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Structure, Chemical Synthesis, and Biosynthesis of Prodiginine Natural Products

Dennis X. Hu[†], **David M. Withall**[‡], **Gregory L. Challis**[‡,*], and **Regan J. Thomson**[†,*]

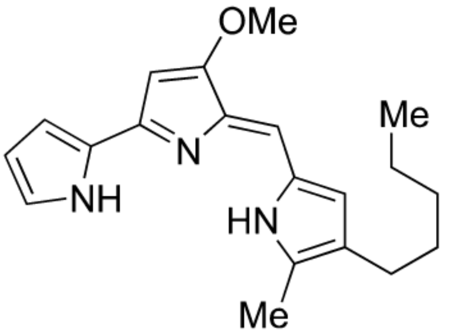
[†]Department of Chemistry, Northwestern University, Evanston, Illinois 60208, United States

[‡]Department of Chemistry, University of Warwick, Coventry CV4 7AL, United Kingdom

Abstract

The prodiginine family of bacterial alkaloids is a diverse set of heterocyclic natural products that have likely been known to man since antiquity. In more recent times, these alkaloids have been discovered to span a wide range of chemical structures that possess a number of interesting biological activities. This review provides a comprehensive overview of research undertaken toward the isolation and structural elucidation of the prodiginine family of natural products. Additionally, research toward chemical synthesis of the prodiginine alkaloids over the last several decades is extensively reviewed. Finally, the current, evidence-based understanding of the various biosynthetic pathways employed by bacteria to produce prodiginine alkaloids is summarized.

Graphical Abstract



prodiginine natural products

- Structure elucidation
- Chemical synthesis
- Biosynthesis

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[*]Corresponding Authors: g.l.challis@warwick.ac.uk (G.L.C.). r-thomson@northwestern.edu (R.J.T.).

Notes

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1. INTRODUCTION

In the summer of 1819, the apparently spontaneous, brilliant reddening of a farmer's polenta (boiled cornmeal) created a stir in Padua, Italy.[1] Local peasants called the occurrence "bloody polenta", believing it to be of diabolical origin, and implored priests to banish the evil spirits behind the event. The hype over "bloody polenta" achieved such a state that the Paduan police department appointed a committee, composed mostly of professors from the University of Padua, to investigate the reports. Among the committee members was the pharmacist Bartolomeo Bizio, who demonstrated through experiment that the development of red color on polenta was dependent on humidity, that the presence of "foul air" accelerated the development of red color, and that the cause of red color was transmissible both by air and by contact.

Bizio therefore concluded that the discoloration was not supernatural but caused by the growth of a microorganism, erroneously classifying the bacterium as a fungus due to its ability to reproduce even after exposure to camphor, turpentine, or tobacco vapors and high temperature. Bizio named the organism *Serratia marcescens*. The descriptor marcescens, derived from the Latin word meaning "decaying," was chosen due to his observation that the organism dissolved into a red fluid and mucus-like matter upon reaching maturity. Bizio's deductions were remarkable as they were made before the birth of Pasteur. The brilliant red pigment that Bizio attempted to isolate from *Serratia* would be identified much later and named prodigiosin (Figure 1).

Today, it is speculated that because of its ability to grow on cereal grains, its dripping fluid-like appearance, and its ability to produce the bright red pigment prodigiosin, *S. marcescens* is responsible for the numerous accounts of "bleeding bread" phenomena in history, akin to the aforementioned 19th-century event in Padua.[2] Recorded events date as far back as 322 B.C., when soldiers under Alexander the Great believed they saw blood inside a piece of bread, an omen interpreted as foreshadowing victory in a coming battle. Perhaps the best known example of such events is the Eucharistic miracle of Bolsena. In a 1263 celebration of Mass, a Roman Catholic priest skeptical of the Catholic doctrine of transubstantiation is believed to have found blood smattered over the bread he broke as the host for Communion. It was only after the investigation of this incident that Pope Urban IV issued a papal bull in 1264 establishing Corpus Christi as the first universal feast throughout the Latin Rite. The event is immortalized in the Vatican by one of Raphael's Stanze, "The Mass at Bolsena" (1512). The role played by prodigiosin in such "prodigious" events is aptly reflected in its name.

Interest in the prodiginines is not limited to their role in human culture. As early as 1823, Bizio thought to apply the red alcohol extract of *Serratia* in the dyeing of silk and wool.[1] Though prodigiosin itself was found to be too light-sensitive for practical application as a dye, in more recent times, the red color of prodigiosin has made its biosynthetic pathway a useful model system for bacterial quorum sensing research[3] as well as a useful educational tool for university-level biology students.[4]

The prodiginines have been continuously investigated for medically relevant properties including antimalarial activity,[5–9] a distinct mechanism of immunosuppression from cyclosporine A, FK506, and rapamycin,[10–19] and their ability to selectively induce apoptosis in many human cancer cell lines.[8,20–27] A synthetic analogue based on the prodiginine family, obatoclax, was used in multiple phase I and II combination cancer chemotherapy studies.[28–33] The quorum sensing control of prodigiosin biosynthesis and medicinal properties of the prodiginines have been previously reviewed.[3,8,24,34,35]

In this review, efforts in the isolation, structural elucidation, total syntheses, and biosyntheses of the prodiginine family of natural products will be discussed, placing in context the relationships between discoveries in the aforementioned fields. In particular, the role of total synthesis in the structural elucidation and structural revision of the prodiginines will be highlighted, updating and elaborating on a prior review from 2003 by Fürstner.[36]

2. ISOLATION AND STRUCTURAL ELUCIDATION

The structures of the prodiginines have consistently elicited curiosity in the chemical and biological communities, and new structural questions about these natural products have continued to arise even since the complete identification of prodigiosin in 1962. The following section recounts efforts to isolate and elucidate the structures of prodiginines between 1920 and 2015.

2.1. Prodigiosin

Prodigiosin was first isolated in pure form from *Serratia marcescens* (then called *Bacillus prodigiosus*) in 1929 by Wrede and Hettche[37] at the University of Greifswald. On the basis of degradation studies, Wrede determined that prodigiosin contained three pyrrole groups linked in an unknown fashion: pyrrole, 3-methoxypyrrole, and 2-methyl-3-amylpyrrole.[38] In 1933, Wrede and Rothhaas[39,40] suggested structures **1**, **2**, and **3** for prodigiosin, arbitrarily favoring the tripyrrylmethene structure **3** (Figure 2).

For the next 20 years, synthetic efforts focused on preparing tripyrrylmethene structures related to **2** and **3**, but comparisons of synthetic model compounds (e.g., **4**, Figure 3) with isolated prodigiosin could neither definitively confirm nor refute Wrede's favored structure **3**, since the synthetic tripyrrylmethenes differed considerably in substitution from prodigiosin.

It was not for another three decades after Wrede's proposal that definitive evidence against structures **2** and **3** became available. In 1956, Santer and Vogel[41] isolated a compound with the formula $C_{10}H_{10}O_2N_2$ from an *S. marcescens* mutant blocked in prodigiosin biosynthesis and demonstrated that this compound could be converted to prodigiosin by a second mutant. Wasserman et al. realized that prodigiosin ($C_{20}H_{25}ON_3$) was the formal condensation product of Vogel's compound and Wrede's degradation product 2-methyl-3-amylpyrrole ($C_{10}H_{17}N$, **7**), and in 1960[42] they reported the partial synthesis of prodigiosin through treatment of Vogel's compound with **7** under acidic conditions. Further degradation studies on Vogel's compound by the same group suggested that it contained two α,α'-linked pyrroles and an aldehyde group, implying that tripyrrylmethene structures **2** and **3** for prodigiosin were highly unlikely.

In 1962, Rapoport and Willson[43] reported the syntheses of bipyrrole aldehydes **5** and **6**, as well as the results of condensation of both materials with 2-methyl-3-amylpyrrole **7** and isomers of **7**. They found that only the condensation of 4-methoxy-2,2′-bipyrrole-5-carbaldehyde (MBC, **6**) with pyrrole **7** provided material that was analytically identical to naturally occurring prodigiosin, firmly establishing the identity of Vogel's $C_{10}H_{10}O_2N_2$ compound as **6** and of prodigiosin as **1** (Figure 4).

2.2. Close Analogues of Prodigiosin

Since Rapoport and Willson's[43] 1962 structural elucidation of prodigiosin, several closely related natural products have been discovered (Figure 5). Among them are alkyl-chain homologues, such as propylprodigiosin (**8**),[44] butylprodigiosin (**9**),[44] hexylprodigiosin (**11**),[44,45] and heptylprodigiosin (**12**).[46] Such metabolites likely arise through incorporation of a different number of malonyl-CoA extender units during fatty acid biosynthesis or the utilization of propionyl-CoA instead of acetyl-CoA as a starter unit by the fatty acid synthase (see section 4.4).[47]

To date, only mass spectrometric data have been reported in support of the structures of the alkyl-chain homologues other than heptylprodigiosin.[48] The O-desmethyl analogue of prodigiosin, norprodigiosin, was isolated from *S. marcescens* mutants and chemically characterized in 1964 by Hearn et al.[49] Norprodigiosin (**13**) likely arises through the condensation of 4-hydroxy-2,2′-bipyrrole-5-carbaldehyde, MBC, with 2-methyl-3-amylpyrrole, MAP (see section 4.5).

In 2008, Hemscheidt and co-workers[50] reported the isolation of the first prodigiosin analogue with a substituent at C-2 of ring A, 2-(*p*-hydroxybenzyl)prodigiosin (**14**), from *Pseudoalteromonas rubra* (Figure 6). Taken from the surface of a nudibranch obtained from the waters off Oahu, Hawaii, this compound was found to have broad-spectrum activity against *Escherichia coli*, *Staphylococcus aureus*, methicillin-resistant *S. aureus*, *Candida albicans*, and human ovarian adenocarcinoma cells. This represents the first member of a new class of acyclic 2-substituted prodiginines. On the basis of the currently proposed biosynthetic pathway for *S. marcescens* (see section 4.2), it is not clear how such a compound would arise. Hemscheidt and co-workers have speculated that **14** may be the result of nonribosomal peptide synthase incorporation of *p*-hydroxybenzyl-substituted proline into pyrrole ring A in place of proline (see section 4.2).

2.3. Cycloprodigiosin

In 1979, nearly 20 years after the structural elucidation of prodigiosin, Gerber and Gauthier[51] isolated a unique cyclic analogue of prodigiosin from *Alteromonas rubra*, a marine bacterium from Mediterranean coastal waters. Notably, this new prodigiosin was not produced by strains previously found to produce prodigiosin, including *S. marcescens*. On the basis of mass spectrometric and ^{1}H NMR data, Gerber assigned the cyclopentane structure **15** to the new compound (Figure 7).

Four years later, Lattasch and Thomson[52] reisolated the cyclic prodigiosin analogue and came to the conclusion that this compound, which they called cycloprodigiosin, should be assigned the structure **16** instead of **15**. Lattasch and Thomson attributed Gerber's misassignment to the presence of an aliphatic impurity in Gerber's original sample, which caused a triplet to appear at 0.95 ppm in the ^1H NMR spectrum, leading Gerber to incorrectly conclude that a methylene group was present between the methyl group and the aliphatic ring. Simultaneously, Gerber[53] discovered a salt-water marsh bacterium, *Beneckea gazogenes*, which provided much higher yields of cycloprodigiosin than *A. rubra*. With greater quantities of cycloprodigiosin in hand, Gerber was able to procure ^{13}C NMR data that allowed her to reassign its structure to **16**. To resolve lingering confusion regarding the structure of cycloprodigiosin, Wasserman and Fukuyuma[54] completed a short total synthesis of the racemate of **16**. Wasserman's synthetic material proved to be "identical with samples of the natural product derived from both *B. gazogenes* and *A. rubra*", confirming the structure of cycloprodigiosin as **16**.

It was a further three decades after the gross structural assignment of cycloprodigiosin (**16**) was confirmed that information regarding its stereochemical configuration came to light. In 2015, Sarpong and co-workers[55] reported the synthesis of both enantiomers of cycloprodigiosin (**16**) through an efficient synthetic route (see section 3.2). Isolation of natural cycloprodigiosin (**16**) from *Pseudoalteromonas rubra* (Gauthier) (ATCC 29570) enabled subsequent determination that the natural material was produced by the microorganism as a scalemic mixture of (*R*)-**16** and (*S*)-**16** in a ratio of 83:17 (Figure 8).

2.4. Undecylprodigiosin

In 1961, shortly after Wasserman reported a partial synthesis of prodigiosin, Perry[56] reported the identification of a red pigment from an actinobacterium belonging to the *Streptomyces* genus. Perry claimed that the isolated pigment was identical to that isolated from *S. marcescens*, based on UV–vis spectra recorded in acid and alkaline solutions and on thin-layer chromatographic (TLC) analysis. In 1966, however, Wasserman et al.[58] at Yale and Nagatsu and co-workers[57] in Tokyo independently disclosed the isolation of the same pigment from two strains of *Streptomyces* and determined that it possessed the chemical formula $C_{25}H_{35}N_3O$. Nagatsu and co-workers assigned structure **17** to this compound and named it "prodigiosin-25 C" on the basis of NMR, mass spectrometric, and degradation studies (Figure 9).

The Wasserman group found that the isolated pigment could be synthesized by condensation of 2-undecylpyrrole with previously synthesized aldehyde **6**, and thus named the compound undecylprodigiosin. Since 1966, undecylprodigiosin has been isolated from numerous other actinobacterial genera, including *Streptoverticillium*, *Actinomadura*, and *Saccharopolyspora*, but it has not been isolated from proteobacteria.[3] Similarly, prodigiosin has been isolated from several different genera of proteobacteria (*Serratia*, *Hahella*, *Pseudomonas*, *Vibrio*), but it has not been reported to be produced by an actinobacterium. An evolutionary explanation for these observations has yet to be proposed.

2.5. Metacycloprodigiosin

When Wasserman et al.[58] first elucidated the structure of undecylprodigiosin from *Streptomyces longisporus ruber*, they noted that a second, more complex, 25-carbon pigment with an additional element of unsaturation ($C_{25}H_{33}N_3O$, vs $C_{25}H_{35}N_3O$ for undecylprodigiosin) was also produced. Several years earlier, Wasserman et al.[59] had suggested that this compound might be a prodigiosin analogue derived from 2-methyl-3-heptyl-4-propylpyrrole (Figure 10). Shortly thereafter, they showed this hypothesis was false by synthesizing 2-methyl-3-heptyl-4-propylpyrrole (**18**) and condensing it with MBC **6** (Figure 10). This reaction yielded a prodiginine (**19**) with properties that differed from those of the natural product in many respects.[60]

Three years after they reported the structural elucidation of undecylprodigioisn, Wasserman et al.[61] deduced the correct structure for the desaturated undecylprodigiosin derivative produced by *S. longisporus ruber*. The structure was confirmed by total synthesis of a racemic sample and the compound was named metacycloprodigiosin (**20**, Figure 11).[62] With the benefit of hindsight, this was a confusing choice of nomenclature, because other meta-disubstituted prodiginines were subsequently discovered (e.g., see section 2.6).

Wasserman et al.[62] also noted that metacycloprodigiosin is optically active. This was significant because it indicated that carbocyclic prodiginine derivatives, such as metacycloprodigiosin and cycloprodigiosin, are enzymatically biosynthesized and do not simply result from adventitious oxidation of the corresponding precursors (**1** and **17**, respectively). Indeed, in 2001, Challis and co-workers[63] postulated that an orthologue of the *Streptomyces coelicolor* Rieske oxygenase-like enzyme RedG is likely responsible for the oxidative cyclization of undecylprodigiosin (**17**) to metacycloprodigiosin (**20**). A decade later, Challis and co-workers[64] reported that RedG catalyzes the conversion of undecylprodigiosin (**17**) to streptorubin B (**22**) and that McpG, a RedG orthologue from *S. longisporus ruber*, catalyzes the conversion of undecylprodigiosin (**17**) to metacycloprodigiosin (**20**) (see section 4.6).

In 2009, 40 years after metacycloprodigiosin’s discovery, Clift and Thomson[65] completed the first enantioselective total synthesis of metacycloprodigiosin in an effort to determine the absolute configuration of the natural product (see section 3.3). Although a natural sample of metacycloprodigiosin could not be obtained at the time of the report, subsequent circular dichroic (CD) spectroscopic comparison of synthetic (*R*)-metacycloprodigiosin with natural metacycloprodigiosin isolated from *S. longisporus ruber* showed that the absolute configuration of natural metacycloprodigiosin is *R* (as shown in Figure 12).[66]

2.6. Streptorubin B

In 1975, Gerber[67] reported the isolation and structural elucidation of two pink pigments from *Streptomyces* sp. Y-42, isolated from leaf and grass compost. One pigment was easily identified as undecylprodigiosin (**17**) on the basis that its chromic acid oxidation product was undecanoic acid. The physical properties of the second pigment strongly resembled those of metacycloprodigiosin (**20**), but the two compounds had different TLC R_f values and produced significantly different fragment ions in mass spectrometric analyses. On the basis of chemical degradation of the second pigment and the observation that it possessed an additional element of unsaturation compared to undecylprodigiosin ($m/z = 391$ for the unidentified pigment versus 393 for undecylprodigiosin-H^+), Gerber narrowed down the structure of the mysterious pigment to ortho- and meta-bridged isomers of butylcycloheptylprodigiosin, **21** and **22**, respectively (Figure 13).

