100 mL). The filtrate was concentrated under reduced pressure to give a crude residue, which was further chromatographed on silica gel, with ethyl acetate/hexanes as eluent, to afford **22** (101 mg, 51%), and **23** (31 mg, 15%).

*10′-Ketoundecylpyrrole (**22**):* mp 58−60 °C; R_f = 0.65 (20% EtOAc/hexanes); ^1H NMR ($CDCl_3$, 400 MHz) δ 7.94 (br s, 1H), 6.65 (dd, *J* = 1.4, 3.8 Hz, 1H), 6.12 (dd, *J* = 3.8, 5.7 Hz, 1H), 5.90 (br s, 1H), 2.59 (t, *J* = 7.8 Hz, 2H), 2.41 (t, *J* = 7.6 Hz, 2H), 2.13 (s, 3H), 1.64−1.54 (m, 4H), 1.35−1.27 (m, 10H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 209.5, 132.8, 116.0, 108.2, 104.8, 43.8, 29.9, 29.7, 29.3 (4C), 29.1, 27.7, 23.8; HRMS (ESI) calcd for $C_{15}H_{26}NO$ $(M + H)^+$ 236.2009, found 236.2002; IR (KBr) ν_{max} 3290, 2951, 2847, 1704, 1404 cm^{-1}.

*5-(10-Oxoundecylidene)-1,5-dihydropyrrol-2-one (**23**):* mp 38−40 °C; R_f = 0.25 (20% EtOAc/hexanes); ^1H NMR ($CDCl_3$, 400 MHz) δ 9.67 (br s, 1H), 6.89 (dd, *J* = 1.6, 5.5 Hz, 1H), 6.09 (dd, *J* = 0.8, 5.5 Hz, 1H), 5.25 (t, *J* = 8.0 Hz, 1H), 2.37 (t, *J* = 7.4 Hz, 2H), 2.33 (m, 2H), 2.10 (s, 3H), 1.54−1.42 (m, 4H), 1.32−1.24 (m, 8H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 209.4, 173.5, 138.7, 138.5, 123.6, 118.6, 43.7, 29.8, 29.2 (2C), 29.1 (2C), 29.0, 27.8, 23.8; HRMS (ESI) calcd for $C_{15}H_{24}NO_2$ $(M + H)^+$ 250.1802, found 250.1794; IR (KBr) ν_{max} 3290, 2951, 2847, 1704, 1404 cm^{-1}.

Synthesis of 23-Ketoundecylprodiginine (3). Compound **3** (32.1 mg, 50%) was synthesized by the same procedure as described for (23*S*)-**2**: mp 97−99 °C; R_f = 0.32 (70% EtOAc/hexanes); ^1H NMR (acetone-d_6, 400 MHz) δ 6.91 (br s, 1H), 6.76 (br s, 2H), 6.55 (br s, 1H), 6.20 (br s, 2H), 5.96 (br s, 1H), 3.93 (s, 3H), 2.55 (t, *J* = 6.2 Hz, 2H), 2.43 (t, *J* = 7.3 Hz, 2H), 2.05 (s, 3H), 1.59−1.49 (m, 4H), 1.36−1.23 (m, 10H); ^{13}C NMR (acetone-d_6, 100 MHz) δ 208.1, 169.3, 160.0, 143.2, 141.3, 130.1, 129.6, 123.0, 120.3, 115.6, 113.3, 110.9, 109.5, 96.2, 58.8, 43.8, 30.6, 30.2−29.7 (six carbons are merged), 28.5, 24.5; HRMS (ESI) calcd for $C_{25}H_{34}N_3O_2$ $(M + H)^+$ 408.2646, found 408.2635; IR (KBr) ν_{max} 3127, 2927, 2848, 1702, 1576, 1490 cm^{-1}.

Synthesis of 5-Ethylidene-1,5-dihydropyrrol-2-one (25a), 3-Methyl-5-methylene-1,5-dihydropyrrol-2-one (25b), 4-Ethyl-3-methyl-5-methylene-1,5-dihydropyrrol-2-one (25c), and 1-(5-Ethyl-1-methylpyrrol-2-yl)propan-1-one (25d). Compounds **25a** (92 mg, 32%), **25b** (100 mg, 35%), **25c** (108 mg, 39%), and **25d** (220 mg, 89%) were synthesized by the same procedure as described for **22**.

*5-Ethylidene-1,5-dihydropyrrol-2-one (**25a**):* mp 106−108 °C; R_f = 0.45 (50% EtOAc/hexanes); ^1H NMR ($CDCl_3$, 400 MHz) δ 10.09 (br s, 1H), 6.89 (dd, *J* = 1.4, 5.5 Hz, 1H), 6.10 (d, *J* = 5.3 Hz, 1H), 5.34 (q, *J* = 7.5 Hz, 1H), 1.96 (d, *J* = 7.5 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.9, 139.7, 138.4, 123.5, 113.5, 13.4; HRMS (ESI) calcd for C_6H_8NO $(M + H)^+$ 110.0600, found 110.0596; IR (KBr) ν_{max} 3165, 2809, 1685, 1652, 1400 cm^{-1}.

*3-Methyl-5-methylene-1,5-dihydropyrrol-2-one (**25b**):* mp >200 °C; R_f = 0.40 (70% EtOAc/hexanes); ^1H NMR ($CDCl_3$, 400 MHz) δ 9.15 (br s, 1H), 6.62 (t, *J* = 1.4 Hz, 1H), 4.92 (s, 1H), 4.71 (s, 1H), 1.94 (d, *J* = 1.4 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.7, 142.8, 135.5, 132.1, 96.7, 10.6; HRMS (ESI) calcd for C_6H_8NO $(M + H)^+$ 110.0600, found 110.0598; IR (KBr) ν_{max} 3156, 2807, 1679, 1648 cm^{-1}.

*4-Ethyl-3-methyl-5-methylene-1,5-dihydropyrrol-2-one (**25c**):* mp 90−92 °C; R_f = 0.52 (50% EtOAc/hexanes); ^1H NMR ($CDCl_3$, 400 MHz) δ 8.93 (br s, 1H), 4.87 (d, *J* = 1.2 Hz, 1H), 4.77 (s, 1H), 2.40 (q, *J* = 7.6 Hz, 2H), 1.85 (s, 3H), 1.09 (t, *J* = 7.6 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.4, 146.0, 143.6, 129.1, 93.2, 17.7, 14.3, 8.3; HRMS (ESI) calcd for $C_8H_{11}NaNO$ $(M + Na)^+$ 160.0733, found 160.0736; IR (KBr) ν_{max} 3163, 2812, 1678 cm^{-1}.

*1-(5-Ethyl-1-methylpyrrol-2-yl)propan-1-one (**25d**):* mp 32−34 °C; R_f = 0.75 (20% EtOAc/hexanes); ^1H NMR ($CDCl_3$, 400 MHz) δ 6.93 (d, *J* = 4.0 Hz, 1H), 5.94 (d, *J* = 4.0 Hz, 1H), 3.86 (s, 3H), 2.77 (q, *J* = 7.5 Hz, 2H), 2.58 (q, *J* = 7.5 Hz, 2H), 1.26 (t, *J* = 7.5 Hz, 3H), 1.17 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 191.6, 144.2, 130.3, 118.6, 106.0, 32.8, 32.1, 19.8, 12.4, 9.4; HRMS (ESI) calcd for $C_{10}H_{16}NO$ $(M + H)^+$ 166.1226, found 166.1222; IR (KBr) ν_{max} 2974, 1644, 1485 cm^{-1}.

Synthesis of 10-Hydroxyundecanoic Acid (27). Au(IPr)Cl (136 mg, 0.22 mmol), AgO_2CCF_3 (49 mg, 0.22 mmol), Shvo's catalyst (239 mg, 0.22 mmol), 10-undecynoic acid (**26**; 2.0 g, 10.98 mmol), H_2O (7 mL, 373.6 mmol), and isopropyl alcohol (IPA) (43 mL) were placed in a sealed tube with a Teflon-lined cap under an argon atmosphere at room temperature. The reaction mixture was stirred and heated for 48 h at 70 °C. The mixture was filtered through Celite and washed with dichloromethane (200 mL). The filtrate was concentrated under reduced pressure to give a crude orange residue, which was further chromatographed on silica gel, with ethyl acetate/hexanes as eluent, to afford the pure 10-hydroxyundecanoic acid (**27**) as a white solid (1.82 g, 82%): mp 46−48 °C; R_f = 0.35 (70% EtOAc/hexanes); ^1H NMR ($CDCl_3$, 400 MHz) δ 3.79 (m, 1H), 2.32 (t, *J* = 7.4 Hz, 2H), 1.59 (m, 2H), 1.44−1.24 (m, 12H), 1.17 (d, *J* = 6.2 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 179.4, 68.3, 39.2, 34.0, 29.5, 29.3, 29.2, 29.0, 25.6, 24.7, 23.3; HRMS (ESI) calcd for $C_{11}H_{22}NaO_3$ $(M + Na)^+$ 225.1461, found 225.1463; IR (KBr) ν_{max} 3450, 2934, 2853, 1728, 1220 cm^{-1}.