On the basis of comparisons of the NMR spectra for other ortho- and meta-substituted pyrroles with those for the desaturated undecylprodigiosin derivative, Gerber assigned structure **21** to the isolate. A few months later, Gerber[67] reported the isolation of two pink pigments from *Streptoverticillium rubrireticuli*, a bacterium known to cause problematic pink staining of PVC plastics, and assigned them the same structures (**17** and **21**) as the Y-42 pigments. Interestingly, in 1964, Thirumalachar et al.[68] isolated a pink pigment from a *Streptomyces* species that they called “streptorubrin B”. Detailed structural characterization of this pigment was not, however, reported. In 1976, Gerber and Lechevalier[69] found that butylcycloheptylprodigiosin was identical to streptorubrin B, which has been arbitrarily renamed as streptorubin B in recent literature. In an effort to remain consistent, we will henceforth refer to streptorubrin B as streptorubin B.

Curiously, in the course of subsequent biosynthetic studies, Gerber et al.[70] reassigned the structure of streptorubin B from the ortho-bridged isomer **21** to the meta-bridged isomer **22**, without commenting on the reasons for the reassignment, which appeared to go unnoticed. In 1985, Floss and co-workers[71] reported the isolation of two pigments from *S. coelicolor* A3(2): undecylprodigiosin (**17**), and a pink pigment of molecular mass 391 Da with spectral data “closely match[ing] those reported [by Gerber] for butylcycloheptylprodigiosin”. Thus, Floss assigned structure **21** to his pigment, apparently unaware that Gerber had reassigned the structure of streptorubin B to **22**.

In 1991, Weyland and co-workers[72] isolated an actinomycete strain (B 4358) that was found also to produce a prodiginine alkaloid with the molecular formula $C_{25}H_{33}N_3O$. On the basis of extensive NMR spectroscopic analysis, structure **22** was assigned to this compound, which had strikingly similar NMR data to that reported by Gerber for streptorubin B. Thus, it was proposed that streptorubin B should also be assigned structure **22**, in agreement with Gerber’s 1978 reassignment. Moreover, an additional signal with a chemical shift of −1.55 ppm was noted in the ^1H NMR spectrum of streptorubin B that had not been previously reported. This provided further evidence that streptorubin B is the meta isomer of butylcycloheptylprodigiosin, because force field calculations predicted that one of the hydrogen atoms attached to C-4′ would lie within the anisotropy cone of the ansa-bridged pyrrole (Figure 14).

In 2005, the structure of butylcycloheptylprodigiosin isolated by Gerber and Floss was called into question by Fürstner et al.,[73] who noted that neither had reported a signal at −1.55 ppm in the ^1H NMR spectra of the pigments they isolated (note, however, that Floss and co-workers[71] did not collect data below −0.5 ppm). Because Gerber, Floss, and Weyland had isolated their pigments from different strains of bacteria, Fürstner argued that there was a possibility that *o*-butylcycloprodigiosin **21** could, in fact, be a natural product. Thus, Fürstner et al.[73] undertook a total synthesis of **21** (see section 3.5), reporting that the ^1H

NMR spectrum of this material was an excellent match with the corresponding spectrum of the natural product from *S. coelicolor* recorded by Floss. On this basis, Fürstner concluded that *o*-butylcycloheptylprodigiosin (**21**) is a distinct natural product from streptorubin B (**22**), as originally suggested by Gerber in 1975. In 2007, Reeves[74] also published a synthesis of racemic **21** (see section 3.5) and came to the same conclusion, following correspondence with Floss and Fürstner.

In 2008, during the course of prodiginine biosynthetic studies in *S. coelicolor* A3(2) (the same species from which Floss isolated undecylprodigiosin and butylcycloheptylprodigiosin in 1985), Challis and co-workers[75] isolated the carbocyclic undecylprodigiosin derivative produced by *S. coelicolor* and showed, by an extensive array of 1D and 2D NMR experiments, that this possesses the meta-bridged structure **22**. Moreover, there was no evidence for production of a metabolite with structure **21.** In 2011, Thomson and co-workers[66] completed the first enantioselective total synthesis of streptorubin B (**22**) (see section 3.4) and compared its ^{1}H NMR spectrum with that provided by Floss for the carbocyclic derivative of undecylprodigiosin he had originally isolated from *S. coelicolor*. Due to significant impurities in the material isolated by Floss, Thomson was unable to draw any firm conclusions from this comparison. However, a short total synthesis of **21** was devised (see section 3.5), and comparison of the fragment ions observed in electron ionization (EI) mass spectra of synthetic **21** and **22** showed significant, reproducible differences.[76] Further comparison of the fragment ions observed for the synthetic compounds with both Gerber's and Floss's original fragmentation data revealed an excellent match between the natural isolates and synthetic **22** but not synthetic **21**. Thus, Thomson and co-workers confirmed that *o*-butylcycloheptylprodigiosin (**21**) is a structural misassignment, as originally proposed by Gerber and Weyland.

Thomson and co-workers[76] speculated that structural changes between *o*-butylcycloheptylprodigiosin **21** and streptorubin B (**25**) might alter the energetic differences between conformational isomers resulting from potential rotation of the bis(pyrrole) side arm relative to the carbocyclic core (Figure 15). The cis isomer (**23** or **25b**) is believed to possess an optimal juxtaposition of functional groups for activities such as anion transport and cation binding.[77,78] An evolutionary advantage may therefore be conferred on bacteria that have evolved oxidative carbocyclization enzymes (e.g., RedG and McpG) that allow the C ring of prodigiosin and undecylprodigiosin to be functionalized at the carbon atom directly adjacent to the azafulvene core. Such functionalization generates significant 1,3-allylic strain in the trans isomer. This is lacking in the cis isomer (see **23** vs **24**, Figure 15), and thus it is energetically favored. Conversion of undecylprodigiosin **17** to *o*-butylcycloheptylprodigiosin **21** would be unlikely to significantly affect the position of equilibrium between the cis isomer **23** and the trans isomer **24**. In contrast, conversion of undecylprodigiosin to the meta-bridged product **22** would greatly increase the concentration of the cis isomer **25** relative to the trans isomer **26**. In this context, it is striking that numerous carbocyclic derivatives of prodigiosin and undecylprodigiosin in which the cis conformation is enforced (e.g., cycloprodigiosin, cyclomethyldecylprodigiosin, cyclononylprodigiosin, streptorubin B, metacycloprodigiosin, and roseophilin) have been isolated, whereas derivatives that do not enforce a cis conformation have yet to be identified.

The resolution of the ambiguity surrounding the structure of streptorubin B set the stage for elucidating the stereochemistry of this fascinating metabolite. In 2011, Thomson and co-workers[66] and Challis and co-workers[79] independently reported that streptorubin B exists as a pair of slowly interconverting atropisomers (Figure 16). The major atropisomer at equilibrium possesses an anti relationship between the 4-methoxypyrrolyldipyrromethene core and the *n*-butyl side chain. The barrier for interconversion of the atropisomers was estimated to be ~20.5 kcal·mol^{-1} (see section 3.4).

Thomson and co-workers[66] utilized X-ray crystallographic analysis to determine the absolute configuration of streptorubin B prepared by enantioselective total synthesis (see section 3.4) and then compared the CD spectrum of the synthetic material with natural streptorubin B isolated by Challis and co-workers from *S. coelicolor*. In a complementary approach (Figure 17), Challis and co-workers[79] stereoselectively synthesized both enantiomers of [4′-^{2}H]2-undecylpyrrole (**27**) and fed them separately to a mutant of *S. coelicolor* unable to produce 2-undecylpyrrole (a key intermediate in streptorubin B biosynthesis). ^{1}H and ^{2}H NMR spectroscopic analysis of the resulting stereoselectively deuterium-labeled streptorubin B hydrochloride salt led to assignment of the 7′*S* configuration for the natural product,[79] in complete agreement with the absolute stereochemical assignment made on the basis of the total synthesis studies.[66]

HPLC comparisons on a homochiral stationary phase of racemic synthetic streptorubin B with the natural product isolated from *S. coelicolor* showed that the latter is in fact a 95:5 mixture of 7′*S* and 7′*R* enantiomers.[79] Remarkably, CD spectroscopic comparisons of metacycloprodigiosin prepared by Clift and Thomson[65] via enantioselective total synthesis with metacycloprodigiosin isolated by the Challis group from *S. longisporus ruber* revealed that the natural product has the 9′*R* absolute configuration;[66] that is, it is the antipode of streptorubin B (Figure 18).

2.7. Nonylprodigiosin, Cyclononylprodigiosin, and Analogues

In 1969, three years after the structural elucidation of undecylprodigiosin, Gerber[80] isolated 13 strains of *Actinomadura (Nocardia) pelletieri* and three strains of *Actinomadura madurae* that produced prodiginines (it was at this time that the use of the generic name “prodiginine” to describe prodigiosin-like compounds was first suggested). From a structural and biosynthetic standpoint, Gerber’s 1969 isolation experiments were significant for the identification of a new 23-carbon prodiginine, nonylprodigiosin (**30**), from *A. madurae* (Figure 19). Nonylprodigiosin is closely related to undecylprodigiosin; it differs only in the number of methylene groups in the hydrocarbon chain. This difference presumably arises because the RedJ homologue in *A. madurae* preferentially hydrolyzes decanoyl-ACP rather than dodecanoyl-ACP and/or the adenylation domain at the N-terminus of the *A. madurae* RedL homologue is selective toward decanoic acid (see section 4.4).

In her 1969 report, Gerber also noted that *A. pelleteri* produces a 25-carbon pigment (“pelletrin”) with an additional element of unsaturation compared to undecylprodigiosin, indicating that it may contain a carbocycle. The spectroscopic data for this compound differed from that reported for metacycloprodigiosin and its identity could thus not be unambiguously established. A year later, Gerber[81] reported that *A. madurae* also makes a carbocyclic derivative of nonylprodigiosin (**30**) under different growth conditions. Degradation and spectroscopic studies led to the identification of this new carbocyclic pigment as cyclononylprodigiosin (**31**, Figure 20), the first member of the prodiginine family with additional functionalization of the A ring. Having elucidated the structure of cyclononylprodigiosin, Gerber returned to the "pelletrin" compound reported in 1969 and, through spectroscopic and degradation studies, assigned structure **32** to the carbocyclic undecylprodigiosin derivative, which she named methylcyclodecylprodigiosin. No signals were observed in the CD spectrum of cyclononylprodigiosin (**31**), whereas methylcyclodecylprodigiosin (**32**) exhibited a clear Cotton effect, indicating that it is enantiomerically enriched.[82] However, the absolute configuration of the stereogenic center in methylcyclodecylprodigiosin remains undefined.

While preparing larger quantities of carbocyclic prodiginines **31** and **32** for antimalarial testing, Gerber[83] discovered that *A. madurae* and *A. pelletieri* produce the minor congeners methylcyclooctylprodigiosin (**33**) and ethylcyclononylprodigiosin (**34**), respectively (Figure 21).

In 1999, 30 years after the report of its isolation, Fürstner et al.[84] completed the first total synthesis of cyclononylprodigiosin (**31**) using ring-closing metathesis methodology (see section 3.6), and were able to obtain an X-ray structure of a key intermediate (**35**) in its synthesis. From this it was concluded that the major tautomer of cyclononylprodigiosin (as well as other prodiginines) has a central rather than a peripheral azafulvene (i.e., **35** as opposed to **36** in Figure 22).

2.8. Roseophilin, Dechlororoseophilin, and Prodigiosin R1

In 1992, Seto and co-workers[85] disclosed the structure of a new antibiotic, roseophilin (**37**), from *Streptomyces griseoviridis* (Figure 23). Roseophilin shows significant resemblance to the carbocyclic prodiginines yet possesses numerous distinct features. First, it contains two (rather than one) C–C bonds between the hydrocarbon chain and the conjugated heterocyclic ring system, resulting in a tricyclic cyclopentylpyrrolophane. Second, the characteristic central azafulvene of the prodiginines is replaced by a furan. Consequently, the pyrrole linked to the hydrocarbon chain becomes an azafulvene. Finally, roseophilin possesses a chlorine substituent on the A-ring pyrrole.

The planar structure and relative stereochemistry of roseophilin were confirmed in 1998 through total synthesis of the racemate by Fürstner and Weintritt[86] (see section 3.7). Although these authors were able to separate the two enantiomers of synthetic roseophilin by HPLC, they were unable to determine the absolute configuration of the natural product. In 2001, Boger and Hong[87] and Harrington and Tius[88] disclosed independent enantioselective total syntheses of (22*S*,23*S*)-**37** and (22*R*,23*R*)-**37**, respectively (see section 3.7). Comparison of CD spectroscopic data for Tius's synthetic (22*R*,23*R*)-roseophilin and the natural product established that they had the same absolute stereochemistry.

Despite several clear differences in the chromophore of roseophilin and the carbocyclic prodiginines discussed previously, roseophilin appears to share a common biosynthetic origin, likely originating from oxidative modifications to a hitherto unidentified dimethylated undecylprodigiosin analogue (**39**, Figure 24). Circumstantial evidence for the existence of 11′-dimethylundecylprodigiosin (**39**) and its role as a roseophilin precursor is provided by the recently reported isolation of prodigiosin R1 (**40**)[89] and dechlororoseophilin (**38**)[90] from the roseophilin producer *S. griseoviridis* (see section 4.8).

2.9. Marineosins A and B

Marineosins A and B were isolated from a marine-derived *Streptomyces*-related actinobacterium (strain CNQ-617) by Fenical and co-workers in 2008[91] (Figure 25). The structure and relative stereochemistry of marineosins A (**41**) and B (**42**) were assigned on the basis of an extensive series of NMR experiments. Although the marineosins are structurally distinct from other prodiginines, they appear to derive from undecylprodigiosin via a series of redox transformations, including one that is similar to the oxidative carbocyclization reactions involved in streptorubin B and metacycloprodigiosin biosynthesis (see section 4.7). A total synthesis of the two compounds remains elusive, but recent studies by Reynolds and co-workers[92] have established the absolute stereochemistry to be as shown in Figure 25.

3. TOTAL SYNTHESES

Due to the diverse and fascinating structures of the prodiginines, as well as their intriguing biological activities, numerous total and formal syntheses have been reported over the last 60 years. Here, we provide a comprehensive overview of the major chemical syntheses to date.

3.1. Prodigiosin and Undecylprodigiosin

In 1962, Rapoport and Willson[43] reported the syntheses of several isomers of prodigiosin in order to determine its complete structure (Scheme 1).

Synthesis of *iso*-**1** initiated with a tandem conjugate addition/Dieckmann cyclization with diethyl fumarate **43** and sodium glycinate **44** to yield 3-oxoprolinate **45**.[43,93] Decarboxylation of **45** under acidic conditions, followed by dimethylsulfite-mediated ketalization, provided pyrrolidine **46**. Treatment of **46** with catalytic palladium on carbon in a dehydrogenation vessel at high temperature led to a pyrrole ethyl ester, which was converted to the corresponding methyl ester (**47**) under standard conditions. Aromatic substitution of **47** with dehydropyrrolidine **48** at elevated temperature provided amine **49**, which was again aromatized with catalytic palladium at high temperature. The resulting bis(pyrrole) ester **50** was subjected to a three-step McFayden–Stevens reduction to provide aldehyde **5**, which was summarily condensed with pyrrole **7** to provide prodigiosin *iso*-**1** after alumina chromatography.

Unfortunately, spectral data of *iso*-**1** did not match that of the prodigiosin from *Serratia*. Rapoport and Willson[43] proceeded to synthesize (Scheme 2) the next most likely isomer, based on previous studies by Wasserman et al.[42] (i.e., **1**).

Synthesis of **1** began with a similar tandem conjugate addition/Dieckmann condensation sequence to that employed in the synthesis of *iso*-**1**.[43] Sodium glycinate **44** was combined with vinylogous ester **51** to afford hydroxypyrrole **52**. O-Methylation of **52**, followed by a two-step decarboxylation sequence, provided methoxypyrrole **54**. Aromatic substitution of

54 with cyclic imine **48** provided amine **55**, which was dehydrogenated at high temperature under palladium catalysis. A three-step McFayden–Stevens reduction revealed aldehyde **6**, which could be condensed with amylpyrrole **7** under acidic conditions to provide prodigiosin (**1**). Prodigiosin (**1**) was found to be identical in all aspects to naturally isolated prodigiosin, fully confirming the natural product's elusive structure more than three decades after its isolation.

Since this first synthesis of prodigiosin (**1**), numerous other syntheses of the key bis(pyrrole) aldehyde **6** have been reported, with applications toward the syntheses of many prodiginine natural products. In 1988, Boger and Patel[94] disclosed the total synthesis of prodigiosin and several prodigiosin analogues for structure–activity relationship (SAR) studies via azadiene Diels–Alder methodology (Scheme 3).