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Synthesis of 10-Oxoundecanoic Acid (28). To a stirred solution of **27** (1.0 g, 4.95 mmol) in anhydrous CH_2Cl_2 (50 mL) was added Dess−Martin periodinane (DMP; 3.14 g, 7.42 mmol) at 0 °C. The mixture was stirred at room temperature for 10 h, and then the solvent was evaporated, and diethyl ether was added to the crude product, and the reaction mixture was stirred until a white solid was formed. The reaction mixture was filtered through Celite and washed with diethyl ether (200 mL). The filtrate was concentrated under reduced pressure to give a crude residue, which was further chromatographed on silica gel, with ethyl acetate/hexanes as eluent, to afford the pure product **28** as a white solid (881 mg, 89%): mp 51−53 °C; R_f = 0.45 (70% EtOAc/hexanes); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.40 (t, J = 7.4 Hz, 2H), 2.32 (t, J = 7.4 Hz, 2H), 2.11 (m, 3H), 1.64−1.50 (m, 4H), 1.27 (m, 8H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 209.6, 180.0, 43.7, 34.0, 29.8, 29.1, 29.0 (2C), 28.9, 24.6, 23.8; HRMS (ESI) calcd for $C_{11}H_{21}O_3$ $(M + H)^+$. 201.1485, found 201.1486; IR (KBr) ν_{max} 2921, 2845, 1726, 1701, 1235 cm^{-1}.

Synthesis of [10-*d*]-10-Hydroxyundecanoic Acid ([10-*d*]-27). To a stirred solution of **28** (850 mg, 4.25 mmol) in anhydrous methanol (15 mL) was added slowly $NaBD_4$ (209 mg, 5.10 mmol) at 0 °C, and the reaction mixture was stirred at the same temperature for 2 h. The resulting solution was quenched with 1 N HCl and extracted with ethyl acetate (3 × 50 mL), and the combined extracts were washed with water and brine, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The crude residue was further chromatographed on silica gel, with ethyl acetate/hexanes as eluent, to afford the pure product [10-*d*]-**27** as a white solid (690 mg, 80%): mp 45−47 °C; R_f = 0.35 (70% EtOAc/hexanes); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.32 (t, J = 7.4 Hz, 2H), 1.59 (m, 2H), 1.44−1.24 (m, 12H), 1.17 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 179.4, 67.8 (t, C-23), 39.0, 34.1, 29.5, 29.3, 29.1, 29.0, 25.6, 24.7, 23.2; HRMS (ESI) calcd for $C_{11}H_{21}DNaO_3$ $(M + Na)^+$ 226.1522, found 226.1523; IR (KBr) ν_{max} 3450, 2934, 2853, 1728, 1220 cm^{-1}.

Representative Procedure for the Synthesis of [10-*d*]-10-Acetoxyundecanoic Acid ([10-*d*]-29). Acetic anhydride (Ac_2O; 1.88 g, 18.47 mmol) was added to a stirred solution of [10-*d*]-**27** (1.5 g, 7.39 mmol) in pyridine (10 mL), and the reaction mixture was stirred for 12 h at room temperature. The reaction mixture was diluted with ethyl acetate (200 mL), the solution was washed with 2 N HCl (3 × 50 mL) and brine, dried over anhydrous Na_2SO_4, and filtered, and the solvent was removed by rotary evaporation to furnish the crude product. The crude compound was chromatographed on silica gel using hexane/ethyl acetate as the mobile phase to afford [10-*d*]-**29** (1.70 g, 94%) as a syrup: R_f = 0.50 (40% EtOAc/hexanes); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.42 (m, 2H), 2.00 (s, 3H), 1.65−1.26 (m, 14H), 1.17 (s, 3H); HRMS (ESI) calcd for $C_{13}H_{24}DO_4$ $(M + H)^+$ 246.1810, found 246.1811; IR (KBr) ν_{max} 2931, 2857, 1742, 1710, 1264 cm^{-1}.

Representative Procedure for the Synthesis of 11-Oxo-11-(pyrrol-2-yl)undecan-2-yl-2-*d* Acetate ([10′-*d*]-30) and 11-Oxo-11-(pyrrol-3-yl)undecan-2-yl-2-*d* Acetate ([10′-*d*]-31). To a stirred solution of [10-*d*]-**29** (1.5 g, 6.12 mmol) and dimethylformamide (DMF; catalytic amount) in toluene (10 mL) was added thionyl chloride ($SOCl_2$; 1.32 mL, 18.36 mmol). The resulting solution was stirred at 80 °C for 2 h. After all solvents were removed in vacuo, the residue was dissolved in toluene (50 mL), and this solution was transferred into a stirred solution of pyrrole (250 mg, 3.73 mmol) and Zn powder (485 mg, 7.46 mmol) in toluene (100 mL). After completion of the reaction (monitored by TLC, 2 h), the reaction mixture was filtered through Celite and washed with dichloromethane (200 mL). The filtrate was concentrated under reduced pressure to give a crude residue, which was further chromatographed on silica gel, with ethyl acetate/hexanes as eluent, to afford the pure [10′-*d*]-**30** (625 mg, 57%), and [10′-*d*]-**31** (88 mg, 8%).

*11-Oxo-11-(pyrrol-2-yl)undecan-2-yl-2-d acetate ([10′-d]-**30**):* syrup; R_f = 0.55 (20% EtOAc/hexanes); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 10.00 (br s, 1H), 7.02 (m, 1H), 6.90 (m, 1H), 6.25 (m, 1H), 2.74 (t, J = 7.4 Hz, 2H), 2.01 (s, 3H), 1.70 (m, 2H), 1.54 (m, 1H), 1.42 (m, 1H), 1.35−1.26 (m, 10H), 1.19 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 191.3, 170.8, 132.0, 124.8, 116.3, 110.5, 71.7 (t, C-23), 38.0, 35.8, 29.4 (4C), 25.3 (2C), 21.4, 19.9; HRMS (ESI) calcd for $C_{17}H_{27}DNO_3$ $(M + H)^+$ 295.2127, found 295.2126; IR (KBr) ν_{max} 3283, 2930, 2856, 1734, 1639, 1404, 1265 cm^{-1}.

*11-Oxo-11-(pyrrol-3-yl)undecan-2-yl-2-d acetate ([10′-d]-**31**):* syrup; R_f = 0.25 (20% EtOAc/hexanes); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.49 (br s, 1H), 7.41 (m, 1H), 6.75 (dd, J = 2.5, 4.7 Hz, 1H), 6.32 (dd, J = 2.6, 4.0 Hz, 1H), 2.73 (t, J = 7.4 Hz, 2H), 2.01 (s, 3H), 1.68 (m, 2H), 1.52 (m, 1H), 1.44 (m, 1H), 1.32−1.26 (m, 10H), 1.17 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 197.2, 171.0, 125.8, 123.4, 119.5, 108.6, 70.8 (t, C-23), 39.7, 35.8, 29.4 (4C), 25.3, 25.1, 21.4, 19.8; HRMS (ESI) calcd for $C_{17}H_{27}DNO_3$ $(M + H)^+$ 295.2127, found 295.2125; IR (KBr) ν_{max} 3281, 2929, 2853, 1729, 1664, 1404.

Representative Procedure for the Synthesis of [10′-*d*]-Hydroxyundecylpyrrole ([10′-*d*]-9). To a stirred solution of [10′-*d*]-**30** (500 mg, 1.70 mmol) in 100 mL of isopropyl alcohol (IPA) at 25 °C was added slowly sodium borohydride ($NaBH_4$) (755 mg, 20.40 mmol), and the reaction mixture was heated at reflux for 12 h. The hot reaction mixture was poured into 100 mL of ice water, and the solution was acidified with 2 N HCl. The suspension was extracted with dichloromethane (3 × 50 mL), and the combined organic extracts were washed with water and brine and dried over anhydrous Na_2SO_4. The solvent was evaporated under reduced pressure, and the crude product was chromatographed on silica gel to afford the title compound ([10′-*d*]-**9** (271 mg, 67%) as a white solid: mp 46−48 °C; R_f = 0.55 (20% EtOAc/hexanes); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.99 (br s, 1H), 6.66 (dd, J = 2.7, 4.0 Hz, 1H), 6.13 (dd, J = 2.7, 5.7 Hz, 1H), 5.91 (m, 1H), 2.59 (t, J = 7.4 Hz, 2H), 1.64−1.58 (m, 2H), 1.47−1.27 (m, 14H), 1.18 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 132.9, 116.0, 108.2, 104.8, 67.7 (t, C-23), 39.2, 29.7, 29.6 (2C), 29.5, 29.4 (2C), 27.7, 25.7, 23.4; HRMS (ESI) calcd for $C_{15}H_{27}DNO$ $(M + H)^+$ 239.2228, found 239.2227; IR (KBr) ν_{max} 3511, 3243, 2923, 2849, 1574, 1470 cm^{-1}.