The synthesis of Boger and Patel[94] began with the tandem [4 + 2]/retro-[4 + 2] cycloaddition reaction of azadiene **57** and vinyl ether **58** to generate pyrazine **59** in excellent yield. Reductive pyrrole formation followed by selective hydrolysis of a methyl ester provided carboxylic acid **60**, which could be decarboxylatively iodinated to form diiodide **61**. Hydrogenolysis of the carbon–halogen bonds, followed by N-acylation of resulting pyrrole **62** with acyl chloride **63**, provided carbonyldipyrrole **64**, which could be cleanly converted to fused compound **65** by action of stoichiometric, polymer-supported palladium(II). Methanolysis of **65** followed by a McFayden–Stevens reduction of ester **66** generated the key prodigiosin intermediate, bis(pyrrole) aldehyde **6**, which could be converted to the natural product as previously reported.

In 1989, Wasserman and Lombardo[95] disclosed a new total synthesis of prodigiosin (**1**) based on newly developed vicinal tricarbonyl methodology (Scheme 4). The first key transformation in Wasserman and Lombardo's synthesis was a twostep condensation between pyrrole aldehyde **67** and acetoacetate dianion **68** to form unsaturated ketone **69**.[95] Oxidation of the β-keto ester in **69** by action of nitrosoaniline **70** provided tricarbonyl **71**, which provided bis(pyrrole) ester **73** upon condensation with benzylamine **72**. Methylation and deprotection of **73** revealed ester **56**, which was subjected to a McFayden–Stevens reduction to provide the key bis(pyrrole) aldehyde (**6**). Aldehyde **6** was finally condensed with amylpyrrole **7** to provide prodigiosin (**1**).

In 1999, Wasserman et al.[96] reported a new, shorter synthesis of the bis(pyrrole) aldehyde based on singlet-oxygen methodology developed in their group (Scheme 5). Wasserman et al. found that pyrrole **74** could be converted to bis(pyrrole) ester **75**, on a small scale, by sequential exposure of **74** to singlet oxygen at low temperature in the presence of excess pyrrole. Compound **75** could then be converted to bis(pyrrole) aldehyde **6** by a McFayden–Stevens reduction, as used in numerous previous syntheses. Aldehyde **6** was identical in all aspects to the naturally occurring aldehyde and was used by Wasserman et al.[96] to synthesize prodiginines bearing simple substitutions on the Aring pyrrole.

In 1996, driven by interest in undecylprodigiosin as an immunosuppressive agent, D'Alessio and Rossi[97] at Pharmacia & Upjohn in Italy disclosed a synthesis of undecylprodigiosin which did not rely on the condensation of a pyrrole with bis(pyrrole) aldehyde **6**, as many of the previously reported synthetic routes to **6** were not suitable for scaling up in case of possible lead development. Their synthesis began with the conversion of undecylpyrrole **76** to pyrrole aldehyde **77** under Vilsmeier– Haack conditions (Scheme 6). Aldehyde **77** was then subject to a base-mediated condensation with lactam **78** to provide conjugated lactam **79**. Treatment of **79** with triflic anhydride provided the corresponding triflate **80**, which could be cross-coupled to pyrroleboronic acid **81** with concomitant *t*-butyloxycarbonyl (Boc) deprotection under Suzuki–Miyaura reaction conditions to provide the natural product (**16**). D'Alessio et al.[11] later applied this method to the synthesis of several undecylprodigiosin derivatives for SAR studies for immunosuppressive activity. Similar reaction sequences were later employed by Fürstner et al.[73,98] in their synthesis of butylcycloheptylprodigiosin (see section 3.5) and by Clift and Thomson[65] in their synthesis of metacycloprodigiosin (see section 3.3).

Most recently, in 2006, Lavallée and co-workers[99] at Gemin X found that the method developed by D'Alessio and Rossi could be applied to synthesis of the commonly used bis(pyrrole) aldehyde **6** (Scheme 7). They found that lactam **78** could be converted to bromoenamine **82** by action of a Vilsmeier–Haack reagent. Bromoenamine **82** could then be employed in a Suzuki– Miyaura cross-coupling with boronic acid **81** under basic, aqueous conditions to provide bis(pyrrole) aldehyde **6**. This process has been used by Gemin X to prepare multikilogram quantities of a synthetic prodigiosin analogue, obatoclax, for clinical trials.[33]

3.2. Cycloprodigiosin

In 1984, to confirm the structure of cycloprodigiosin after revisions by Gerber and Laatsch, Wasserman and Fukuyama[54] at Yale undertook its total synthesis. Their synthesis began with a condensation of methylcyclohexanone (**83**) with sulfonylhydrazine **84** to afford hydrazone **85** (Scheme 8). Treatment of **85** with *sec*-butyllithium generated a vinyl anion, which was trapped with *N*,*N*-dimethylformamide (DMF) to form aldehyde **86**. Reaction of **86** with thioacetal anion **87** at low temperature generated **88**, the deprotection of which revealed 1,4-dicarbonyl **89**. Treatment of **89** with ammonium carbonate at high temperature allowed the formation of pyrrole **90**, which was condensed with bis(pyrrole) aldehyde **6** to afford a compound that was identical with samples of the natural product derived from both *B. gazogenes* and *A. rubra*, establishing the structure of cycloprodigiosin as **16**.

The first enantioselective synthesis of cycloprodigiosin was reported in 2013 by Schultz and Sarpong[100] (Scheme 9). Their approach centered upon an application of a novel method they had developed for the construction of fused pyrroles. The requisite methyl stereogenic center was set at the beginning of the synthesis by way of a Myers auxiliary-controlled enolate alkylation to afford amide **93**. Functional group interconversions then allowed for synthesis of terminal alkyne **95**, which was further processed to provide propargylic alcohol **96** over five steps. Generation of the 1,2-disubstituted allene **97** was achieved by a Myers allene synthesis, which set the stage for the key rhodium-catalyzed cycloaddition cascade to fused pyrrole **98**. Removal of the N-tosyl group, followed by condensation with bis(pyrrole) **99** and in situ Boc group cleavage, provided cycloprodigiosin in 71% yield over three steps.

In a subsequent publication in 2015, Sarpong and coworkers[55] reported an efficient second-generation route to cycloprodigiosin (**15**) that enabled access to both enantiomers of the natural product (Scheme 10).

Application of the Schöllkopf–Barton–Zard pyrrole synthesis using enantiopure menthol-derived isonitrile **101** in conjunction with racemic **100** allowed direct access to both diasteromeric pyrroles **102** and **103** in a combined yield of 62%. Separation of the diastereomers allowed further processing to either of the methyl-substituted pyrroles, **104** or **105**, through a three-step procedure of bromination, pyrrole protection, and palladium-catalyzed methylation. Cleavage of the chiral auxiliary within **104** or **105** was achieved with concomitant decarboxylation under basic conditions, allowing for an acid-promoted condensation with pyrrole **6** to deliver either enantiomeric form of the natural product in good yield.

3.3. Metacycloprodigiosin

The first total synthesis of metacycloprodigiosin (**20**) was conducted by Wasserman and co-workers in 1969[61] as part of a sustained effort to elucidate the structures of the prodigiosin family.[101] Their synthesis (Scheme 11) began with cyclododecanone (**106**), which was alkylated and protected as ketal **107**. Regioselective bromination of ketal **107** under mildly acidic conditions provided bromide **108** in quantitative yield. Elimination at high temperature followed by acid-catalyzed deprotection revealed cyclododecenone **109**, which was epoxidized to provide keto-epoxide **110** as a mixture of diastereomers. Wharton fragmentation of epoxide **110** with hydrazine afforded allylic alcohol **111**, the oxidation and cyanation of which provided nitrile **112.** Nitrile **112** was then converted to the corresponding 1,4-dicarbonyl **113** through trivial functional-group manipulations. Condensation of **113** with an ammonia equivalent produced pyrrole **114**, which was further condensed with bis(pyrrole) aldehyde **6** to provide the natural product as the hydrochloride salt. The final condensation step in Wasserman's synthesis of metacycloprodigiosin, cycloprodigiosin, and other prodiginines is often referred to as "biomimetic",[102] but in the true biosynthesis of cyclic prodiginines such as metacycloprodigiosin, the condensation first occurs with 2-methyl-3-amylpyrrole (MAP) or 2-undecylpyrrole (2-UP) before an enzyme-catalyzed oxidative radical cyclization forms the ring[64] (e.g., **114** and related cyclic pyrroles are not true biosynthetic precursors of **20** and corresponding prodiginines—see section 4.5).

Three decades after Wasserman's first synthesis of metacycloprodigiosin, two elegant nonenantioselective formal total syntheses based on the success of Wasserman's condensation were reported by Fürstner et al. in 1998[102] and 1999.[103] Fürstner's 1998 formal synthesis of metacycloprodigiosin (Scheme 12A) was based on the development of an enynemetathesis reaction. To begin the synthesis, cyclodecene **115** was treated with an in situ-generated diiminoselenium reagent to afford aminated cyclodecene **116**.[102] N-Alkylation of **116** with propargyl bromide provided alkyne **117**, which could be acylated via the intermediacy of an alkynylzinc reagent. Treatment of the resulting ynone **118** with either catalytic platinum(II) or stoichiometric boron trifluoride resulted in a rearrangement to form bridged pyrrolidine **119**. Reduction of the alkene in **119** provided ketone **120**, which could be reduced and thionylated to provide thionocarbonate **121**. Barton–McCombie dehydroxylation of **121** then generated pyrrolidine **122**. The choice of a radical dehydroxylation process was critical, as any pathway involving the generation of a carbocation at the hydroxyl position led to significant rearrangement in the molecule. Final elimination of the tosyl group from **122** was achieved with excess KAPA (potassium anion of 1,3-diaminopropane), providing Wasserman's pyrrole **114** in 5% overall yield from cyclodecene.

Fürstner and Krause's 1999 formal synthesis of metacycloprodigiosin[103] (Scheme 12B) employed methodology similar to that used in Fürstner and Weintritt's 1998 total synthesis of roseophilin[86] (see section 3.7). The synthesis was initiated with a Corey–Chaykovsky reaction between sulfonium **124** (available in five steps from allylic chloride **123**) and 8-bromooctanal.[103] The formed epoxide **125** was then used to alkylate potassium enolate **126**, generating **127**. Subjection of **127** to palladium(0) catalysis under high dilution conditions resulted in an intramolecular Tsuji–Trost allylation to form cyclododecanene **128** as an inconsequential mixture of diastereomers. Protecting group removal and oxidation with Dess–Martin periodinane (DMP) provided enone **130**, which rearranged with loss of phenylsulfinate under basic conditions to form pyrone **131**. Methanolysis of **131** provided α,β-unsaturated aldehyde **132**, which was condensed with benzylamine under acidic conditions to generate pyrrole **133**. Removal of the acetyl group followed by ruthenium-mediated oxidation provided β-keto ester **134**, which in turn revealed ketone **135** after subjection to Krapcho decarboxylation conditions. Finally, Wittig olefination of **135** and hydrogenation of the resulting alkene with Crabtree's catalyst produced benzylpyrrole **136**. Removal of the benzyl group would afford pyrrole **114** and thence **20**, but the actual transformation was not reported.

In 2009, 40 years after metacycloprodigiosin's discovery, Clift and Thomson[65] at Northwestern University completed the first enantioselective total synthesis of metacycloprodigiosin in an effort to finally identify the absolute configuration of the natural product (Scheme 13). Their synthesis employed an asymmetric Cu-catalyzed conjugate addition to *trans*-dienone **137** as the enantioselective step. The nascent magnesium enolate was then trapped with an equivalent of chloroenolsilane **138** to afford silylbis(enol) ether **139**. Treatment of unpurified **139** with ceric ammonium nitrate (CAN) provided the 1,4-dicarbonyl **140**, which underwent ring-closing metathesis to provide substituted dodecanone **141**. Upon hydrogenation and condensation with ammonium acetate, **141** was converted to 2-methylpyrrole **142**. In the final steps of the synthesis, an alteration of the prodigiosin core synthesis of D'Alessio and Rossi[97] was employed (see section 3.1). After oxidation of **142**, aldehyde **143** was treated with trimethylsilyl trifluoromethanesulfonate (TMSOTf) and lactam **78** to afford the vinylogous aldol product **144**. Acidic fragmentation of **144** provided lactam **145**, which was converted to the corresponding triflate and cross-coupled with Bocprotected pyrroleboronic acid **81** under Suzuki–Miyaura reaction conditions. Deprotection under basic conditions furnished enantiopure metacycloprodigiosin (93:7 enantiomeric ratio, er). Although a natural sample could not be obtained at the time of the report, two years later, in 2011,[66] comparisons of CD spectra of synthetic (*R*)-metacycloprodigiosin with natural metacycloprodigiosin isolated from *S. longisporus ruber* were used to assign the absolute configuration of natural metacycloprodigiosin as *R* (as it appears in Scheme 13).

In 2015, Thomson and co-workers reported a second enantioselective total synthesis of metacycloprodigisoin (**20**) that cut the number of operations required to complete the synthesis from 12 to nine steps (Scheme 14). Their new route took advantage of the late-stage union of pyrrole **114** with bispyrrole aldehyde **99**, in a fashion analogous to that described by Wasserman and co-workers in their inaugural synthesis of **20**. Thomson's enantioselective synthesis of Wasserman's pyrrole (**114**) began with the synthesis of aldehyde **149** in four steps from diol **146**. As in their previous synthesis, enantioenrichment was achieved through the use of an asymmetric Feringa conjugate addition. Oxidative coupling of aldehyde **149** with enol silane **150** was then carried out via enamine catalysis to generate 1,4-dicarbonyl **152** in 22% yield. Ring-closing metathesis once again forged the requisite 12-membered ring. Hydrogenation and Paal–Knorr pyrrole condensation delivered Wasserman's pyrrole **114**, which was then converted in one step to the natural product upon acid-catalyzed condensation with **99**. The brevity of this route, which is currently the shortest synthesis of metacycloprodigiosin (**20**), goes some way toward making up for the disappointingly low yield obtained during the key oxidative coupling event (**149** → **152**).

3.4. Streptorubin B

Although it was first isolated in 1964, the first synthetic studies toward streptorubin B were not reported until 1998, when Fürstner et al.[102] disclosed a synthesis of streptorubin's pyrrole core (Scheme 15A).[104] Fürstner's work was based on their development of an enyne-metathesis reaction and paralleled their synthetic studies toward metacycloprodigiosin. To begin the synthesis, cyclooctene **154** was treated with an intermediate generated in situ from chloramine T and elemental selenium to afford aminated cyclooctene **155**. N-Alkylation of **155** with propargyl bromide provided alkyne **156**, which could be acylated via the intermediacy of an alkynylzinc reagent. Treatment of the resulting ynone **157** with catalytic platinum(II) resulted in a highly efficient rearrangement to form bridged pyrrolidine **158**. Radical reduction of the alkene in **158** provided ketone **159**, which could be reduced and thionylated to provide thionocarbonate **160**. Radical dehydroxylation of **160** then generated pyrrolidine **161**. Final elimination of the tosyl group from **161** was effected with excess KAPA, affording synthetic pyrrole **162** for the first time. The conversion of **162** into streptorubin B (**22**) was not reported, though related condensations had been previously reported for other prodigionines.

Seven years after Fürstner's publication, Chang et al.[105] at the National University of Kaohsiung in Taiwan reported an approach to streptorubin B based on Fürstner's pyrrolidine intermediate **161** (Scheme 15B). Chang's synthesis centered on a ring-closing metathesis reaction to create the cyclodecane backbone. Pyrrolidine **163** (available in four steps from *trans*-4-hydroxyproline) was oxidized and homologated with ylide **164** to provide ester **165**. A two-step reduction of both the alkene and ester functionalities provided alcohol **166**, which was oxidized and converted to terminal alkene **167** by Wittig methodology. Deprotection and oxidation of ether **167** in one pot revealed ketone **168**, which was converted to requisite diene **170** upon treatment with Grignard reagent **169**. Exposure of **170** to Grubbs' first-generation catalyst under high dilution conditions provided cycloalkene **171** as an inconsequential mixture of diastereomers and geometric isomers. Hydrogenation of the double bond followed by dehydration provided pyrrolidine **161** again as a mixture of diastereomers, which may be converted to **162** according to the procedure of Fürstner et al.[102]

In 2011, Thomson and co-workers[66] disclosed the first enantioselective total synthesis of streptorubin B (Scheme 15C). To begin the synthesis, heptanedial **172** was treated with catalytic (*S*)-proline according to a procedure developed by List to induce an enantioselective *exo*-enol-6-*exo*-trig aldol reaction. The transient aldehyde **173** was trapped in one pot with Wittig reagent **159** to afford cyclohexanol **174**. Oxidation of **174** under Swern conditions, followed by the 1,2-addition of organolithium reagent **175**, generated dienol **176**. Treatment of the dienol with potassium hexamethyldisilazide, in the presence of stoichiometric 18-crown-6 ether, induced an anionic oxy-Cope rearrangement to cyclodecanone **178** with excellent transfer of stereochemistry through a chair-like transition state (**177**). Tandem hydrogenation and benzyl group hydrogenolysis afforded alcohol **179**, which was oxidized with Dess–Martin periodinane and condensed with ammonium acetate to afford the pyrrole core **162**. The synthesis was completed with an acidpromoted condensation between pyrrole **162** and Boc-protected bis(pyrrole) aldehyde **99**, followed by protecting group removal under basic conditions in one pot. Analysis of the bright-red material thus obtained revealed an approximately 10:1 mixture of two compounds in which the major compound did not match the natural product. Reexamination of the NMR sample after 10 days, however, revealed that the mixture had transformed almost completely to streptorubin B (**22**). The synthetic HCl salt was identical to the natural product as determined by ^{1}H and ^{13}C NMR spectroscopy and mass spectrometry.

Intrigued by the initial formation of an apparent isomer of the natural product, Thomson and co-workers[66] conducted a series of nuclear Overhauser effect spectroscopic (NOESY) NMR experiments to determine its identity. They realized that the pyrrole precursor **162** had been formed atropdiastereoselectively from alcohol **179** as the syn atropisomer **181** (Figure 26), which was favored in equilibrium with the anti atropisomer **180**. Condensation of the isomeric mixture led to a mixture of streptorubin B atropisomers **22**·HCl and **182**·HCl, initially favoring **182**·HCl. Relaxation over a period of several days at room temperature then led to a mixture strongly favoring isomer **22**·HCl. Kinetic measurements of the equilibrium allowed the torsional barrier to be approximated as ca. 20.5 kcal·mol^{-1}.[66]

3.5. Butylcycloheptylprodigiosin

In 2005, Fürstner et al.[73] conducted a total synthesis of butylcycloheptylprodigiosin (**21**) in an effort to determine whether or not it had in fact been previously isolated in nature (Scheme 16).

Cyclononadienone **184** (available in six steps from cyclooctanone) was reduced and acetylated under standard conditions to provide acetate **185**. Exposure of allyl acetate **185** to sodium methyl acetoacetate with catalytic Pd(0), followed by thermal decarboxylation, led to diene **186** as the major product. Condensation of **186** with hydroxylamine, followed by acylation with pentafluorobenzoyl chloride, afforded oxime ester **187**. A unique Narasaka–Heck cyclization was then conducted on a multigram scale with Pd(0) and P(*o*-tolyl)^3 to provide bicyclic imine **188**, which further rearranged under strongly basic conditions to a conjugated pyrrole (Boc-protected as **189**). The protected pyrrole **189** was then subjected to a hydroboration– oxidation sequence, providing alcohol **190**, which could be further oxidized with Dess–Martin periodinane to reveal ketone **191**. Wittig olefination of **191** at elevated temperature provided olefin **192** in good yield. Catalytic hydrogenation of the double bond, along with oxidation of the pyrrole methyl substituent, proceeded smoothly to generate aldehyde **193**, which could be condensed under basic conditions with lactam **78** to afford lactam **194**.

Formation of the corresponding triflate and Suzuki–Miyaura cross-coupling with boronic acid **81** then furnished the desired *o*-butylcycloheptylprodigiosin **21**. On the basis of comparisons of synthetic **21** to unpublished spectral data obtained from Floss, Fürstner and co-workers concluded that butylcycloheptylprodigiosin was in fact a natural product (see section 2.6).

In 2007, Reeves[74] at Boehringer Ingelheim Pharmaceuticals reported a short synthesis of butylcycloheptylprodigiosin based on a method for the synthesis of 2-formyl-4,5-disubstituted pyrroles that he had recently disclosed (Scheme 17). The synthesis began with cyclononenone (available in three steps from cyclooctanone). Treatment of cyclononenone **195** with *n*-butylmagnesium chloride under copper catalysis at low temperature generated a magnesium enolate, which could be diastereoselectively trapped with formyloxazole **196** to provide keto alcohol **197**. Elimination of the hydroxyl group in **197** by action of mesyl chloride under basic conditions provided 2-formylpyrrole **198**, which could be extended to lactam **194** by condensation with **78**. As per Fürstner's synthesis, lactam **194** could be converted to the appropriate triflate and coupled to boronic acid **81** under Suzuki–Miyaura conditions to provide butylcycloheptylprodigiosin **21**.

In 2013, Thomson and co-workers[76] developed a short synthesis of butylcycloheptylprodigiosin using a tandem catalytic approach in order to provide material for comparisons with their synthetic streptorubin B.[66] In the synthesis (Scheme 18), a copper-catalyzed conjugate addition of butylmagnesium bromide to cyclononenone **195** generated a transient magnesium enolate, which could be efficiently allylated by allyl bromide in the presence of catalytic Pd(0). Alkene **199** was then subjected to Lemieux–Johnson oxidation conditions to afford 1,4-dicarbonyl **200**, which could be smoothly condensed with ammonium acetate to afford pyrrole **201**. Condensation with protected bis(pyrrole) aldehyde **99** under dry acidic conditions, followed by basic deprotection in situ, afforded the desired prodigiosin **21**. Thomson and co-workers[76] were able to use the same route to synthesize alkyl-chain analogues of butylcyclohepylprodigiosin for mass spectrometric studies that invalidated the identity of **21** as a natural product (see section 2.6).

3.6. Cyclononylprodigiosin

In 1999, 30 years after cyclononylprodigiosin's isolation, Fürstner's research group at the Max Planck Institute completed its first total synthesis using ring-closing metathesis (RCM) methodology (Scheme 19).[84]

Fürstner's synthesis began with the assembly of formylpyrrole **203** in four trivial steps from 5-hexenoic acid (**202**). Condensation of **203** with lactam **78** in basic dimethyl sulfoxide (DMSO) according the method of D'Alessio resulted in the smooth formation of oxodipyrrylmethene **204**, which could be easily converted to corresponding triflate **205**. Suzuki coupling of **205** with pyrroleboronic acid **206** with concomitant deprotection generated the tripyrrylmethene **207**. At this point, the Fürstner group faced a synthetic dilemma. Compound **207** was not a suitable substrate for ring-closing metathesis, as free amines were known to shut down the metal catalysts. While one solution would be to protect the amine as the hydrochloride salt, it was known that the protonated forms of prodiginines such as undecylprodigiosin and PNU-156804 (**208**) favored the *trans*-rotomeric configuration (Figure 27).[106]

At high pH, **208** had been shown to exist exclusively in the cis configuration as opposed to the trans configuration (i.e., as **208**, not **209**)[84] due to the existence of a N–H–N hydrogen bond in the dipyrrylmethene moiety. Upon protonation with strong acid, however, the equilibrium shifted in the opposite direction (i.e., **211** was favored over **210**) due to an O–H–N hydrogen bond (which had been previously observed in the crystal structure of a prodigiosin analogue).[107] Fortunately, however, Fürstner's group was able to take advantage of conformational dynamics to close the macrocyclic ring (Scheme 20). Since **212**·HCl would not react intramolecularly, only **213**·HCl would be converted to the macrocycle. Evolution of ethylene gas would thus slowly drive the intramolecular reaction to completion. By performing the RCM with catalyst **214** at high dilution (1.4 mM), they were able to recover macrocycle **215** as the HCl salt in good yield. Finally, catalytic hydrogenation of **215** with Wilkinson's catalyst furnished the natural product as its HCl salt (**31**·HCl).

3.7. Roseophilin

Due to its unique structure and corresponding biological activity, roseophilin (**37**) was a popular synthetic target from the moment of its reported isolation.[85] Beginning in 1995 with the report of Terashima and co-workers[108] on synthesis of the pyrrolylfuran moiety of roseophilin (**216**, Figure 28), numerous creative synthetic studies have been reported in the last two decades. Major total synthesis contributions have been made by groups including Fuchs and co-workers (1997, formal),[109,110] Terashima and co-workers (1998, formal),[108,111] Fürstner and Weintritt (1998),[86] Robertson et al. (1999, formal),[112,113] Hiemstra and Speckamp and co-workers (2000, enantioselective formal),[114,115] Trost and Doherty (2000, enantioselective formal),[116] Boger and Hong (2001, enantioselective),[87] Harrington and Tius (2001, enantioselective),[88,117] Bitar and Frontier (2009, formal),[118] and Frederich and Harran (2013).[119] As our review is primarily concerned with the structure of the prodiginines, we will focus our coverage on the works of Fürstner, Boger, and Tius and their co-workers, whose syntheses first shed new light on the structure and properties of roseophilin. We then conclude with a summary of the most recent synthesis by the Harran lab, due to its unique synthetic strategy, which does not employ ketone **217** as an intermediate (unlike every other synthesis to date).

The first total synthesis of roseophilin was completed in 1998 by Fürstner and Weintritt,[86] who developed a convergent approach involving the coupling of a pyrrylfuran fragment (**216**) and a macrotricyclic core (**217**) (Scheme 21). The first key step in Fürstner and Weintritt's synthesis of roseophilin was a Corey– Chaykovsky epoxidation of 9-bromononanal (**219**) with sulfonium **124** (available in three steps from alcohol **218**) to afford the allylic epoxide **220**. Displacement of the bromide in **220** with potassium sulfonate **221** provided α-sulfonyl ester **222**. Treatment of **222** with a palladium(0) catalyst caused an intramolecular Tsuji–Trost-type macrocyclization to afford allylic alcohol **223**. Fluoridic deprotection of **223** with concomitant esterification under basic conditions resulted in the formation of lactone **224**, which could be oxidized with DMP and treated with benzylamine and catalytic palladium(0) to afford pyrrole **225**. Activation of the carboxylic acid of **225** with Vilsmeier–Haack reagent **226**, followed by exposure to stannous chloride, allowed an intramolecular Friedel–Crafts reaction to take place, forming α-sulfonyl ketone **227**. Elimination of the sulfinate group followed by a zincate-conjugate addition reaction provided the benzyl-protected roseophilin core structure, **228**. Reductive debenzylation and reprotection of the pyrrole nitrogen with 2-(trimethylsilyl)ethoxymethyl chloride (SEMCl) resulted in tricyclic species **229**, which was treated with a carefully prepared furanyl cerate, **230**, to afford tertiary alcohol **231**. While Terashima and co-workers[108] had reported the acid condensation of pyrrylfuran **216** with simple ketones, Fürstner and Weintritt[86] found that such a condensation was ineffective on more complex substrates. Finally, desilylation and dehydration of **231** afforded the natural product (**37**) in racemic form (1% yield after 18 linear steps from alcohol **218**). A comparison of the synthetic product to an authentic sample of natural roseophilin confirmed the structure and relative stereochemistry originally postulated in 1992 (see section 2.8).

Boger and Hong's synthesis[87] involved oxidation of alcohol **233** (available in three steps from oxazolidinone **232**) followed by Wittig homologation of the corresponding aldehyde to enol ether **234** (Scheme 22A). A [4 + 2]/retro-[4 + 2] cycloaddition sequence with tetrazine **235**, with concomitant elimination of methanol, afforded diazine **236**. Reductive ring contraction of **236** with zinc under acidic conditions, followed by benzyl group hydrogenolysis and acid-catalyzed lactonization, provided **237**. A series of trivial functional-group interconversions from **237** provided lactone **238**, which could be subjected to oxidation, Wittig olefination, and hydrogenation to furnish alcohol **239**. Oxidation and olefination of **239** led to alkene **40**, which could be hydrolyzed and similarly olefinated to afford triene **241**. Ring closing metathesis with Grubbs' first-generation catalyst provided a macrocyclic diene, which was further elaborated to acyl selenide **242**. Treatment of **242** with azobis- (isobutyronitrile) (AIBN) and tributyltin hydride led to the formation of an acyl radical, which cyclized in an intramolecular fashion to afford the tricyclic ketone **229** after catalytic hydrogenation over a platinum catalyst. The enantioenriched ketone **229** could then be converted to (22*S*,23*S*)-**37** according to Fürstner's cerate-addition procedure.

Tius and Harrington's synthesis[88,117] targeted the opposite enantiomer to that of Boger and Hong, and it used a method they had developed to introduce asymmetry at a later stage. Their synthesis initiated with an amination of 5-hexenal with *tert*-butylamine to afford imine **244** (Scheme 22B). C-Silylation of **244**, followed by a Peterson-type olefination and imine hydrolysis, afforded enal **245**. Pinnick oxidation of **245**, followed by amidation of the resulting carboxylic acid, led to morpholine amide **246**. Monoaddition of lithiated allene **247** to **246**, followed by transfer of the solution to a cold acidic bath, led to the enantioselective formation of cyclopentenone **248** through a Nazarov cyclization process. Benzoylation of **248** under standard conditions provided benzoate **249**, which was subject to Stetter reaction conditions with 6-heptenal to generate 1,4-dicarbonyl **252**. Ring-closing metathesis with Grubbs' first-generation catalyst provided macrocycle **253** as a mixture of *E*/*Z* isomers, which were simultaneously hydrogenated to diketone **254**. Paal– Knorr condensation and deprotection of **254**, followed by SEM protection of the resulting pyrrole, afforded the roseophilin core structure **229**, which was converted to enantiopure (22*R*,23*R*)-roseophilin according to the method of Fürstner.

A comparison of the CD spectrum of enantiopure, synthetic (22*S*,23*S*)-roseophilin from Boger and Hong[87] to that of an authentic sample provided by Seto and co-workers[85] revealed that natural roseophilin was the enantiomer of Boger and Hong's synthetic roseophilin (Figure 29). Indeed, a direct comparison of Tius's synthetic (22*R*,23*R*)-roseophilin to the natural product revealed a perfect spectroscopic match, setting the absolute configuration of roseophilin as (22*R*, 23*R*).[88]

Based on the structure of roseophilin and the fact that *S. griseoviridis* produces both natural products, the absolute configuration of prodigiosin R1 might be expected to be as shown in Figure 29. However, as mentioned in the section discussing the structure of streptorubin B (see section 2.6), streptorubin B and its very similar congener metacycloprodigiosin are pseudoenantiomeric in nature,[66] and the situation may in fact be the same for prodigiosin R1 and roseophilin. Clift and Thomson[65] completed an enantioselective synthesis of both enantiomers of prodigiosin R1 using the same strategy as that used to prepare metacycloprodigiosin (see section 3.3). However, without natural material for comparison, the absolute configuration of prodigiosin R1 remains unknown.

Most recently, Frederich and Harran[119] reported a concise synthesis of (+)-roseophilin (**37**) through a unique strategy (Scheme 23). Unlike all previous syntheses of **37**, which used Fürstner's end-game, Frederich and Harran utilized a completely different series of end-game bond constructions. Their synthesis involved initial generation of alkene **262** in a short number of steps from furan **260** (for which they developed an improved synthesis). Phosphorylation of the pyrrole nitrogen within **262**, and subsequent formation of ketone **263** by cross-metathesis and conjugate reduction, set the stage for the first critical cyclization to generate pyrrolophane **267**. In this cleverly designed step, enolization of ketone **263** with potassium bis(trimethylsilyl)-amide (KHMDS) allows for reversible formation of the thermodynamically disfavored macrocyclic aldol adduct **265**, which is kinetically trapped by a subsequent N-to-O migration of the phosphoryl group to generate **266**. In situ elimination affords the pyrrolophane **267** in a remarkable 66% yield. Enantioselective hydrogenation of the thus-formed tetrasubstituted alkene with JosiPhos ligand **268** afforded ketone **269** in

92% yield and 67% enantiomeric excess (ee), with a diastereomeric ratio (dr) > 25:1. Completion of the synthesis was achieved through an intramolecular Friedel–Crafts condensation that initially generates azafulvene **270**. In situ treatment with acid cleaves the SEM protecting group and facilitates isomerization of the azafulvene subunit to the fully conjugated isomer, which in this case is the desired natural product, (+)-roseophilin (**37**).

4. BIOSYNTHESIS

Due to their fascinating structures and wide range of biological activities, numerous studies of prodiginine alkaloid biosynthesis have been reported over the last century. Here, we give a historical perspective of these studies and summarize modern understanding of the natural mechanisms for prodiginine assembly.

4.1. Precursor Incorporation Experiments and Early Genetic Studies

Due to the original tripyrrylmethene structure proposed for prodigiosin (see section 2.1) and the proposed involvement of a tripyrrylmethene intermediate in porphyrin biosynthesis, prior to 1960 it was hypothesized that prodigiosin (**1**) and porphyrin (**274**) are biosynthesized via similar pathways (Figure 30).[120] Supporting this notion, in 1950 Hubbard and Rimington[121] found that, as is the case for porphyrins, radiolabeled glycine and acetate were incorporated into prodigiosin (**1**). In 1958, Gibson et al.[122] found that eukaryotic cells converted glycine (**271**) and succinyl-CoA to 5-aminolevulinic acid (5-ALA, **272**), a key intermediate in porphobilinogen (**274**) biosynthesis.

In 1960, however, Marks and Bogorad[123] fed radiolabeled 5-aminolevulinic acid (5-ALA) to *S. marcescens* and found that it was not incorporated into prodigiosin (**1**), indicating that the 4-methoxypyrrolyldipyrromethene moiety of **1** does not derive from porphobilinogen, despite the fact that glycine is a common precursor of both **1** and porphyrins. While investigations into a possible biosynthetic relationship between prodiginines and porphyrins were being carried out, evidence that prodigiosin derives from a bifurcated pathway that is distinct from porphyrin biosynthesis began to emerge. In 1956, Santer and Vogel[41] isolated 4-methoxy-2,2′-bipyrrole-5-carboxaldehyde (MBC, **6**) from an *S. marcescens* mutant (9-3-3) blocked in prodigiosin biosynthesis and demonstrated that it could be converted to prodigiosin by a second strain also blocked in prodigiosin production. In 1960, Wasserman et al.[124] found that exposure of *S. marcescens* strain 9-3-3 to vapors of synthetic methylamylpyrrole (MAP, **7**) resulted in the formation of prodigiosin within a few minutes. Evidence that the condensation of **6** and **7** (Figure 31) is enzyme-catalyzed was reported by Williams et al. in 1965,[125] who found that the condensation of MBC and MAP by a cell lysate of strain 9-3-3 is significantly less efficient at slightly elevated temperatures (>35 °C).