Synthesis of [23-*d*]-23-Hydroxyundecylprodiginine ([23-*d*]-2). Compound [23-*d*]-**2** (113 mg, 70%) was synthesized by the same procedure as described for (23*S*)-**2**: mp 94−96 °C; R_f = 0.70 (20% EtOAc/hexanes); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.86 (s, 1H), 6.67 (dd, J = 1.1, 3.6 Hz, 1H), 6.61 (dd, J = 1.1, 2.7 Hz, 1H), 6.45 (d, J = 3.6 Hz, 1H), 6.11 (dd, J = 2.7, 3.6 Hz, 1H), 6.08 (s, 1H), 5.82 (d, J = 3.6 Hz, 1H), 3.97 (s, 3H), 2.05 (t, J = 7.6 Hz, 2H), 1.46−1.15 (m, 19H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 169.1, 160.2, 144.7, 138.7, 128.3, 128.2, 123.1, 121.2, 116.0, 112.9, 110.0, 108.6, 95.8, 67.7 (t, C-23), 58.4, 39.4, 29.7, 29.6 (3C), 29.4, 29.2, 27.3, 25.8, 23.5; HRMS (ESI) calcd for $C_{25}H_{35}DN_3O_2$ $(M + H)^+$ 411.2865, found 411.2860; IR (KBr) ν_{max} 3368, 3227, 2925, 2851, 1606, 1574, 1192 cm^{-1}.

Synthesis of 7-Hydroxyoctanoic Acid (42). Compound **42** (1.62 g, 80%) was synthesized from 7-oxooctanoic acid by the same procedure as described for [10-*d*]-**27** using $NaBH_4$: syrup; R_f = 0.25 (60% EtOAc/hexanes); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.77 (m, 1H), 2.31 (t, J = 7.4 Hz, 2H), 1.61 (m, 2H), 1.43−1.30 (m, 6H), 1.16 (t, J = 6.2 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 179.1, 68.1, 38.8, 34.0, 29.0, 25.3, 24.6, 23.2; HRMS (ESI) calcd for $C_8H_{16}NaO_3$ $(M + Na)^+$ 183.0992, found 183.0987; IR (KBr) ν_{max} 3417, 2935, 2861, 1731, 1713 cm^{-1}.

Synthesis of 10-Acetoxyundecanoic Acid (29). Compound **29** (1.72 g, 95%) was synthesized by the same procedure as described for [10-*d*]-**29**: syrup; R_f = 0.50 (40% EtOAc/hexanes); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.85 (m, 1H), 2.31 (t, J = 7.5 Hz, 2H), 2.00 (s, 3H), 1.64−1.53 (m, 3H), 1.44 (m, 1H), 1.33−1.22 (m, 10H), 1.16 (d, J = 6.2 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 180.0, 170.9, 71.1, 35.9, 34.0, 29.3, 29.2, 29.1, 29.0, 25.3, 24.6, 21.4, 19.9; HRMS (ESI) calcd for $C_{13}H_{25}O_4$ $(M + H)^+$ 245.1747, found 245.1740; IR (KBr) ν_{max} 2931, 2857, 1743, 1730, 1245 cm^{-1}.

Synthesis of 10-Methoxyundecanoic Acid (43). To a stirred solution of **27** (1.0 g, 4.95 mmol) in DMF (20 mL) was added gradually NaH (237 mg, 9.90 mmol) at 0 °C. The resulting suspension was stirred for 30 min, and methyl iodide (CH_3I) (1.05 g, 7.42 mmol) was added over 10 min at the same temperature. Then the reaction mixture was warmed to room temperature and stirring was continued for 12 h. After completion of the reaction, the reaction mixture was gradually poured into ice cold water and extracted with ethyl acetate (3

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× 100 mL). The combined organic layers were washed with water and brine. The solvent was evaporated under reduced pressure to give the crude product, which was dissolved in THF (20 mL), and aqueous 5 N NaOH (10 mL) was added. The resulting solution was stirred at 60 °C for 3 h, and then it was acidified (pH ∼6) by 2 N HCl and extracted with ethyl acetate (3 × 100 mL). The combined organic extracts were washed with water and brine and dried over anhydrous Na_2SO_4. The solvent was evaporated under reduced pressure to afford the pure product **43** (866 mg, 81%) as a syrup: R_f = 0.35 (30% EtOAc/hexanes); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.32 (s, 3H), 3.30 (m, 1H), 2.33 (t, J = 7.5 Hz, 2H), 1.64−1.61 (m, 3H), 1.34−1.18 (m, 11H), 1.12 (d, J = 6.2 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 179.7, 77.0, 55.8, 36.2, 34.1, 29.6, 29.4, 29.2, 29.0, 25.4, 24.7, 18.9; HRMS (ESI) calcd for $C_{12}H_{24}NaO_3$ $(M + Na)^+$ 239.1618, found 239.1619; IR (KBr) ν_{max} 2930, 2855, 1738, 1710, 1463 cm^{-1}.

Synthesis of 10-(Benzyloxy)undecanoic Acid (44). A stirred solution of 2-benzyloxy-1-methylpyridinium triflate (Dudley reagent;[28] 3.45 g, 9.90 mmol), MgO (396 mg, 9.90 mmol), and compound **27** (1.0 g, 4.95 mmol) in toluene (75 mL) was heated at 85 °C for 24 h. The reaction mixture was cooled to room temperature, filtered through Celite, and washed with dichloromethane (100 mL). The filtrate was concentrated under vacuum, the obtained crude product was dissolved in THF (20 mL), and aqueous 5 N NaOH (10 mL) was added. The resulting solution was stirred at 60 °C for 3 h and then acidified (pH ∼6) by 2 N HCl and extracted with ethyl acetate (3 × 100 mL). The combined organic extracts were washed with water and brine and dried over anhydrous Na_2SO_4. The solvent was evaporated under reduced pressure to afford the pure product **44** (1.09 g, 76%) as a syrup: R_f = 0.30 (30% EtOAc/hexanes); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.36−2.28 (m, 5H), 4.59 (d, J = 11.8 Hz, 1H), 4.49 (d, J = 11.8 Hz, 1H), 3.52 (m, 1H), 2.36 (t, J = 7.4 Hz, 2H), 1.67−1.61 (m, 3H), 1.45−1.35 (m, 11H), 1.18 (d, J = 6.2 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 180.0, 139.1, 128.3 (2C), 127.7 (2C), 127.4, 74.9, 70.3, 36.6, 34.1, 29.6, 29.4, 29.2, 29.1, 25.5, 24.7, 19.6; HRMS (ESI) calcd for $C_{18}H_{28}NaO_3$ $(M + Na)^+$ 315.1931, found 315.1930; IR (KBr) ν_{max} 3030, 2929, 2855, 1731, 1454 cm^{-1}.

Synthesis of 7-Acetoxyoctanoic Acid (45). Compound **45** (1.78 g, 94%) was synthesized by the same procedure as described for [10-*d*]-**29**: syrup; R_f = 0.55 (60% EtOAc/hexanes); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.86 (m, 1H), 2.31 (t, J = 7.4 Hz, 2H), 2.00 (s, 3H), 1.62−1.28 (m, 8H), 1.16 (d, J = 6.2 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 179.6, 171.0, 71.0, 35.6, 33.9, 28.8, 25.0, 24.5, 21.3, 19.9; HRMS (ESI) calcd for $C_{10}H_{18}NaO_4$ $(M + Na)^+$ 225.1097, found 225.1088; IR (KBr) ν_{max} 2977, 2939, 2863, 1738, 1710, 1374, 1246 cm^{-1}.

Synthesis of 30, 31, 46a,b, 47a,b, and 48a,b. Compounds **30** (600 mg, 55%), **31** (76 mg, 7%), **46a** (450 mg, 65%), **46b** (62.2 mg, 9%), **47a** (560 mg, 63%), **47b** (71.2 mg, 8%), **48a** (496 mg, 53%), and **48b** (94 mg, 10%) were synthesized by the same procedure as described for [10′-*d*]-**30** and [10′-*d*]-**31**.