In 1966, Morrison[126] was able to further delineate the prodigiosin biosynthetic pathway through the isolation of several hundred *S. marcescens* mutants blocked at different points in prodigiosin biosynthesis. These mutants were labeled B or M according to whether they are blocked in MBC or MAP biosynthesis, respectively. He was able to determine the point at which the biosynthesis was blocked in most of the mutants by cosynthesis experiments; mutants blocked at late stages in the biosynthetic pathway could restore prodigiosin production in those blocked at earlier stages (e.g., B2 could restore prodigiosin production in

B1; Figure 32). Through such cosynthesis studies, Morrison was also able to confirm the bifurcated nature of the prodigiosin biosynthetic pathway, because different classes of mutants existed that could restore prodigiosin production in other classes regardless of order (e.g., M2 and M1 could restore prodigiosin biosynthesis in both B2 and B1). Morrison[126] also noted the appearance of single mutants that appeared to be simultaneously blocked in both MAP and MBC biosynthesis. On the basis of this observation, he speculated that there might be a step common to both MAP and MBC biosynthesis, though current knowledge suggests such mutations were in regulatory genes (see sections 4.2 and 4.3).

In the early 1970s, Wasserman et al.[127,128] conducted a series of incorporation experiments utilizing ^{13}C and ^{14}C labeled precursors to determine the metabolic origins of prodigiosin (**1**) and undecylprodigiosin (**17**) (Figure 33). Both A-rings were found to derive from L-proline and both B-rings were found to originate from L-serine, a unit of acetate, and the *S*-methyl group of methionine. The early observation that glycine is incorporated into prodigiosin can be explained by the well-known metabolic interconversion of glycine and serine. The precursor incorporation pattern was found to diverge for the C-ring, which was found to derive from acetate and L-alanine in prodigiosin (**1**), but from acetate and glycine in undecylprodigiosin (**17**).

These studies led to the hypothesis that undecylprodigiosin is biosynthesized in *Streptomyces* species via a pathway analogous to that for prodigiosin biosynthesis in *Serratia* species, in which the common intermediate MBC (**6**) is condensed with 2-undecylpyrrole (2-UP, **76**) instead of MAP (**7**). In 1980, Rudd and Hopwood[129] used genetic analysis of nonpigmented mutants to identify and map the prodiginine biosynthetic gene cluster (the *red* cluster) in a mutant of *Streptomyces coelicolor* A3(2) deficient in actinorhodin biosynthesis. A decade later, Hopwood and co-workers[130] reported cloning of the complete *red* cluster from *S. coelicolor* and expression in a heterologous host (*Streptomyces lividans*). In 2001, the sequence of the entire *red* cluster became available through the *S. coelicolor* genome sequencing project (Figure 34),[63] facilitating elucidation of the biosynthetic pathway to undecylprodigiosin (**17**) and streptorubin B (**22**) via molecular genetics and biochemical studies (vida infra).

In 2000, while screening a *Serratia* sp. ATCC 39006 chromosomal cosmid library in *Erwinia carotovora* mutants deficient in carbapenem biosynthesis for genes that could restore carbapenem production, Salmond and co-workers[131] inadvertently created red-pigmented *E. carotovora* transformants that were found to produce prodigiosin (**1**). Four years later, Salmond and co-workers[132] reported sequencing and bioinformatics analysis of the cosmid responsible for inducing red pigment production upon introduction into *E. carotovora*, leading to identification of the cluster of genes (the *pig* cluster) that directs prodigiosin biosynthesis in *Serratia* species (Figure 34). Subsequent molecular genetic studies established the role played by each of the genes within the *pig* cluster in prodigiosin biosynthesis.[133]

4.2. 4-Methoxy-2,2′-bipyrrole-5-carboxaldehyde (MBC) Biosynthesis

The biosynthesis of MBC (**6**) in both *Serratia* and *Streptomyces* begins with the activation of L-proline as a thioester and subsequent conversion to the corresponding pyrrole. In 2001, Challis and co-workers[63] hypothesized that the *redM*, *redO*, and *redW* genes, respectively encoding a standalone nonribosomal peptide synthetase (NRPS) adenylation (A) domain, a peptidyl carrier protein (PCP), and a flavin adenine dinucleotide (FAD)-dependent dehydrogenase, are responsible for these transformations, in analogy to the initial steps of pyoluteorin biosynthesis proposed by Gould and co-workers.[134] The following year, Walsh and co-workers[135] experimentally confirmed that oxidation of the pyrrolidine of a L-prolyl thioester to the corresponding pyrrole is common to pyoluteorin and undecylprodigiosin biosynthesis and is catayzed by the PltF/RedM, PltL/RedO, and PltE/RedW enzymes. The *pigI*, *pigG*, and *pigA* genes in *Serratia* species were subsequently assigned similar roles in prodigiosin biosynthesis,[133] and Walsh, Kelleher, and co-workers[136] later verified the functions of the corresponding proteins. Independent deletion of the *redM* and *redW* genes in *S. coelicolor* abolished the biosynthesis of undecylprodigiosin (**17**) and streptorubin B (**22**).[137] Production of these metabolites could be restored by feeding synthetic MBC to the mutants, confirming that RedM and RedW are involved in MBC assembly.[137] Thus, the initial steps of MBC biosynthesis in *S. coelicolor* and *Serratia* species consist of L-proline activation by RedM/PigI, loading onto the peptidyl carrier protein RedO/PigG, and four-electron oxidation of the resulting prolyl thioester **277** by the FAD-dependent oxidase RedW/PigA to generate the pyrrole-2-carboxyl thioester **278** (Scheme 24).

A plausible mechanism for oxidation of thioester **277** to pyrrolyl thioester **278** can be proposed, based on mechanisms of other FAD-dependent dehydrogenases (Scheme 25).[138] Removal of the acidic α-proton of thioester **277**, followed by net elimination of the β-hydride (presumably via single-electron transfer from enolate **283** to the FAD cofactor **284**, followed by abstraction of the β-hydride by the resulting flavin radical) would result in Δ^2-pyrrolinyl-2-carboxyl-PCP **285**. Conversion of the $FADH_2$ formed in this process back to FAD by molecular oxygen would allow subsequent oxidation of Δ^2-pyrrolinyl-2-carboxyl-PCP **285** to pyrrole-2-carboxyl-PCP **278** via removal of the acidic γ-proton, followed by net δ-hydride elimination from **286**.

PCPs such as RedO and PigG must undergo post-translational phosphopantetheinylation, catalyzed by a phosphopantetheinyl transferase (PPTase), to convert them to their active holo forms. RedU and PigL show significant sequence similarity to known PPTases. Challis and co-workers[137] reported that deletion of *redU* in *S. coelicolor* abolishes the biosynthesis of undecylprodigiosin (**17**) and streptorubin B (**22**). Production of these metabolites could be restored by feeding synthetic MBC (**6**) to the mutant, indicating that *redU* is involved in the phosphopantetheinylation of carrier proteins involved in MBC biosynthesis. Feeding pyrrole-2-carboxylic acid *N*-acetylcysteamine (NAC) thioester **288**, which mimics the pyrrole-2-carboxyl-PCP intermediate in MBC biosynthesis (Figure 35), to the *redU* mutant also restored production of undecylprodigiosin (**17**) and streptorubin B (**22**), indicating that RedU is required only for the phosphopantetheinylation of the RedO PCP and not the Red N acyl carrier protein (ACP) domains (Scheme 24).

The pyrrole-2-carboxyl-PCP intermediate (**279**) in MBC biosynthesis is proposed to be elongated by decarboxylative condensation with a malonyl-CoA-derived thioester to yield β-keto thioester **280** (Scheme 24). RedX and RedN in *S. coelicolor*, and their homologues PigJ and PigH in *Serratia* species, are hypothesized to be involved in this step. RedN/PigH possesses two ACP domains, but in-frame deletion experiments have shown that either one is sufficient to support MBC biosynthesis. RedX/PigJ contains two ketosynthase-like domains, though only the C-terminal domain is predicted to possess ketosynthase activity because the active-site cysteine residue in the N-terminal domain is replaced by aspartate. The transfer of the pyrrole-2-carboxyl intermediate from RedO/PigG to the ketosynthase domain of RedX/PigJ has been inferred to occur by direct transthioesterificaton rather than via hydrolysis, reactivation, and acylation. This is because production of undecylprodigiosin (**17**) and streptorubin B (**22**) in *redM* and *redW* mutants of *S. coelicolor* was restored when the NAC thioester of pyrrole-2-carboxylic acid **289** (Figure 35) was added to the culture, but not when pyrrole-2-carboxylic acid was fed.[137]

RedN/PigH possesses a putative α-oxoamine synthase (OAS) domain at its C-terminus, which is predicted to catalyze chain release. OASs are pyridoxal 5′-phosphate (PLP)-dependent enzymes that catalyze the decarboxylative condensation of amino acids with acyl-CoA thioesters.[139] The OAS domain of RedN/PigH is therefore proposed to catalyze decarboxylative condensation of L-serine with RedN/PigH-bound β-keto thioester intermediate **280** to form α,γ-dioxoamine **281**, which likely undergoes spontaneous cyclization, dehydration, and tautomerization to yield 4-hydroxyl-2,2′-bipyrrole-5-methanol (HBM) **282** (Scheme 24).

A plausible mechanism for the reaction catalyzed by the RedN/PigH OAS domain can be proposed, based on the well-studied catalytic mechanism of 7-oxo-8-aminononanoate synthase (Scheme 26).[140] In the resting state, the PLP cofactor is bound to the OAS domain via an iminium linkage to an activesite lysine residue. Transimination with L-serine forms the external aldimine **290**, which is deprotonated by the liberated lysine amino group to form **291**. β-Keto thioester **280** acylates **291** to form **292**. Decarboxylation of **292** and subsequent protonation yields the product aldimine **293**, which undergoes transimination with the active-site lysine residue to release α,γ-dioxoamine **281**. Subsequent cyclization, dehydration, and tautomerization of **281** to yield HBM **282** can likely occur spontaneously, but catalysis of these transformations by RedN/PigH cannot be excluded.

Mutants of *Serratia* lacking *pigM* were shown to produce alcohol **282** instead of MBC (**6**), implicating PigM/RedV in the oxidation of **282** to aldehyde **275**. PigM and RedV show modest sequence similarity to flavin mononucleotide (FMN)-dependent bacterial nitroreductases,[133] suggesting that the oxidation of alcohol **282** to aldehyde **275** utilizes a flavin cofactor.

The final step in MBC (**6**) biosynthesis is methylation of the hydroxyl group of HBC **275** (Scheme 24). PigF and PigN have both been implicated in this transformation in *Serratia* species, but RedI is the only enzyme known to be involved in this reaction in *S. coelicolor* (note, however, that RedF is a homologue of PigN). Sequence comparisons suggest that the homologous RedI and PigF proteins function as *S*-adenosylmethionine (SAM)-dependent *O*-methyltransferases (OMTs). Deletion of the genes encoding these proteins in *S. coelicolor* and *Serratia* species results in the formation of O-demethylated analogues of undecylprodigiosin and prodigiosin, respectively.[64,133] Although PigN appears to be necessary for the efficient conversion of aldehyde **275** to MBC **6**, its precise function remains unknown.[133]

4.3. Methylamylpyrrole (MAP) Biosynthesis in *Serratia* Species

The same researchers[133] have reported that only three genes are required for MAP (**7**) biosynthesis in *Serratia: pigD*, *pigE*, and *pigB* (Figure 34). Sequence comparisons of the proteins encoded by these genes with proteins of known function led initially to a proposed pathway for MAP biosynthesis from 2-octenal and pyruvate. However, this proposal was subsequently revised in light of further experimental data.[141,142] (Scheme 27).

The first step of MAP biosynthesis was originally hypothesized to involve decarboxylative condensation of pyruvate with 2-octenal catalyzed by PigD, a thiamine diphosphate (TDP)-dependent enzyme.[133] This hypothesis explains the pattern of acetate incorporation into the MAP-derived moiety of prodigiosin **1** observed in earlier feeding experiments (Figure 33)[141] and is consistent with derivation of the *n*-pentyl chain and C-3/C-4/C-5 of the pyrrole from an eight-carbon fatty acid or polyketide precursor.[142] However, investigations of the substrate tolerance and product profile of purified recombinant PigD suggest that it catalyzes the condensation of pyruvate with a 2-octenoyl-ACP or CoA thioester (**294**) rather than 2-octenal.[143]

The catalytic mechanism of PigD likely involves addition of the heterocyclic carbene resulting from deprotonation of TDP to the keto group of pyruvate, followed by decarboxylation to form a C-1 acetaldehyde anion equivalent that adds to C-3 of 2-octenoyl thioester **294** (Scheme 28). Protonation of the resulting enolate, followed by deprotonation of the tertiary alcohol and elimination of the TDP carbene, would yield 3-acetyloctanoyl thioester **295**. Such a mechanism explains the 1968 observation of Goldschmidt and Williams[144] that thiamin promotes the formation of MAP in *Serratia*. The incorporation of C-3 of L-alanine into the methyl group attached to C-2 of the MAP pyrrole (Figure 33) is also consistent with this catalytic mechanism, because L-alanine is known to act as an amino donor in vivo in numerous PLP-mediated transamination reactions that yield pyruvate as a byproduct.

The second step in MAP biosynthesis was originally hypothesized to involve reductive amination of the aldehyde derived from PigD-catalyzed condensation of octenal with pyruvate by PigE, a putative PLP-dependent transaminase, followed by spontaneous cyclization of amino ketone **296** to generate dihydro-MAP **297**.[133] Consistent with this hypothesis, deletion of *pigB* (which encodes the putative final enzyme in MAP biosynthesis) resulted in accumulation of a metabolite that gave rise to ions in liquid chromatographic–mass spectrometric (LC-MS) analyses with $m/z = 152.1$, corresponding to the protonated form of **297**.[133] Treatment of mycelial extracts of this mutant with sodium cyanoborohydride ($NaBH_3CN$), an imine-specific reducing agent, converted the accumulated metabolite to a new species, which gave rise to ions with $m/z = 154.1$ in LC-MS analyses, corresponding to the protonated form of the pyrrolidine resulting from reduction of **297**.[133] More recently, however, it has been proposed that PigE is a bifunctional thioester reductase/PLP-dependent transaminase that catalyzes reduction of γ-keto thioester

295 to the corresponding aldehyde, followed by transamination to yield aminoketone **296** (Scheme 27).[145]

It has been proposed that the enamine tautomer of **297** is oxidized to MAP (**7**) by PigB, which shows sequence similarity to FAD-dependent amine oxidases. Interestingly, PigB possesses three putative transmembrane helices at its N-terminus and is thus likely to be membrane-associated. This is presumably due to the hydrophobic nature of the PigB substrate, which causes it to partition preferentially into the membrane bilayer.[133]

4.4. 2-Undecylpyrrole (2-UP) Biosynthesis in *Streptomyces* Species

Various genetic and biochemical experiments have implicated *redP*, *redQ*, *redR*, *redJ*, *redK*, and *redL* in the biosynthesis of 2-UP **76** (Scheme 29).[75,146,147] RedP, a homologue of the fatty acid biosynthetic enzyme FabH, was proposed to initiate 2-UP biosynthesis by catalyzing the decarboxylative condensation of a malonyl group, attached via a thioester linkage to the RedQ ACP, with acetyl-CoA (Scheme 29).[63] Consistent with this, deletion of *redP* in *S. coelicolor* strongly impaired prodiginine biosynthesis and led to the production of several undecylprodigiosin (**17**) analogues with altered alkyl chains.[146] These analogues are believed to result from FabH-mediated condensation of alternative starter units, such as isobutyryl-CoA and isovaleryl-CoA, with malonyl-RedQ. The keto group in acetoacetyl-RedQ resulting from RedP-catalyzed condensation is reduced to a methylene group, presumably by ketoreductase (KR), dehydratase (DH) and enoylreductase (ER) components of the core metabolic fatty acid synthase (FAS), because no genes encoding such enzymes are present in the *red* gene cluster (Figure 34; Scheme 29).[63] The butanoyl group generated by this process is then proposed to translocate onto the active-site cysteine residue of the FabF homologue RedR, where it undergoes chain elongation via a further round of decarboxylative condensation with malonyl-RedQ and subsequent ketoreduction, dehydration, and enoylreduction, again presumably catalyzed by the requisite components of the core metabolic FAS (Scheme 29).[63] Three further iterations of this cycle result in the formation of dodecanoyl-RedQ,[63] which is hydrolytically cleaved by the RedJ thioesterase to generate dodecanoic acid **298**.[147] Deletion of *redR* in *S. coelicolor* strongly impaired undecylprodigiosin production, which could be restored to wild-type levels by feeding of dodecanoic acid.[75] These results are consistent with the proposed role of RedR and RedJ in 2-UP biosynthesis. Purified recombinant RedJ has a preference for decanoyl-and dodecanoyl-ACP thioester substrates but is also able to catalyze the hydrolysis of acetyl-ACPs. The X-ray crystal structure of RedJ reveals a large hydrophobic pocket adjacent to its Ser-His-Asp catalytic triad that is capable of accommodating the hydrophobic alkyl chains of decanoyl- and dodecanoyl-ACPs,[147] explaining the substrate preference of RedJ. However, levels of prodiginine production are approximately 4-fold lower in a *redJ* mutant of *S. coelicolor* than in the wild type.[147] Feeding of dodecanoic acid to the mutant does not increase the amount of undecylprodigiosin **17**/streptorubin B **22** produced, but in-trans expression of *redJ* in the mutant restores wild-type production levels.[147] These data indicate that RedJ may also play a role in deacetylation of acetylated prodiginine biosynthetic ACPs/PCPs, which arise from utilization of acetyl-CoA in place of coenzyme A by the PPTase(s) responsible for converting them from apo to holo forms. Such partial acetylation of the phosphopantetheine thiols of these ACPs/PCPs would be expected to reduce the overall efficiency of prodiginine biosynthesis.

Deletion of *redL* in *S. coelicolor* abrogates the biosynthesis of undecylprodigiosin **17** and streptorubin B **22**.[75] Production of both metabolites is restored by feeding chemically synthesized 2-UP **76** to the *redL* mutant, demonstrating that RedL plays a role in the biosynthesis of 2-UP **76**, but not MBC **6**, and that streptorubin B **22** arises from functionalization of the alkyl chain of either 2-UP **76** or undecylprodigiosin **17** (see section 4.6).[75] Sequence analysis of RedL indicates that it contains an adenylation (A) domain, two ACP domains, a KS domain, an acyltransferase (AT) domain, and an OAS domain (Scheme 29). The A domain is proposed to catalyze adenylation of dodecanoic acid, prior to transfer of the dodecanoyl chain to the phosphopantetheine thiol of the adjacent ACP domain. Similarly, the AT domain is proposed to catalyze malonylation of the ACP domain appended to its C-terminus. Decarboxylative condensation of the malonyl thioester with the dodecanoyl thioester, catalyzed by the KS domain, would yield the corresponding β-keto tetradecanoyl thioester. Condensation of this thioester with glycine and subsequent decarboxylation is proposed to be catalyzed by the PLP-dependent OAS domain, via a mechanism analogous to that employed by the RedN OAS domain in MBC biosynthesis (Schemes 26 and 29). Cyclodehydration of the resulting α-oxoamine yields 5-undecylpyrrolin-3-one **299**, which is proposed to undergo reduction to **300** catalyzed by RedK, a putative NAD(P)H-dependent oxidoreductase, and subsequent dehydration to yield 2-UP **76**. Consistent with this hypothesis, the production of both undecylprodigiosin **17** and streptorubin B **22** was abolished in a *redK* mutant of *S. coelicolor*, and feeding of synthetic 2-UP **76** to the mutant restored the production of both metabolites.[75] LC-MS analyses showed that the *redK* mutant accumulated two new metabolites with m/z = 238.0 and 410.3, corresponding to the $[M + H]^+$ ions for 5-undecylpyrrolin-3-one **299** and a hydroxylated derivative of undecylprodigiosin, respectively.[75] The instability of these shunt metabolites precluded their isolation and structural elucidation by NMR spectroscopy. However, the hydroxylated undecylprodigiosin derivative was assigned structure **301** on the basis of UV–vis and high-resolution and tandem mass spectrometric (HRMS and MS/MS) data, biosynthetic considerations, and the result of the feeding experiment described above.[75] Compound **301** presumably arises from condensation of MBC **6** with 5-undecylpyrrolin-3-one **299** (Figure 36).

4.5. Condensation of 4-Methoxy-2,2′-bipyrrole-5-carboxaldehyde (MBC) with Methylamylpyrrole (MAP) or 2-Undecylpyrrole (2-UP)

The final step in biosynthesis of prodigiosin (**1**) and undecylprodigiosin (**17**) is the condensation of MAP (**7**) and 2-UP (**76**), respectively, with MBC (**6**, Scheme 24). These reactions were shown to be catalyzed by PigC in *Serratia* species and RedH in *S. coelicolor*.[148,149] Mutants in which *redH* and *pigC* have been deleted are unable to produce prodiginines, even when fed with MBC, MAP, or 2-UP.[148,149] Moreover, feeding of 2-UP and MBC to *Streptomyces venezuelae* expressing *redH* resulted in the production of undecylprodigiosin **17**.[148] Similarly, when MAP and MBC were fed to *E. coli* expressing *pigC*, prodigiosin **1** was produced.[149] Both RedH and PigC are able to utilize a variety of MBC analogues with alterations to the monosubstituted pyrrole as substrates. This results in production of the corresponding undecylprodigiosin and prodigiosin analogues. [148,149] However, RedH cannot tolerate an MBC analogue in which the trisubstituted pyrrole is replaced with a furan, indicating that the NH group of the trisubstituted pyrrole may play an important role in the condensation reaction.[148]

Sequence analyses indicated that RedH contains three functional domains (Scheme 30).[63] Two of these domains show significant sequence similarity to the ATP-binding and phosphotransfer domains of phosphoenolpyruvate synthase (PEPS) and pyruvate phosphate dikinase (PPDK), suggesting that RedH is an ATP-dependent enzyme that transfers a phosphate group to one of its substrates.[63] PigC has an identical domain architecture to RedH.[133] The third domain of RedH/PigC is not similar to any proteins of known function and has been proposed to be an MBC-binding domain.[133,148] More recently it has been shown that PigC partitions to the membrane bilayer when it is overproduced in *E. coli*, and it has been confirmed that ATP is required for the condensation of MBC and MAP by PigC-containing membrane preparations.[145]

A plausible mechanism for the RedH-catalyzed condensation reaction has been proposed, based on the previously studied catalytic mechanisms of PEPS and PPDK (Scheme 30).[148,150] Thus, a conserved histidine residue in the phosphotransfer domain reacts with the γ-phosphate group of ATP. The resulting phosphoryl-histidine intermediate then phosphorylates the carbonyl group of MBC **6** to form doubly vinylogous iminium ion **302**. Nucleophilic attack of **302** by C-5 of 2-undecylpyrrole **76**, followed by elimination of phosphate and concomitant deprotonation to rearomatize the pyrrole, yields undecylprodigiosin **17** (Scheme 30). A similar mechanism for PigC-catalyzed condensation of MBC **6** and MAP **7** to form prodigioisin **1** has also been proposed, and site-directed mutagenesis of the conserved histidine residue in the phosphotransfer domain has shown it plays an essential role in catalysis (Scheme 30).[133,145]

4.6. Oxidative Carbocyclizations of Undecylprodigiosin

Carbocyclic derivatives of undecylprodigiosin **17**, such as streptorubin B **22** and metacycloprodigiosin **20**, have been shown to result from oxidative C–C bond-forming reactions catalyzed by enzymes belonging to the Rieske non-heme iron-dependent oxygenase family.[64,151] RedG, which shares conserved [2Fe-2S] cluster and non-heme iron-binding residues with the well-studied Rieske oxygenase naphthalene dioxygenase (NDO), is responsible for the conversion of undecylprodigiosin **17** to streptorubin B **22**.[64] RedG was first implicated in this oxidative carbocyclization reaction via deletion of its encoding gene, which abrogated the production of streptorubin B **22**, but not undecylprodigiosin **17**.[64] Furthermore, a *redO* mutant of *S. coelicolor* accumulated desmethylundecylprodigiosin **303** (Figure 37) but not desmethylstreptorubin B, implying that undecylprodigiosin **17**, rather than 2-undecylpyrrole **76**, is the substrate of RedG.[64]

Feeding of undecylprodigiosin **17** to *S. venezuelae* in which *redG* or *redHG* have been constitutively expressed resulted in the production of streptorubin B **22**, demonstrating that RedG alone is responsible for the oxidative carbocyclization reaction. Streptorubin B **22** production levels were higher in the strain expressing both *redG* and *redH* than in the strain expressing *redG* alone, suggesting that RedG may form a complex with RedH that results in enhanced catalytic efficiency. No transformation to a carbocyclic derivative was observed when 2-undecylpyrrole **76** was fed to the strains.[64]

Orthologues of *redH* and *redG*, named *mcpH* and *mcpG*, respectively, were identified in the genome of the metacycloprodigiosin **20** producer *S. longisporus ruber* and sequenced. Expression of *mcpG* in the *redG* mutant of *S. coelicolor* led to the exclusive production of metacycloprodigiosin **20**, demonstrating that RedG and McpG catalyze the regio- and stereodivergent oxidative carbocyclizations of undecylprodigiosin **17** to form streptorubin B **22** and metacycloprodigiosin **20**, respectively (Scheme 31).[64]

A mechanism for the RedG-catalyzed oxidative carbocyclization reaction has recently been proposed, based on what is known about the catalytic mechanism of the well-studied Rieske oxygenase NDO (Figure 38).[151] Binding of undecylprodigiosin **17** to the active site is hypothesized to trigger loss of a water ligand from the non-heme iron center, allowing dioxygen to bind. Transfer of an electron from the [2Fe-2S] cluster to the nonheme iron center, coupled with loss of a second water ligand and proton transfer, leads to formation of Fe(III)OOH complex **304**. Abstraction of a hydrogen atom (or hydride ion) from C-7′ of enzyme-bound undecylprodigiosin **17** by either the Fe(III)OOH complex, or an Fe(V)O(OH) complex resulting from rearrangement of the Fe(III)OOH complex, would result in formation of a carbon-centered radical **305** (or the corresponding cation in the case of hydride abstraction) and an Fe(IV)O(OH_2) complex **306**. Addition of the C-7′ radical (or cation) to C-4 would result in formation of the C-5 radical **307** (or the corresponding cation), either of which can be stabilized by delocalization into the adjacent π-system. Abstraction of a hydrogen atom from C-4 of **307** by the Fe(IV)O(OH_2) complex **306** (or a proton in the case of the cationic intermediate) would yield enzyme-bound streptorubin B **22**, the release of which is coupled to transfer of a second electron from the [2Fe-2S] cluster to the non-heme iron center and protonation of the hydroxide ligand.

Very recently, the use of 2-(5-pentoxypentyl)-pyrrole **308** to probe the mechanism of RedG-catalyzed conversion of undecylprodigiosin (**17**) to streptorubin B (**22**) has been reported.[152] Feeding of 2-(5-pentoxypentyl)pyrrole **308**, along with MBC **6**, to *Streptomyces albus* expressing *redH* and *redG* resulted in production of the expected 6′-oxa undecylprodigiosin analogue **309**. However, none of the corresponding 6′-oxa streptorubin B analogue could be detected. Instead, a small quantity of 5-hydroxypentylprodigioisn **310** was produced (Scheme 32).

5-Hydroxypentylprodigiosin **310** presumably arises via abstraction of a hydrogen atom from C-7′ of **309** by Fe(III)OOH complex **304**. The resulting radical/cation is stabilized by the adjacent oxygen lone pair, preventing it from adding to C-4. Instead it reacts with the Fe(IV)O(OH_2) complex **306** to form the hemiketal complex **311**, which collapses with loss of *n*-pentanal **312** to yield 5-hydroxypentylprodigiosin **310** (Scheme 33).

The same study[152] also investigated the stereochemical course of RedG-catalyzed conversion of undecylprodigiosin **17** to streptorubin B **22**. Feeding of [7′-^{2}H](7′*R*)-2-undecylpyrrole **314**, along with MBC **6**, to *S. albus* expressing *redH* and *redG* resulted in substantial loss of the deuterium label from the resulting streptorubin B **22** (Scheme 34). In contrast, the deuterium label was largely retained when [7′-^{2}H](7′*S*)-2-undecylpyrrole **315** and MBC **6** were fed to *S. albus* expressing *redH* and *redG* (Scheme 34).[152]

These results show that RedG abstracts predominantly the *pro-R* hydrogen atom from C-7′ of undecylprodigiosin (Scheme 34). Streptorubin B **22** has predominantly the 7′*S* configuration (see section 2.6). Thus, the conversion of undecylprodigiosin **17** to streptorubin B **22** proceeds with inversion of configuration at C-7′. This contrasts with the functionalization of unactivated carbon centers via oxidative heterocyclization reactions catalyzed by non-heme iron-dependent oxygenases such as isopenicillin N synthase and clavaminate synthase. In such cases the reactions proceed with retention of configuration at the carbon atom undergoing functionalization.[153,154]

4.7. Marineosin Biosynthesis

In their paper reporting the isolation and structural elucidation of marineosins A and B (**41** and **42**), Fenical and co-workers[91] noted that both structures contain a retron for an inverse electron-demand Diels–Alder reaction. This led them to propose that the marineosins may be biosynthesized via an intramolecular [4 + 2] cycloaddition reaction of **316** (Scheme 35).

In early 2010, Lindsley and co-workers[155] reported the synthesis of **316** and attempts to complete a biomimetic synthesis of the marineosins inspired by Fenical's biosynthetic proposal. Despite trying numerous different reaction conditions, they were unable to observe the conversion of **316** to either marineosin A (**41**) or marineosin B (**42**). Although this did not disprove Fenical's biosynthetic proposal, because the Diels– Alder reaction may require enzymatic catalysis, these findings called its feasibility into question.

Later the same year, Snider and co-workers[156] reported a different route toward the synthesis of marineosins and put forward an alternative biosynthetic proposal, involving abstraction of a hydrogen atom from C-8′ of undecylprodigiosin **17** to create a radical that undergoes cyclization to generate intermediate **317** (Scheme 36). Intramolecular hydrogen transfer via a six-membered transition state would convert intermediate **317** to the C-10′ radical **318**, which could be trapped by enzyme-mediated hydroxylation to give **319**. Tautomerization of **319** followed by nonselective spirocyclization would yield marineosins A and B (**41** and **42**; Scheme 36).

In 2014, Reynolds and co-workers[157] reported the cloning, sequencing, and heterologous expression of the *mar* gene cluster responsible for marineosin biosynthesis in *Streptomyces* CNQ-617. The gene content and organization of the *mar* cluster are identical to those of the *red* cluster responsible for streptrorubin B biosynthesis in *S. coelicolor*. The only exception is that the *mar* cluster contains an additional gene, *marA*, which was shown to be involved in the last step of marineosin biosynthesis, the reduction of premarineosins **321** to **41** and **42** (Scheme 37). Deletion of *marG*, which encodes a RedG homologue (see section 4.6), from the heterologous expression construct abrogated marineosin production and resulted in the accumulation of 10′-hydroxyundecylprodigiosin. Feeding of (10′*S*)-10′-hydroxyundecylprodigiosin **320** deuterated at C-10′ to *S. venezuelae* expressing *marG* resulted in the production of premarineosins **321** in which the deuterium label had been retained, whereas an analogous experiment employing (10′*R*)-10′-hydroxyundecylprodigiosin deuterated at C-10′ resulted in loss of the deuterium label from premarineosins **321**.[92] Taken together, these data suggest that the marineosins are biosynthesized via the pathway shown in Scheme 37.

On the basis of their recent mechanistic studies of RedG, Challis and co-workers[152] have proposed a MarG catalytic mechanism involving abstraction of a hydrogen atom from C-8′ of (10′*S*)-10′-hydroxyundecylprodigiosin **320** by the Fe(III)-OOH complex **304** to yield a methylene radical that cyclizes onto C-1″. The resulting delocalized radical **322** can then react with Fe(IV)O(OH$_2$) complex **306** to form the hydroxylated product **323** (Figure 39).

The product **323** of the MarG-catalyzed reaction is hypothesized by Challis and co-workers[152] to undergo elimination of water to form **324**, followed by addition of the C-10 hydroxyl group to afford the premarineosins **321** (Scheme 38).

4.8. Roseophilin Biosynthesis

Although roseophilin has been the target of numerous synthetic studies over the last two decades, relatively little has been known about its biosynthesis until recently. It seems likely that chlorination is the final step in roseophilin biosynthesis, because dechlororoseophilin **38** was isolated by Kawasaki and coworkers[90] from the roseophilin producer *S. griseoviridus* in 2009. Prodigiosin R1 **40** has also been isolated from this strain and it seem likely that both **38** and **40** are derived from the common precursor 11′-dimethylundecylprodigiosin **39**.[89]

In 2009, Kawasaki et al.[158] reported identification of the probable dechlororoseophilin **38** and prodigiosin R1 **40** biosynthetic gene cluster in *S. griseoviridis*. This gene cluster is very similar to the *red* cluster in *S. coelicolor*, but it contains four genes (*rphG*, *rphG2*, *rphG3*, and *rphG4*) encoding RedG orthologues. The protein encoded by the *rphG3* gene is likely nonfunctional because its N-terminal Rieske domain lacks the conserved Cys and His residues that ligate the [2Fe-2S] cluster. Two of the other RedG orthologues have recently been proposed by Challis and co-workers[152] to be involved in the assembly of dechlororoseophilin **38**, via a RedG-like oxidative carbocyclization between C-9′ and C-4 of 11′-dimethylundecylprodigiosin **39** to yield **325**, followed by a MarG-like oxidative carbocylization– hydroxylation to yield **326** (Scheme 39). Ring opening of **326**, followed by tautomerization, cyclization, and elimination of ammonia would yield dechlororoseophilin (**38**). The remaining RedG orthologue is hypothesized to catalyze the conversion of 11′-dimethylundecylprodigiosin **39** to prodigiosin R1 (Scheme 39).[152]

5. CONCLUDING REMARKS

The prodiginine family of natural products has stimulated research in diverse fields of science for well over a century due to their wide range of fascinating properties, from their color to their chirality. In writing this review, we aimed to provide a comprehensive overview of developments in the chemistry and biology of the prodiginine alkaloids over the past century, while providing the historical context in which major discoveries were made. We have also taken care to note the last century's rich interplay between natural product structural elucidation and chemical synthesis, between chemical synthesis and biosynthetic theory, and between biosynthetic theory and molecular genetics. Nearly 200 years after the identification of *Serratia*, research into prodiginine chemistry and biology continues to provide leads in fascinating fields including aliphatic C–H functionalization, cellular ion transport, and modular natural product biosynthesis. We hope that our organization of recent research and newly proposed classification of prodiginine natural products will assist modern researchers in natural products chemistry, organic synthesis, and microbial natural product biosynthesis alike.