*11-Oxo-11-(pyrrol-2-yl)undecan-2-yl acetate (**30**):* syrup; R_f = 0.55 (20% EtOAc/hexanes); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 10.18 (br s, 1H), 7.05 (m, 1H), 6.93 (m, 1H), 6.26 (m, 1H), 4.88 (m, 1H), 2.76 (t, J = 7.4 Hz, 2H), 2.02 (s, 3H), 1.73−1.26 (m, 14H), 1.19 (d, J = 6.2 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 191.5, 170.8, 132.0, 125.0, 116.6, 110.5, 71.1, 38.0, 35.9, 29.4 (4C), 29.3, 25.3, 21.4, 19.9; HRMS (ESI) calcd for $C_{17}H_{28}NO_3$ $(M + H)^+$ 294.2064, found 294.2057; IR (KBr) ν_{max} 3282, 2930, 2856, 1736, 1639, 1410, 1247 cm^{-1}.

*11-Oxo-11-(pyrrol-3-yl)undecan-2-yl acetate (**31**):* syrup; R_f = 0.25 (20% EtOAc/hexanes); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.52 (br s, 1H), 7.43 (m, 1H), 6.77 (dd, J = 2.5, 4.9 Hz, 1H), 6.65 (dd, J = 2.5, 4.2 Hz, 1H), 4.88 (m, 1H), 2.75 (t, J = 7.4 Hz, 2H), 2.03 (s, 3H), 1.72−1.28 (m, 14H), 1.20 (d, J = 6.2 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 197.2, 171.0, 125.8, 123.4, 119.5, 108.6, 71.2, 39.7, 35.9, 29.4 (3C), 29.3, 25.4, 25.1, 21.4, 19.9; HRMS (ESI) calcd for $C_{17}H_{28}NO_3$ $(M + H)^+$ 294.2064, found 294.2056; IR (KBr) ν_{max} 3280, 2936, 2851, 1734, 1664, 1244 cm^{-1}.

*10-Methoxy-1-(pyrrol-2-yl)undecan-1-one (**46a**):* syrup; R_f = 0.50 (20% EtOAc/hexanes); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 10.02 (br s, 1H), 7.05 (m, 1H), 6.94 (m, 1H), 6.28 (m, 1H), 3.32 (s, 3H), 3.30 (m, 1H), 2.77 (t, J = 7.5 Hz, 2H), 1.72 (m, 2H), 1.55 (m, 1H), 1.38−1.30 (m, 11H), 1.13 (d, J = 6.2 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 191.5, 132.0, 124.8, 116.5, 110.5, 76.9, 55.9, 38.0, 36.3, 29.7, 29.4 (3C), 25.9 (2C), 19.0; HRMS (ESI) calcd for $C_{16}H_{27}NaNO_2$ $(M + Na)^+$ 288.1934, found 288.1933; IR (KBr) ν_{max} 3282, 2929, 2855, 1738, 1639, 1405 cm^{-1}.

*10-Methoxy-1-(pyrrol-3-yl)undecan-1-one (**46b**):* syrup; R_f = 0.25 (20% EtOAc/hexanes); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.50 (br s, 1H), 7.42 (m, 1H), 6.78 (dd, J = 2.5, 4.8 Hz, 1H), 6.64 (dd, J = 2.5, 4.2 Hz, 1H), 3.32 (s, 3H), 3.30 (m, 1H), 2.75 (t, J = 7.4 Hz, 2H), 1.70 (m, 2H), 1.50 (m, 1H), 1.34−1.26 (m, 11H), 1.12 (d, J = 6.2 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 197.3, 125.8, 123.3, 119.5, 108.7, 77.0, 55.9, 39.7, 36.3, 29.7, 29.5 (2C), 29.4, 25.4, 25.2, 19.0; HRMS (ESI) calcd for $C_{16}H_{27}NaNO_2$ $(M + Na)^+$ 288.1934, found 288.1936; IR (KBr) ν_{max} 3279, 2933, 2848, 1737, 1654, 1402 cm^{-1}.

*10-(Benzyloxy)-1-(pyrrol-2-yl)undecan-1-one (**47a**):* syrup; R_f = 0.65 (20% EtOAc/hexanes); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 10.01 (br s, 1H), 7.37−7.30 (m, 5H), 7.05 (m, 1H), 6.94 (m, 1H), 6.29 (m, 1H), 4.65 (d, J = 11.8 Hz, 1H), 4.49 (d, J = 11.8 Hz, 1H), 3.54 (m, 1H), 2.79 (t, J = 7.4 Hz, 2H), 1.77−1.62 (m, 3H), 1.39−1.31 (m, 11H), 1.21 (d, J = 6.3 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 191.4, 139.2, 132.1, 128.3 (2C), 127.6 (2C), 127.4, 124.7, 116.2, 110.5, 74.9, 70.3, 38.1, 36.7, 29.7, 29.5 (2C), 29.4, 25.5, 25.4, 19.7; HRMS (ESI) calcd for $C_{22}H_{31}NaNO_2$ $(M + Na)^+$ 364.2247, found 364.2243; IR (KBr) ν_{max} 3281, 2928, 2854, 1637, 1546, 1404 cm^{-1}.

*10-(Benzyloxy)-1-(pyrrol-3-yl)undecan-1-one (**47b**):* syrup; R_f = 0.30 (20% EtOAc/hexanes); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.61 (br s, 1H), 7.39−7.30 (m, 6H), 6.71 (dd, J = 2.7, 4.9 Hz, 1H), 6.64 (m, 1H), 4.60 (d, J = 11.8 Hz, 1H), 4.50 (d, J = 11.8 Hz, 1H), 3.55 (m, 1H), 2.77 (t, J = 7.4 Hz, 2H), 1.75−1.52 (m, 3H), 1.47−1.26 (m, 11H), 1.23 (d, J = 6.2 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 197.4, 139.0, 128.4 (2C), 127.8 (2C), 127.5, 125.8, 123.4, 119.6, 108.5, 75.1, 70.4, 39.8, 36.7, 29.7, 29.5 (3C), 25.5, 25.2, 19.7; HRMS (ESI) calcd for $C_{22}H_{31}NaNO_2$ $(M + Na)^+$ 364.2247, found 364.2245; IR (KBr) ν_{max} 3278, 2931, 2848, 1655, 1401 cm^{-1}.

*8-Oxo-8-(pyrrol-2-yl)octan-2-yl acetate (**48a**):* syrup; R_f = 0.55 (50% EtOAc/hexanes); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 10.15 (br s, 1H), 7.03 (m, 1H), 6.90 (m, 1H), 6.24 (m, 1H), 4.85 (m, 1H), 2.74 (t, J = 7.4 Hz, 2H), 2.00 (s, 3H), 1.72−1.19 (m, 8H), 1.17 (d, J = 6.2 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 191.2, 170.8, 131.9, 125.0, 116.5, 110.5, 70.9, 37.8, 35.7, 29.2, 25.2, 25.2, 21.3, 19.9; HRMS (ESI) calcd for $C_{14}H_{22}NO_3$ $(M + H)^+$ 252.1594, found 252.1596; IR (KBr) ν_{max} 3284, 2937, 2861, 1733, 1639, 1404, 1248 cm^{-1}.

*8-Oxo-8-(pyrrol-3-yl)octan-2-yl acetate (**48b**):* syrup; R_f = 0.25 (50% EtOAc/hexanes); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.48 (br s, 1H), 7.41 (m, 1H), 6.75 (dd, J = 2.3, 4.8 Hz, 1H), 6.62 (dd, J = 2.3, 4.2 Hz, 1H), 4.85 (m, 1H), 2.73 (t, J = 7.4 Hz, 2H), 2.00 (s, 3H), 1.71−1.30 (m, 8H), 1.16 (d, J = 6.2 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 197.0, 171.0, 125.8, 123.3, 119.5, 108.6, 71.0, 39.5, 35.7, 29.2, 25.2, 24.9, 21.4, 19.9; HRMS (ESI) calcd for $C_{14}H_{22}NO_3$ $(M + H)^+$ 252.1594, found 252.1589; IR (KBr) ν_{max} 3281, 2931, 2858, 1731, 1654, 1400 cm^{-1}.

Synthesis of 2-(10-Hydroxyundecyl)pyrrole (9), 2-(10-Methoxyundecyl)pyrrole (49), 2-(10-(Benzyloxy)undecyl)-pyrrole (50), and 2-(7-Hydroxyoctyl)pyrrole (51). Compounds **9** (262 mg, 65%), **49** (341 mg, 72%), **50** (359 mg, 75%), and **51** (260 mg, 67%) were synthesized by the same procedure as described for [10′-*d*]-**9**.