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Biographies

Dennis X. Hu graduated from Northwestern University in 2011 with B.A./M.S. degrees in chemistry carried out under the mentorship of Professor Regan J. Thomson. After a year of graduate study at the University of Cambridge with Professor Steven Ley as a Churchill fellow, he moved to Stanford University as an NSF graduate fellow in the lab of Professor Noah Z. Burns. Following the completion of his Ph.D. in 2015, Dennis accepted a position as a medicinal scientist at FLX Biosciences, Inc., South San Francisco, CA.

David M. Withall graduated with an M. Chem. in chemistry with industrial training from the University of Warwick in 2010 and completed Ph.D. studies on the biosynthesis of streptorubin B at the same institution under the supervision of Professor Greg Challis in 2014. He is currently a postdoctoral fellow at Rothamsted Research with Dr. Mike Birkett and Professor John Pickett. He was the recipient of a Briggs scholarship from the Royal Society of Chemistry (2010–2013).

Gregory L. Challis was born in the United Kingdom in 1973. He graduated with a B.Sc. in chemistry from Imperial College London in 1994 and was awarded a Ph.D. by the University of Oxford in 1998, for research carried out under the supervision of Professor Sir Jack Baldwin. After postdoctoral studies as a Wellcome Trust International Prize travelling research fellow with Professor Craig Townsend at Johns Hopkins University and with Professor Keith Chater at the John Innes Center, he joined the faculty of the University of Warwick in 2001. Greg's research interests encompass the discovery, biosynthesis, bioengineering, and mechanism of action of bioactive natural products. He is the recipient of the Royal Society of Chemistry's Meldola Medal (2002) and Hickinbottom Award (2009), the Fleming Prize of the Microbiology Society (2007), and the Gabor Medal (2009) and a Wolfson Research Merit Award (2013–2018) from the Royal Society.

Regan J. Thomson was born in New Zealand in 1976 and received his Ph.D. in 2003 at The Australian National University, working with Professor Lewis N. Mander. Following postdoctoral studies with Professor David A. Evans at Harvard University, he joined the faculty at Northwestern University in 2006. Regan's research interests include reaction development, total synthesis, natural product discovery and biosynthesis, and atmospheric chemistry. He is the recipient of an NSF Career Award (2009), an Amgen Young Investigator Award (2010), an Illinois Division American Cancer Society Research Scholar Award (2012), and a Novartis Chemistry Lectureship (2015–2016).

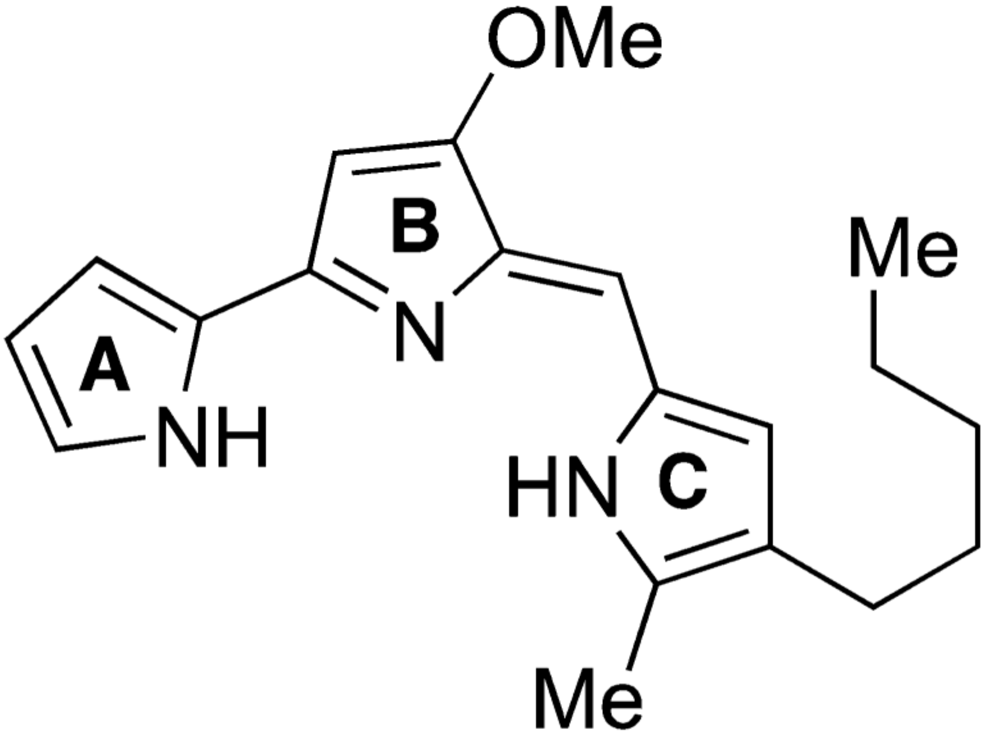

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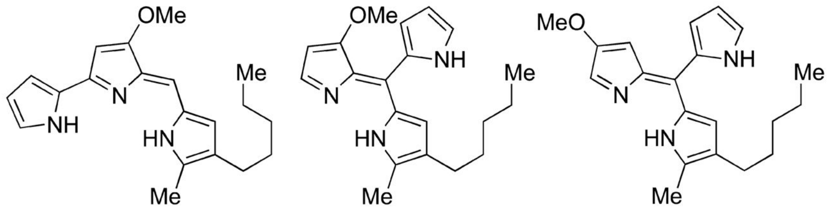
Figure 1.

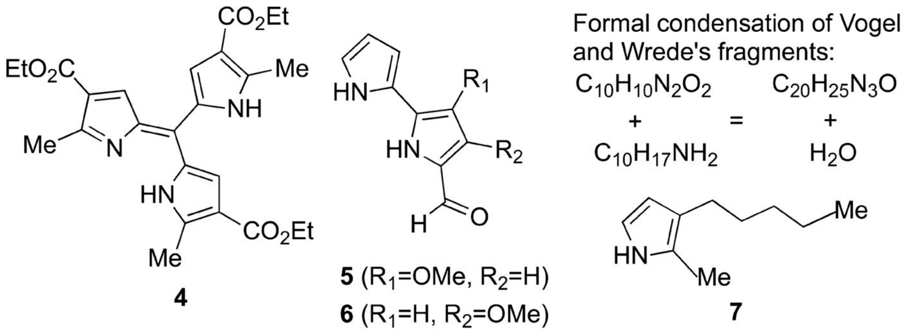
Prodigiosin (**1**), the prototypical prodiginine.



1 **2** **3**

Figure 2.

Structures originally proposed by Wrede and Rothhaas[39,40] for prodigiosin. Stucture **1** is now known to be correct.



4

5 (R_1=OMe, R_2=H)
6 (R_1=H, R_2=OMe)

7

Figure 3.

Early structural studies attempted to match the UV spectrum of prodigiosin to synthetic model compounds such as **4**.

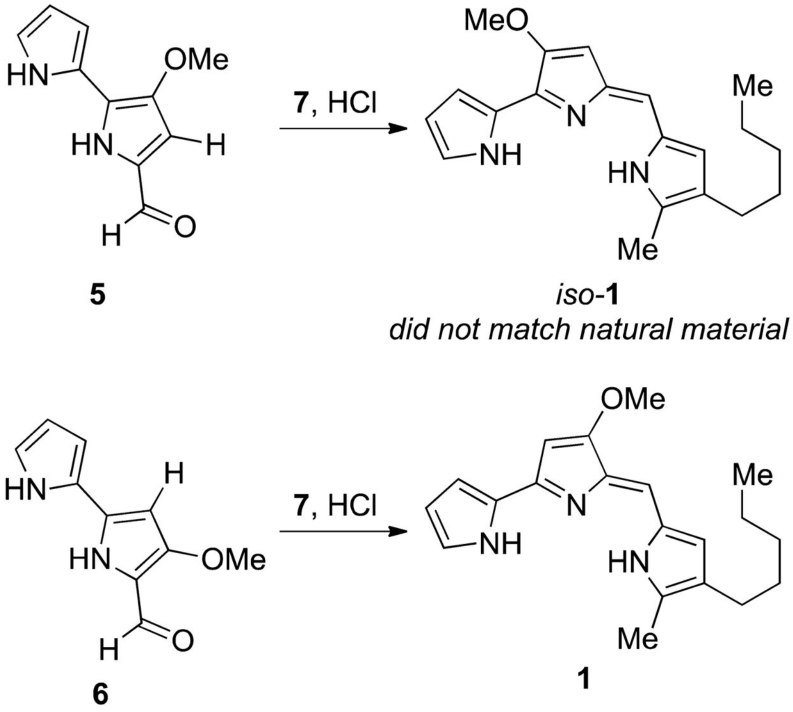
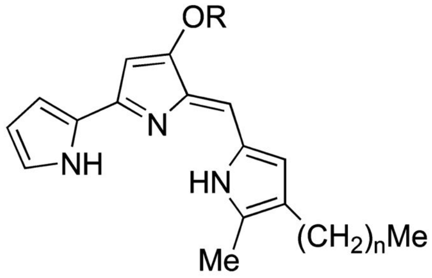


Figure 4.

Rapoport and Willson's[43] condensation of aldehyde **5** with pyrrole **7** yielded a compound, *iso*-**1**, whose UV–vis spectrum differed significantly from natural prodigiosin. Condensation of aldehyde **6** with pyrrole **7** yielded a compound identical to natural prodigiosin (**1**).



8 (n = 2, R = Me)
9 (n = 3, R = Me
10 (n = 4, R = Me)
11 (n = 5, R = Me)
12 (n= 6, R= Me)
13 (n = 4, R = H)

Figure 5.

Structures of alkyl-chain homologues of prodigiosin (**8–12**) and norprodigiosin (**13**), a demethylated prodigiosin analogue.

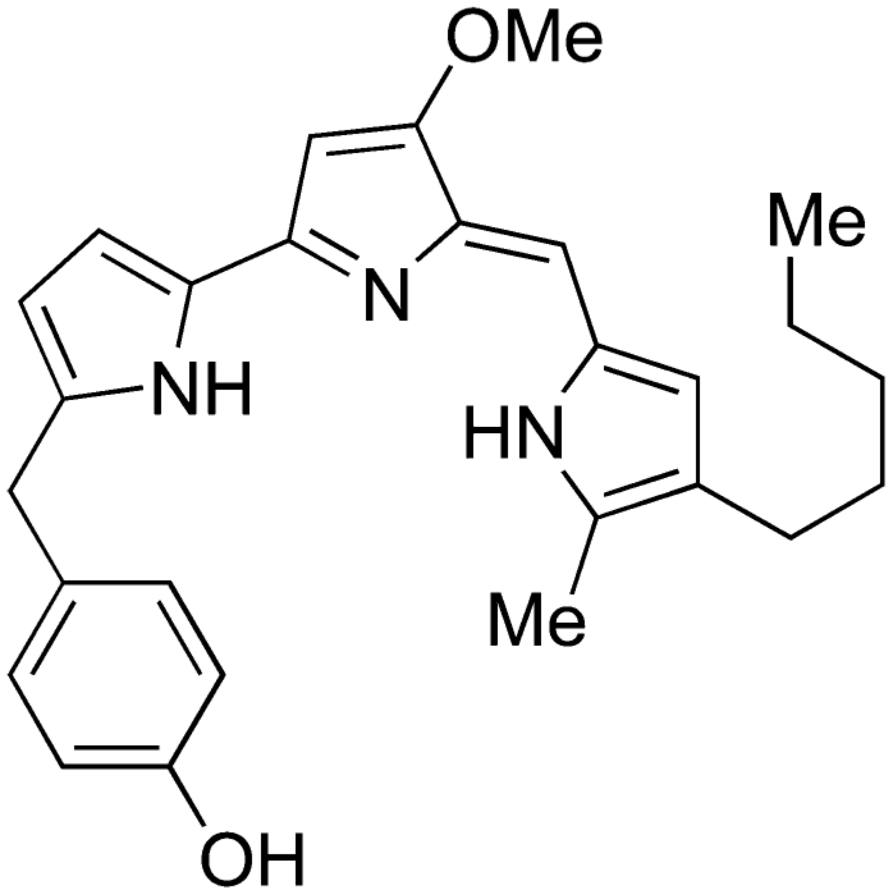


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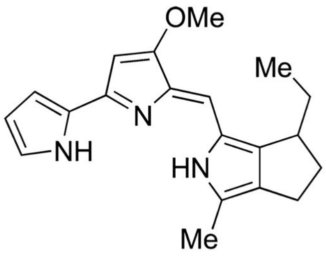
Figure 6.

Structure of a unique prodigiosin analogue (**14**) from *P. rubra* with a substituent at C-2 of ring A.



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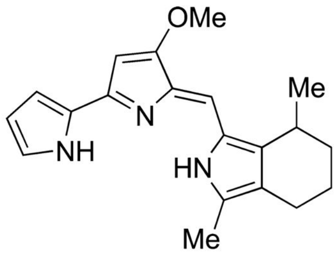

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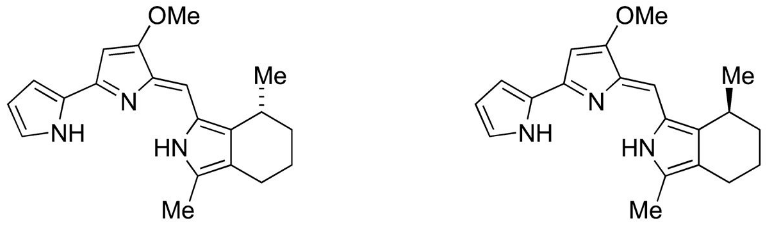
Figure 7.

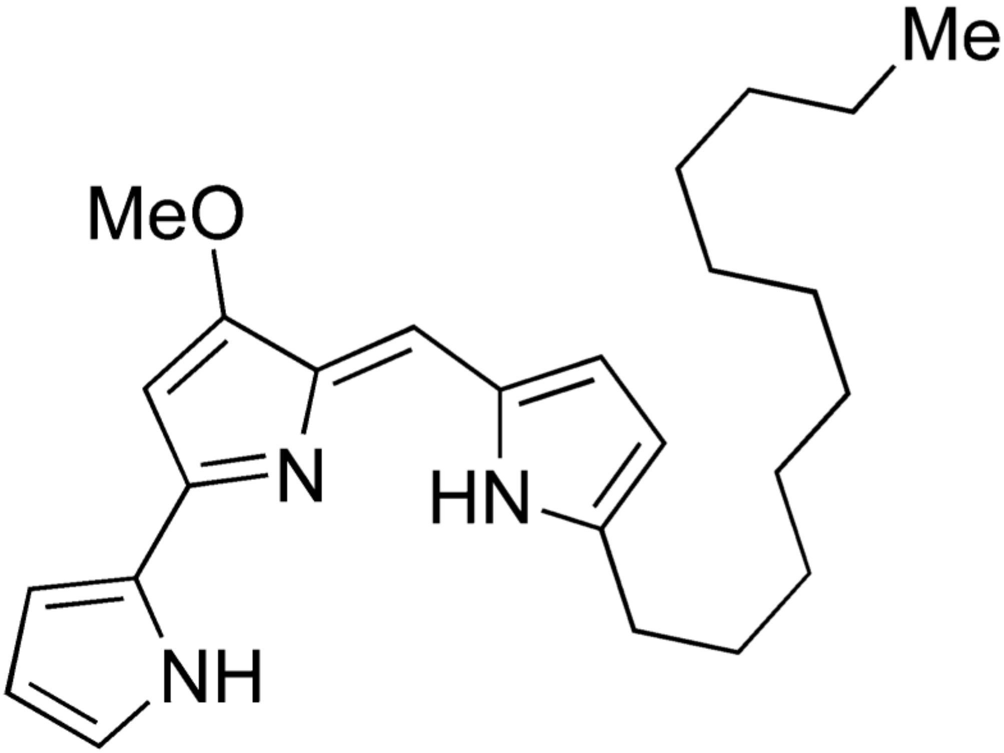
Originally proposed (**15**) and subsequently revised (**16**) structures for cycloprodigiosin.