*2-(10-Hydroxyundecyl)pyrrole (**9**):* compound **9** has the same spectral data as shown for [10′*S*]-**9**.

*2-(10-Methoxyundecyl)pyrrole (**49**):* syrup; R_f = 0.65 (15% EtOAc/hexanes); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.01 (br s, 1H), 6.68 (dd, J = 2.8, 4.2 Hz, 1H), 6.15 (t, J = 2.8 Hz, 1H), 5.94 (m, 1H), 3.35 (s, 3H), 3.32 (m, 1H), 2.62 (t, J = 7.6 Hz, 2H), 1.66−1.51 (m, 3H), 1.38−1.31 (m, 13H), 1.15 (d, J = 6.2 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 132.9, 116.0, 108.2, 104.8, 76.9, 55.9, 36.4, 29.8, 29.7, 29.6, 29.5, 29.4 (2C), 27.7, 25.5, 19.0; HRMS (ESI) calcd for $C_{16}H_{30}NO$ $(M + H)^+$ 252.2322, found 252.2327; IR (KBr) ν_{max} 3297, 2935, 2853, 1693, 1464, 1373 cm^{-1}.

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*2-(10-(Benzyloxy)undecyl)pyrrole (**50**):* syrup; R_f = 0.80 (15% EtOAc/hexanes); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.95 (br s, 1H), 7.41−7.31 (m, 5H), 6.69 (m, 1H), 6.17 (dd, J = 2.8, 5.8 Hz, 1H), 5.96 (m, 1H), 4.61 (d, J = 11.8 Hz, 1H), 4.50 (d, J = 11.8 Hz, 1H), 3.55 (m, 1H), 2.63 (t, J = 7.6 Hz, 2H), 1.67−1.62 (m, 3H), 1.48−1.32 (m, 13H), 1.24 (d, J = 6.3 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 139.2, 132.9, 128.3 (2C), 127.7 (2C), 127.4, 116.0, 108.2, 104.9, 75.0, 70.3, 36.7, 29.7 (2C), 29.6, 29.5 (2C), 29.4, 27.8, 25.6, 19.7; HRMS (ESI) calcd for $C_{22}H_{34}NO$ $(M + H)^+$ 328.2635, found 328.2638; IR (KBr) ν_{max} 3280, 2934, 2847, 1682, 1404 cm^{-1}.

*2-(7-Hydroxyoctyl)pyrrole (**51**):* mp 80−82 °C; R_f = 0.42 (60% EtOAc/hexanes); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.05 (br s, 1H), 6.65 (m, 1H), 6.13 (dd, J = 2.8, 5.6 Hz, 1H), 5.91 (br s, 1H), 3.80 (m, 1H), 2.59 (t, J = 7.6 Hz, 2H), 1.63 (m, 2H), 1.48−1.33 (m, 8H), 1.19 (d, J = 6.2 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 132.7, 116.0, 108.1, 104.8, 68.1, 39.2, 29.6, 29.4, 29.3, 27.7, 25.6, 23.5; HRMS (ESI) calcd for $C_{12}H_{21}NaNO$ $(M + Na)^+$ 218.1515, found 218.1509; IR (KBr) ν_{max} 3374, 2929, 2855, 1712, 1673, 1463, 1378 cm^{-1}.

Synthesis of Prodiginines 2 and 32−38. Compounds **2** (110 mg, 68%), **32** (194 mg, 87%), **33** (199 mg, 76%), **34** (66 mg, 70%), **35** (70 mg, 65%), **36** (75 mg, 75%), **37** (66 mg, 71%), and **38** (74 mg, 71%) were synthesized by the same procedure as described for (23*S*)-**2**.

*23-Hydroxyundecylprodiginine (**2**):* Compound **2** has the same spectral data as shown for [23*S*]-**2**.

*23-Methoxyundecylprodiginine (**32**):* syrup; R_f = 0.55 (40% EtOAc/hexanes); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.88 (s, 1H), 6.69 (dd, J = 1.3, 3.6 Hz, 1H), 6.63 (dd, J = 1.3, 2.6 Hz, 1H), 6.47 (d, J = 3.6 Hz, 1H), 6.14 (dd, J = 2.6, 3.6 Hz, 1H), 6.13 (s, 1H), 5.85 (d, J = 3.6 Hz, 1H), 4.00 (s, 3H), 3.35 (s, 3H), 3.32 (m, 1H), 2.07 (t, J = 7.4 Hz, 2H), 1.50 (m, 1H), 1.39−1.22 (m, 15H), 1.17 (d, J = 6.2 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 169.1, 160.3, 144.7, 138.6, 128.4, 128.3, 123.0, 121.0, 116.0, 112.8, 110.0, 108.6, 95.7, 76.9, 58.4, 56.0, 36.4, 29.8, 29.7, 29.6 (2C), 29.4, 29.2, 27.3, 25.5, 19.1; HRMS (ESI) calcd for $C_{26}H_{38}N_3O_2$ $(M + H)^+$ 424.2958, found 424.2959; IR (KBr) ν_{max} 3318, 2926, 2853, 1625, 1362 cm^{-1}.

*23-Benzoxyundecylprodiginine (**33**):* syrup; R_f = 0.40 (30% EtOAc/hexanes); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.40−7.30 (m, 5H), 6.90 (s, 1H), 6.71 (dd, J = 1.2, 3.6 Hz, 1H), 6.65 (dd, J = 1.2, 2.3 Hz, 1H), 6.50 (d, J = 3.7 Hz, 1H), 6.15 (dd, J = 2.5, 3.6 Hz, 1H), 6.12 (s, 1H), 5.86 (d, J = 3.7 Hz, 1H), 4.62 (d, J = 11.8 Hz, 1H), 4.50 (d, J = 11.8 Hz, 1H), 4.00 (s, 3H), 3.55 (m, 1H), 2.08 (t, J = 7.6 Hz, 2H), 1.67 (m, 1H), 1.49−1.16 (m, 18H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 169.1, 160.3, 144.7, 139.2, 138.5, 128.4 (2C), 128.3 (2C), 127.6 (2C), 127.4, 123.0, 121.1, 116.1, 112.9, 110.0, 108.6, 95.8, 75.0, 70.3, 58.4, 36.8, 29.8, 29.7, 29.6 (2C), 29.4, 29.2, 27.3, 25.6, 19.7; HRMS (ESI) calcd for $C_{32}H_{42}N_3O_2$ $(M + H)^+$ 500.3272, found 500.3271; IR (KBr) ν_{max} 3321, 2931, 2845, 1617 cm^{-1}.

*20-Hydroxyoctylprodiginine (**34**):* mp 88−90 °C; R_f = 0.35 (50% EtOAc/hexanes); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.86 (s, 1H), 6.68 (dd, J = 1.0, 3.5 Hz, 1H), 6.61 (d, J = 1.0 Hz, 1H), 6.47 (d, J = 3.7 Hz, 1H), 6.11 (dd, J = 2.6, 3.5 Hz, 1H), 6.09 (s, 1H), 5.82 (d, J = 3.7 Hz, 1H), 3.97 (s, 3H), 3.78 (m, 1H), 2.05 (t, J = 7.5 Hz, 2H), 1.44−1.28 (m, 13H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 169.1, 160.3, 144.5, 138.6, 128.3 (2C), 123.0, 121.1, 116.0, 112.9, 110.0, 108.6, 95.8, 68.1, 58.4, 39.3, 29.4, 29.3, 29.1, 27.2, 25.7, 23.5; HRMS (ESI) calcd for $C_{22}H_{30}N_3O_2$ $(M + H)^+$ 368.2333, found 368.2319; IR (KBr) ν_{max} 3418, 3125, 2929, 2847, 1622, 1575, 1490 cm^{-1}.

*11-(5-((3′,4′-Dimethyl-[2,2′-bipyrrol]-5′-ylidene)methyl)pyrrol-2-yl)undecan-2-ol hydrochloride (**35**):* mp 119−121 °C; R_f = 0.35 (50% EtOAc/hexanes); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 13.10 (s, 1H), 12.94 (s, 1H), 12.56 (s, 1H), 7.26 (s, 1H), 7.03 (d, J = 2.3 Hz, 1H), 6.83 (br s, 2H), 6.37 (dd, J = 2.5, 3.6 Hz, 1H), 6.20 (d, J = 3.6 Hz, 1H), 3.77 (m, 1H), 2.94 (br s, 2H), 2.21 (s, 6H), 1.73 (m, 2H), 1.45−1.22 (m, 14H), 1.16 (d, J = 6.1 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 154.6, 147.5, 144.5, 130.6, 129.2, 129.0 (2C), 125.0, 122.1, 118.9, 118.2, 113.2, 112.0, 68.1, 39.4, 29.6, 29.5, 29.4 (2C), 29.3, 29.2, 28.5, 25.7, 23.5, 11.8, 10.2; HRMS (ESI) calcd for $C_{26}H_{38}N_3O$ $(M + H)^+$ 408.3009, found 408.2997; IR (KBr) ν_{max} 3397, 3146, 2925, 2855, 1620, 1592, 1400 cm^{-1}.

*11-(5-((5-Ethyl-4′-methoxy-[2,2′-bipyrrol]-5′-ylidene)methyl)-pyrrol-2-yl)undecan-2-ol (**36**):* mp 90−92 °C; R_f = 0.30 (50% EtOAc/hexanes); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.80 (s, 1H), 6.56 (d, J = 3.5 Hz, 1H), 6.42 (d, J = 3.6 Hz, 1H), 6.06 (s, 1H), 5.82 (d, J = 3.5 Hz, 1H), 5.80 (d, J = 3.6 Hz, 1H), 3.96 (s, 3H), 3.81 (m, 1H), 2.16 (q, J = 7.5 Hz, 2H), 2.03 (q, J = 7.6 Hz, 2H), 1.47−1.12 (m, 19H), 0.91 (t, J = 7.6 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 169.2, 160.2, 144.5, 141.3, 138.5, 128.3, 127.1, 120.4, 114.9, 113.7, 108.2, 107.1, 95.8, 68.2, 58.4, 39.4, 29.7 (2C), 29.6 (2C), 29.4, 29.2, 27.1, 25.8, 23.5, 20.8, 13.4; HRMS (ESI) calcd for $C_{27}H_{39}N_3O_2$ $(M + H)^+$ 438.3115, found 438.3105; IR (KBr) ν_{max} 3429, 3129, 2929, 2848, 1618, 1576, 1402 cm^{-1}.

*11-(5-((5-(Furan-2-yl)-3-methoxypyrrol-2-ylidene)methyl)pyrrol-2-yl)undecan-2-ol (**37**):* mp 80−82 °C; R_f = 0.42 (60% EtOAc/hexanes); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.55 (d, J = 1.2 Hz, 1H), 6.97 (d, J = 3.3 Hz, 1H), 6.88 (s, 1H), 6.57 (d, J = 3.6 Hz, 1H), 6.54 (dd, J = 1.8, 3.4 Hz, 1H), 6.01 (d, J = 3.6 Hz, 1H), 5.98 (s, 1H), 3.88 (s, 3H), 3.78 (m, 1H), 2.72 (t, J = 7.5 Hz, 2H), 1.72 (m, 2H), 1.43−1.33 (m, 14H), 1.19 (d, J = 6.2 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 168.1, 156.3, 151.4, 143.9, 143.6, 140.7, 129.9, 120.6, 118.1, 112.2, 110.5, 109.5, 94.8, 68.2, 58.3, 39.4, 29.6 (2C), 29.4 (2C), 29.3, 28.9, 28.4, 25.8, 23.5; HRMS (ESI) calcd for $C_{25}H_{35}N_2O_3$ $(M + H)^+$ 411.2642, found 411.2625; IR (KBr) ν_{max} 3420, 3120, 2929, 2852, 1615, 1574, 1497 cm^{-1}.

*11-(5-((4′-Methoxy-1-methyl-[2,2′-bipyrrol]-5′-ylidene)methyl)-pyrrol-2-yl)undecan-2-ol (**38**):* mp 78−80 °C; R_f = 0.40 (60% EtOAc/hexanes); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.80 (d, J = 2.6 Hz, 1H), 6.76 (s, 1H), 6.70 (dd, J = 1.5, 3.8 Hz, 1H), 6.49 (d, J = 3.6 Hz, 1H), 6.21 (dd, J = 2.6, 3.8 Hz, 1H), 5.98 (d, J = 3.6 Hz, 1H), 5.91 (s, 1H), 4.17 (s, 3H), 3.88 (s, 3H), 3.76 (m, 1H), 2.65 (t, J = 7.5 Hz, 2H), 1.42 (m, 2H), 1.39−1.25 (m, 14H), 1.18 (d, J = 6.2 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 167.4, 159.4, 141.8, 140.9, 129.9, 128.8, 128.2, 118.2, 115.0, 114.7, 108.8, 108.5, 96.6, 68.2, 58.3, 39.4, 37.7, 29.6 (2C), 29.5, 29.4, 29.3, 29.2, 28.5, 25.8, 23.5; HRMS (ESI) calcd for $C_{26}H_{38}N_3O_2$ $(M + H)^+$ 424.2959, found 424.2941; IR (KBr) ν_{max} 3422, 3128, 2928, 2852, 1623, 1574, cm^{-1}.

Synthesis of 54a−c. Compounds **54a** (2.30 g, 94%), **54b** (2.32 g, 96%), and **54c** (2.29 g, 96%) were synthesized by the same procedure as described for [10-*d*]-**29**.

*10-Acetoxydecanoic acid (**54a**):* semisolid, R_f = 0.45 (40% EtOAc/hexanes); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.02 (t, J = 6.7 Hz, 2H), 2.31 (t, J = 7.5 Hz, 2H), 2.01 (s, 3H), 1.60 (m, 4H), 1.27 (m, 10H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 179.9, 171.4, 64.7, 34.0, 29.2, 29.1 (2C), 29.0, 28.5, 25.8, 24.6, 21.0; HRMS (ESI) calcd for $C_{12}H_{22}NaO_4$ $(M + Na)^+$ 253.1410, found 253.1403; IR (KBr) ν_{max} 2916, 2848, 1731, 1689, 1256, cm^{-1}.

*11-Acetoxyundecanoic acid (**54b**):* mp 35−37 °C; R_f = 0.50 (40% EtOAc/hexanes); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.02 (t, J = 6.7 Hz, 2H), 2.32 (t, J = 7.5 Hz, 2H), 2.02 (s, 3H), 1.58 (m, 4H), 1.28 (m, 12H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 180.0, 171.4, 64.7, 34.1, 29.4, 29.3, 29.2 (2C), 29.0, 28.6, 25.9, 24.6, 21.0; HRMS (ESI) calcd for $C_{13}H_{24}NaO_4$ $(M + Na)^+$ 267.1567, found 267.1559; IR (KBr) ν_{max} 2920, 2846, 1728, 1679 cm^{-1}.

*12-Acetoxydodecanoic acid (**54c**):* mp 40−42 °C; R_f = 0.55 (40% EtOAc/hexanes);^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.02 (t, J = 6.7 Hz, 2H), 2.31 (t, J = 7.5 Hz, 2H), 2.02 (s, 3H), 1.58 (m, 4H), 1.28 (m, 14H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 180.0, 171.4, 64.7, 34.1, 29.4 (3C), 29.3, 29.2, 29.0, 28.6, 25.9, 24.6, 21.0; HRMS (ESI) calcd for $C_{14}H_{26}NaO_4$ $(M + Na)^+$ 281.1723, found 281.1718; IR (KBr) ν_{max} 2916, 2850, 1731, 1691, 1256 cm^{-1}.

Synthesis of 55a−c and 56a−c. Compounds **55a** (614 mg, 59%), **55b** (601 mg, 55%), **55c** (641 mg, 56%), **56a** (73 mg, 7%), **56b** (87 mg, 8%), and **56c** (114 mg, 10%) were synthesized by the same procedure as described for [10′-*d*]-**30** and [10′-*d*]-**31**.

*10-Oxo-10-(pyrrol-2-yl)decyl acetate (**55a**):* syrup; R_f = 0.52 (40% EtOAc/hexanes); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 10.15 (br s, 1H), 7.03 (m, 1H), 6.91 (m, 1H), 6.24 (m, 1H), 4.02 (t, J = 6.8 Hz, 2H), 2.74 (t, J = 7.4 Hz, 2H), 2.02 (s, 3H), 1.71−1.55 (m, 4H), 1.28 (m, 10H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 191.5, 171.3, 132.0, 125.0, 116.6, 110.5, 64.6, 38.0, 29.4, 29.3 (2C), 29.2, 28.6, 25.9. 25.3, 21.0;

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HRMS (ESI) calcd for $C_{16}H_{26}NO_3$ $(M + H)^+$ 280.1907, found 280.1902; IR (KBr) ν_{max} 3283, 2929, 2856, 1739, 1639, 1404, 1240 cm^{-1}.

*11-Oxo-11-(pyrrol-2-yl)undecyl acetate (**55b**):* mp 53−55 °C; R_f = 0.65 (30% EtOAc/hexanes);^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.82 (br s, 1H), 7.02 (m, 1H), 6.90 (m, 1H), 6.25 (m, 1H), 4.04 (t, J = 6.6 Hz, 2H), 2.75 (t, J = 7.5 Hz, 2H), 2.03 (s, 3H), 1.70 (m, 2H), 1.60 (m, 2H), 1.27 (m, 12H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 191.3, 171.3, 132.0, 124.6, 116.2, 110.5, 64.7, 38.0, 29.4 (4C), 29.2, 28.6, 25.9. 25.3, 21.0; HRMS (ESI) calcd for $C_{17}H_{28}NO_3$ $(M + H)^+$ 294.2064, found 294.2057; IR (KBr) ν_{max} 3353, 3113, 2930, 2914, 2849, 1716, 1651, 1403 cm^{-1}.

*12-Oxo-12-(pyrrol-2-yl)dodecyl acetate (**55c**):* mp 42−44 °C; R_f = 0.55 (35% EtOAc/hexanes); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 10.14 (br s, 1H), 7.02 (m, 1H), 6.90 (m, 1H), 6.24 (m, 1H), 4.03 (t, J = 6.6 Hz, 2H), 2.74 (t, J = 7.5 Hz, 2H), 2.02 (s, 3H), 1.70 (m, 2H), 1.59 (m, 2H), 1.28 (m, 14H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 191.4, 171.2, 132.0, 124.9, 116.4, 110.4, 64.6, 38.0, 29.4 (5C), 29.2, 28.5, 25.9. 25.3, 21.0; HRMS (ESI) calcd for $C_{18}H_{30}NO_3$ $(M + H)^+$ 308.2220, found 308.2214; IR (KBr) ν_{max} 3349, 2912, 2850, 1714, 1644, 1405 cm^{-1}.

*10-Oxo-10-(pyrrol-3-yl)decyl acetate (**56a**):* syrup; R_f = 0.25 (40% EtOAc/hexanes); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.53 (br s, 1H), 7.40 (dd, J = 1.5, 2.8 Hz, 1H), 6.74 (m, 1H), 6.62 (m, 1H), 4.02 (t, J = 6.8 Hz, 2H), 2.72 (t, J = 7.5 Hz, 2H), 2.02 (s, 3H), 1.71−1.56 (m, 4H), 1.27 (m, 10H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 197.2, 171.4, 125.7, 123.3, 119.5, 108.5, 64.6, 39.7, 29.4, 29.3 (2C), 29.1, 28.5, 25.8, 25.1, 21.0; HRMS (ESI) calcd for $C_{16}H_{26}NO_3$ $(M + H)^+$ 280.1907, found 280.1905; IR (KBr) ν_{max} 3279, 2928, 2848, 1566, 1472, 1400 cm^{-1}.

*11-Oxo-11-(pyrrol-3-yl)undecyl acetate (**56b**):* mp 39−41 °C; R_f = 0.32 (30% EtOAc/hexanes);^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.45 (br s, 1H), 7.41 (m, 1H), 6.75 (dd, J = 2.3, 4.9 Hz, 1H), 6.63 (m, 1H), 4.03 (t, J = 6.6 Hz, 2H), 2.73 (t, J = 7.6 Hz, 2H), 2.02 (s, 3H), 1.69 (m, 2H), 1.59 (m, 2H), 1.28 (m, 12H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 197.2, 171.4, 125.8, 123.3, 119.5, 108.6, 64.7, 39.7, 29.4 (3C), 29.3, 29.2, 28.5, 25.8, 25.1, 21.0; HRMS (ESI) calcd for $C_{17}H_{28}NO_3$ $(M + H)^+$ 294.2064, found 294.2056; IR (KBr) ν_{max} 3353, 3115, 2915, 2853, 1720, 1664, 1401 cm^{-1}.

*12-Oxo-12-(pyrrol-3-yl)dodecyl acetate (**56c**):* mp 44−46 °C; R_f = 0.30 (35% EtOAc/hexanes); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.31 (br s, 1H), 7.42 (m, 1H), 6.75 (dd, J = 2.3, 4.9 Hz, 1H), 6.64 (m, 1H), 4.03 (t, J = 6.6 Hz, 2H), 2.73 (t, J = 7.6 Hz, 2H), 2.03 (s, 3H), 1.69 (m, 2H), 1.59 (m, 2H), 1.28 (m, 14H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 197.4, 171.4, 125.9, 123.2, 119.5, 108.7, 64.7, 39.8, 29.5 (5C), 29.2, 28.6, 25.9, 25.1, 21.0; HRMS (ESI) calcd for $C_{18}H_{30}NO_3$ $(M + H)^+$ 308.2220, found 308.2218; IR (KBr) ν_{max} 3338, 2920, 2846, 1718, 1657, 1401 cm^{-1}.

Synthesis of 57a−c. Compounds **57a** (287 mg, 72%), **57b** (283 mg, 70%), and **57c** (314 mg, 77%) were synthesized by the same procedure as described for [10′-*d*]-**9**.

*2-(10-Hydroxydecyl)pyrrole (**57a**):* mp 57−59 °C; R_f = 0.50 (40% EtOAc/hexanes); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.05 (br s, 1H), 6.64 (m, 1H), 6.14 (dd, J = 2.8, 5.7 Hz, 1H), 5.92 (m, 1H), 3.64 (t, J = 6.6 Hz, 2H), 2.59 (t, J = 7.6 Hz, 2H), 1.64−1.54 (m, 4H), 1.33 (m, 12H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 132.9, 116.0, 108.1, 104.7, 63.0, 32.7, 29.6, 29.5, 29.4 (2C), 29.3 (2C), 27.7, 25.7; HRMS (ESI) calcd for $C_{14}H_{26}NaNO$ $(M + Na)^+$ 247.1907, found 247.1916; IR (KBr) ν_{max} 3356, 2928, 2848, 1566, 1472, 1400 cm^{-1}.

*2-(11-Hydroxyundecyl)pyrrole (**57b**):* mp 63−65 °C; R_f = 0.60 (30% EtOAc/hexanes); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.07 (br s, 1H), 6.66 (dd, J = 2.6, 4.1 Hz, 1H), 6.14 (dd, J = 2.8, 5.7 Hz, 1H), 5.92 (d, J = 1.0 Hz 1H), 3.64 (t, J = 6.5 Hz, 2H), 2.60 (t, J = 7.6 Hz, 2H), 1.65−1.54 (m, 4H), 1.33 (m, 14H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 132.9, 115.9, 108.1, 104.7, 62.9, 32.7, 29.6, 29.5 (3C), 29.4 (2C), 29.3, 27.7, 25.7; HRMS (ESI) calcd for $C_{15}H_{28}NaNO$ $(M + Na)^+$ 261.2063, found 261.2055; IR (KBr) ν_{max} 3355, 3147, 2930, 2847, 1566, 1400 cm^{-1}.

*2-(12-Hydroxydodecyl)pyrrole (**57c**):* mp 69−71 °C; R_f = 0.65 (30% EtOAc/hexanes); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.02 (br s, 1H), 6.66 (m, 1H), 6.13 (dd, J = 2.8, 5.8 Hz, 1H), 5.92 (d, J = 0.7 Hz 1H), 3.64 (t, J = 6.5 Hz, 2H), 2.59 (t, J = 7.6 Hz, 2H), 1.66−1.54 (m, 4H), 1.28 (m, 16H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 132.9, 116.0, 108.1, 104.8, 63.0, 32.8, 29.7 (3C), 29.6 (2C), 29.5 (2C), 29.4, 27.7, 25.7; HRMS (ESI) calcd for $C_{16}H_{30}NaNO$ $(M + Na)^+$ 275.2220, found 275.2209; IR (KBr) ν_{max} 3362, 2928, 2853, 1564, 1402 cm^{-1}.

Synthesis of Podiginines 39−41. Compounds **39** (83 mg, 80%), **40** (85 g, 79%), and **41** (83 g, 75%) were synthesized by the same procedure as described for (23*S*)-**2**.

*10-(5-((4′-Methoxy-[2,2′-bipyrrol]-5′-ylidene)methyl)pyrrol-2-yl)-decan-1-ol (**39**):* mp 118−120 °C; R_f = 0.35 (50% EtOAc/hexanes); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.85 (s, 1H), 6.68 (dd, J = 1.3, 3.6 Hz, 1H), 6.62 (dd, J = 1.3, 2.6 Hz, 1H), 6.46 (d, J = 3.6 Hz, 1H), 6.12 (dd, J = 2.6, 3.6 Hz, 1H), 6.11 (s, 1H), 5.82 (d, J = 3.6 Hz, 1H), 3.97 (s, 3H), 3.64 (t, J = 6.6 Hz, 2H), 2.06 (t, J = 7.7 Hz, 2H), 1.55 (m, 2H), 1.38−1.27 (m, 14H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 169.1, 160.2, 144.6, 138.6, 128.4, 128.3 123.0, 121.0, 116.0, 112.8, 110.0, 108.6, 95.7, 63.0, 58.4, 32.8, 29.6 (2C), 29.5 (2C), 29.3, 29.2, 27.3, 25.8; HRMS (ESI) calcd for $C_{24}H_{34}N_3O_2$ $(M + H)^+$ 396.2646, found 396.2634; IR (KBr) ν_{max} 3367, 3124, 2912, 2846, 1630, 1578, 1490 cm^{-1}.

*11-(5-((4′-Methoxy-[2,2′-bipyrrol]-5′-ylidene)methyl)pyrrol-2-yl)-undecan-1-ol (**40**):* mp 108−110 °C; R_f = 0.35 (50% EtOAc/hexanes); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.86 (s, 1H), 6.67 (dd, J = 1.0, 3.5 Hz, 1H), 6.61 (br s, 1H), 6.47 (d, J = 3.6 Hz, 1H), 6.12 (dd, J = 2.7, 3.5 Hz, 1H), 6.08 (s, 1H), 5.82 (d, J = 3.6 Hz, 1H), 3.97 (s, 3H), 3.64 (t, J = 6.6 Hz, 2H), 2.05 (t, J = 7.6 Hz, 2H), 1.57 (m, 2H), 1.37−1.15 (m, 16H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 169.1, 160.3, 144.7, 138.6, 128.4, 128.3 123.0, 121.1, 116.0, 112.9, 110.0, 108.6, 95.8, 63.0, 58.4, 32.8, 29.7 (3C), 29.6, 29.5, 29.4, 29.2, 27.3, 25.8; HRMS (ESI) calcd for $C_{25}H_{36}N_3O_2$ $(M + H)^+$ 410.2802, found 410.2793; IR (KBr) ν_{max} 3410, 3134, 2920, 2847, 1631, 1566, 1401 cm^{-1}.

*12-(5-((4′-Methoxy-[2,2′-bipyrrol]-5′-ylidene)methyl)pyrrol-2-yl)-dodecan-1-ol (**41**):* mp 121−123 °C; R_f = 0.25 (50% EtOAc/hexanes); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.85 (s, 1H), 6.66 (m, 1H), 6.62 (br s, 1H), 6.46 (d, J = 3.7 Hz, 1H), 6.12 (m, 1H), 6.08 (s, 1H), 5.82 (d, J = 3.7 Hz, 1H), 3.97 (s, 3H), 3.64 (t, J = 6.6 Hz, 2H), 2.07 (t, J = 7.6 Hz, 2H), 1.57 (m, 2H), 1.35−1.16 (m, 18H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 169.1, 160.2, 144.6, 138.6, 128.4, 128.3 122.9, 121.0, 116.0, 112.8, 110.0, 108.6, 95.7, 63.1, 58.4, 32.8, 29.6 (5C), 29.5, 29.4, 29.2, 27.3, 25.8; HRMS (ESI) calcd for $C_{26}H_{38}N_3O_2$ $(M + H)^+$ 424.2959, found 424.2948; IR (KBr) ν_{max} 3397, 3123, 2915, 2848, 1634, 1580, 1489 cm^{-1}.

Bacterial Strains, Plasmids, and Cosmid. *S. venezuelae* ATCC 15439 was used as a heterologous host for *marG* overexpression.[14] *Escherichia coli* One Shot Top10 (Invitrogen) was used as a host strain for the construction of recombinant plasmids. *E. coli* ET12567 (pUZ8002) was used as donor strain in conjugation experiments.[29] pCR8-GW-TOPO (Invitrogen) was used as cloning vector. pSE34 is a *E. coli-Streptomyces* shuttle vector, which contains pIJ101 derivative replication element and the constitutive strong *ermE** promoter. pIJ778 is a plasmid containing an *OriT* transfer element required for conjugation and the spectinomycin-resistant gene *aadA*. All bacterial strains, plasmids, and cosmid used in this study are given in Table S1 (Supporting Information).

General DNA Manipulations. 8A7, a SuperCosl cosmid carrying the entire *mar* gene cluster, was isolated by the standard protocol.[14,30] DNA fragments were recovered from an agarose gel by using the QIAquick Gel Extraction Kit (QIAGEN). Restriction endonucleases were purchased from New England Biolab. Preparation of plasmid DNA was done by using a QIAprep Spin Miniprep Kit (QIAGEN). All other DNA manipulations were performed according to standard protocols.[30,31] PCR was performed in 35 cycles by using a GeneAmp PCR system 2700 (Applied Biosystems). Platinum *Taq* DNA polymerase high fidelity (Invitrogen) was used for amplification of *marG* gene. Oligodeoxyribonucleotides for PCR primers were synthesized by Integrated DNA Technologies, and their sequences are shown in Table S2 (Supporting Information). The nucleotide sequences of the gene fragment were determined at the MMI DNA analysis core facility, Oregon Health and Sciences University (Portland, OR, USA).

Generation of marG Overexpression Strain. The *marG* ORF was amplified using forward primer *marG*-15bF, introducing a unique

NdeI site at the 5′-end of the gene, and the reverse primer *marG*-15bR, introducing a unique *Bam*HI site downstream to the TGA translational stop codon. Cosmid DNA 8A7 was used as a template. The PCR fragment was first cloned into the pCR8-GW-TOPO vector. The 1.4-kbp NdeI-*Bam*HI insert was further subcloned into pET15b to generate pMarG-15b. After sequencing to confirm the inset, this plasmid was digested with XbaI and *Hin*dIII. The fragment containing the *marG* gene, along with the ribosome binding site (RBS), was cloned into the corresponding sites of pSE34 to generate pMarG-34. The ampicillin resistance marker *bla* on the pMarG-34 plasmid was replaced by *aadA-oriT* cassette generated by PCR from pIJ778 with primers *Amp-SpF/Amp-SpR* using the standard method of PCR targeting.[32] The resulting *pMarG-34S* was introduced into *S. venezuelae* strain by conjugation following the established protocol, except mannitol-soy flour agar was replaced with AS1 media containing 10 mM $MgCl_2$.[33] Thiostrepton-resistant exconjugants representing *S. venezuelae* strains host with *marG* overexpression plasmids. The new strain was named *S. venezuelae* MarG.

Media, Culture Techniques, and Feeding Experiments. Recombinant *S. venezuelae* MarG was grown on sporulation agar (SPA; 0.1% yeast extract, 0.1% beef extract, 0.2% tryptose, 0.01% ferrous sulfate, 1.0% glucose, and 2.0% Difco bacto agar) containing 50 μg/mL of spectinomycin at 30 °C for 7 days. Fresh spores were used to inoculate 10 mL of SCM seed cultures (1.5% soluble starch, 2.0% soytone, 0.01% $CaCl_2$, 0.15% yeast extract and 1.0% MOPS, pH 7.2) containing 10 μg/mL of spectinomycin and were incubated at 30 °C for 3 days. Production cultures were prepared with the same medium (150 mL each flask) and inoculated with seed cultures (5.0% v/v). The production cultures were grown in Baffled Erlenmeyer flasks at 30 °C with vigorous shaking (220 rpm). After incubation for 3 days, the cultures were grouped into four groups; the first group was fed with (23*S*)-**2** (2.0 mg in 200 μL of DMSO), the second group was fed with [23-*d*]-**2** (2.0 mg in 200 μL of DMSO), the third group was fed with **3** (2.0 mg in 200 μL of DMSO), and the fourth group (control group) was fed with 200 μL of DMSO. After 4 days of incubation, the cultures were harvested by centrifugation at 5000 rpm, 4 °C for 20 min. The harvested cells were disrupted by the addition of 20 mL of methanol solution containing 1.0% 2 N HCl followed by vortex for 1 min. After filtration using cotton wool, the methanol extract was dried under reduced pressure and stored at −20 °C until further analysis. The same procedures were used for other analogues.

Premarineosin Production Analysis. The crude methanolic extract was dissolved in methanol, centrifuged, and filtered through a 0.22 μm filter to give a clear solution, and this was used for our analysis. Analytical HPLC and LC-MS analyses were performed on a C18 column (2.1 mm × 250 mm, 5 μm) with a linear elution gradient as specified in Table S4 (Supporting Information). The molecular weight of each compound was determined by electrospray mass spectrometry. Extracted ion chromatograms and tandem MS analyses were performed to analyze the consumption of feeding substrates and the production of premarineosins.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Figures giving spectra of all synthesized compounds, tables giving cosmids, strains, and primers, and figures giving HPLC, MS, MS/MS, and LC-MS profiles. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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