(*R*)-**16** (*S*)-**16**

Figure 8.

Sarpong and co-workers[55] determined that naturally occurring cycloprodigiosin (**16**) is a 83:17 mixture of (*R*) and (*S*) enantiomers.



17

Figure 9.

Structure of undecylprodigiosin (**17**), the 25-carbon prodiginine identified independently by Wasserman et al.[58] and Nagatsu and co-workers.[57]

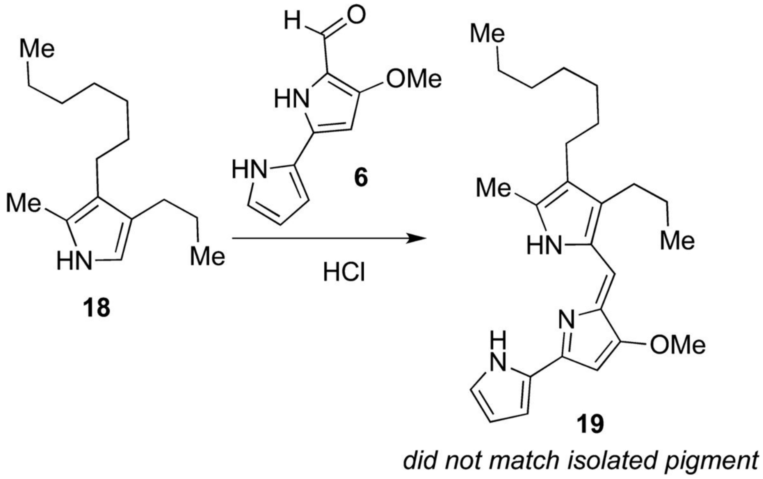


Figure 10.

Wasserman's original structural proposal for metacycloprodigiosin (**19**), based on the incorrect assumption that *Streptomyces* species produce prodigiosin (**1**). The properties of synthetic **19** did not match those of isolated metacycloprodigiosin.

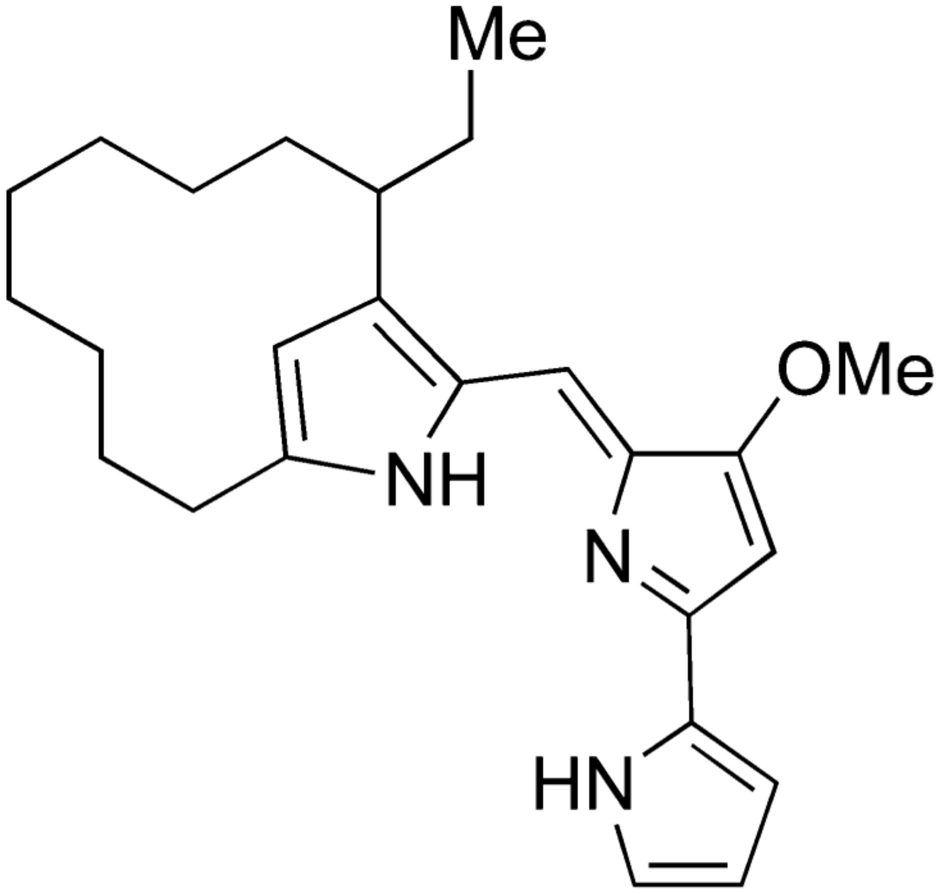


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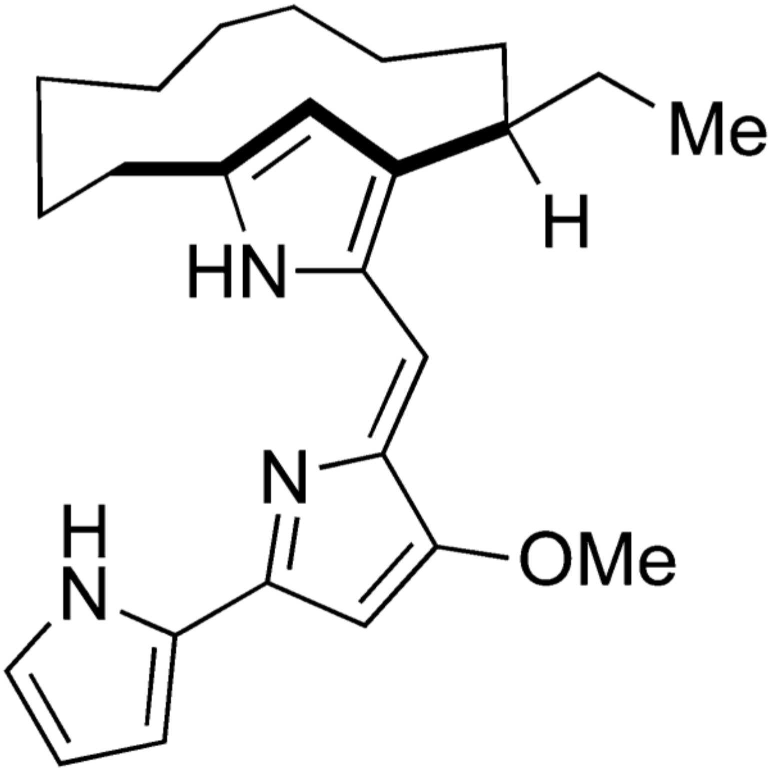
Figure 11.

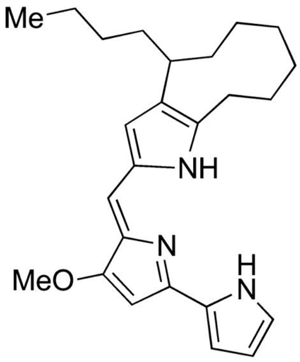
Structure of metacycloprodigiosin (**20**), deduced by Wasserman and co-workers[61] and confirmed by total synthesis.[62] The absolute stereochemistry was not defined.



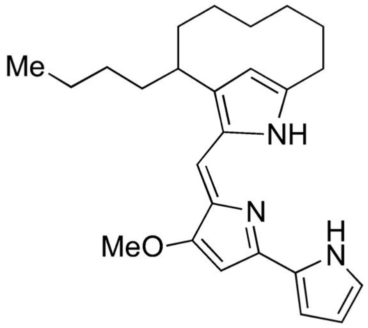
(*R*)-**20**

Figure 12.

Absolute configuration of metacycloprodigiosin was assigned as *R* through total synthesis.

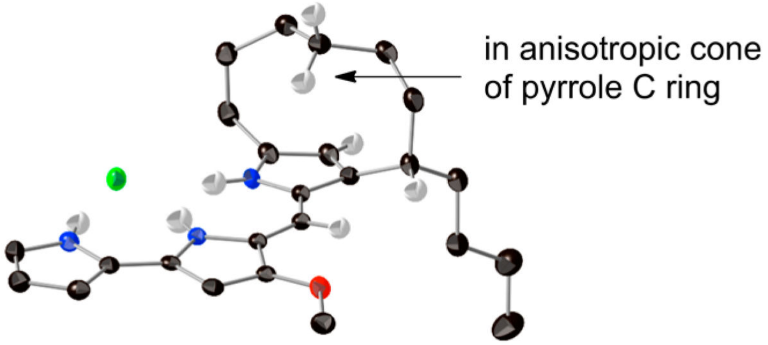


21



22

Figure 13.
Two structures initially suggested by Gerber[67] for the desaturated derivative of undecylprodigiosin produced by *Streptomyces* sp. Y-42. In early work, structure **21** was favored.



22•HCl

Figure 14.

Calculations predict that the meta-bridged isomer **21** has a proton located directly within the anisotropy cone of the ansa-bridged pyrrole. Consistent with this hypothesis, one of the C-4′ protons of streptorubin B is observed to be significantly shielded in the ^{1}H NMR spectrum. This is confirmed by the X-ray structure of **22**·HCl shown.[66]

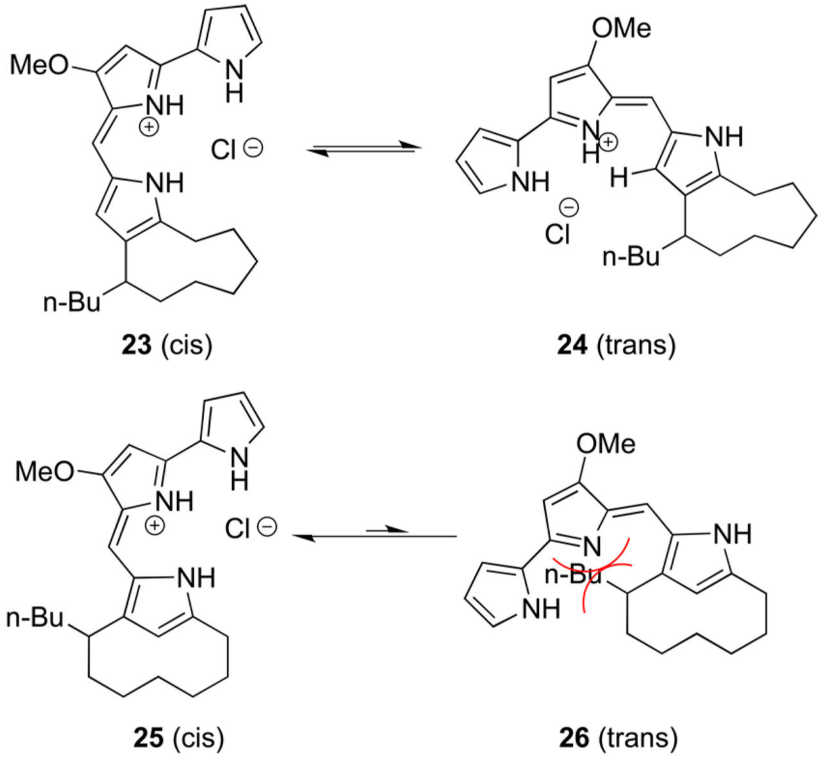


Figure 15.

Bacteria appear to have evolved oxidative cyclization enzymes to enforce the cis configuration, in which counteranion and cation binding is favored by the protonated and neutral forms of the molecules, respectively.

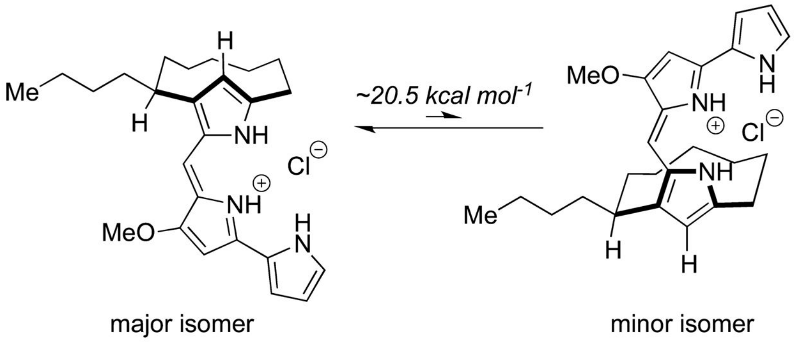
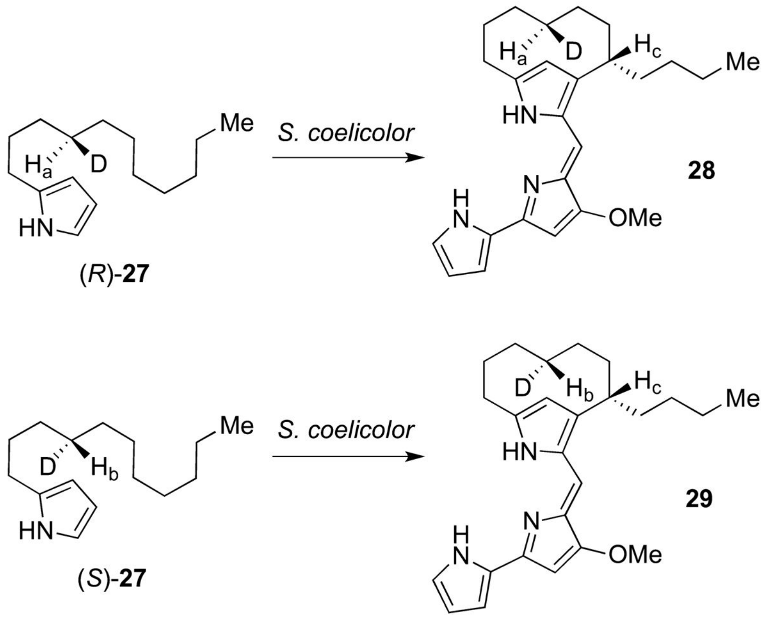


Figure 16.

Structures of the atropisomers of streptorubin B discovered in the course of stereochemical studies by Challis and co-workers[79] and synthetic studies by Thomson and co-workers.[66] The natural product has the 7′*S* absolute configuration as shown, and the *n*-butyl side chain is oriented anti to the exocyclic methine group in the thermodynamically more stable atropisomer.



(*R*)-**27**

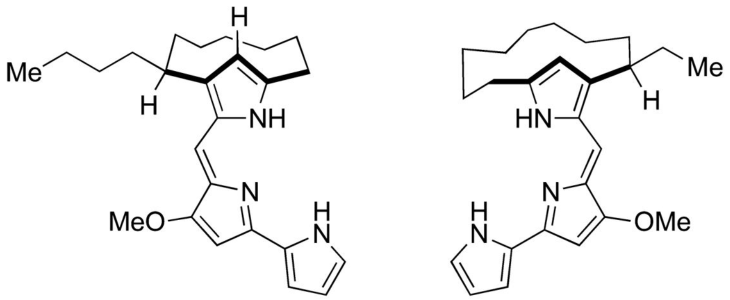
28

(*S*)-**27**

29

Figure 17.

Feeding experiment by Challis and co-workers[79] to elucidate the absolute configuration of streptorubin B.



streptorubin B (**22**) from
S. coelicolor

metacycloprodigiosin (**20**) from
S. longisporus ruber

Figure 18.

Naturally occurring streptorubin B and metacycloprodigiosin are pseudoenantiomeric. It is not clear whether metacycloprodigiosin possesses a great enough torsional barrier about its 12-membered ring to allow observation of atropisomers as in the case of streptorubin B.

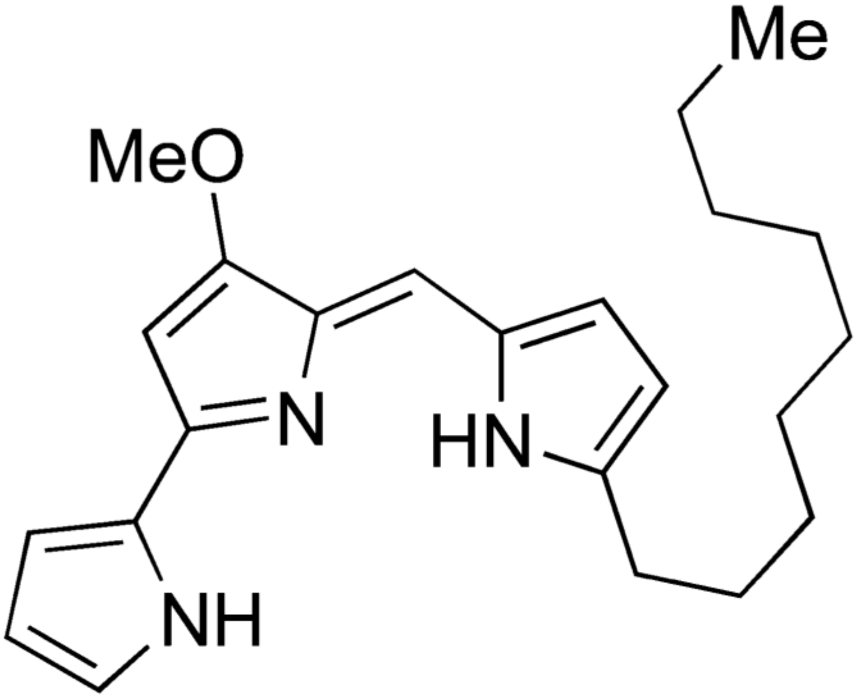


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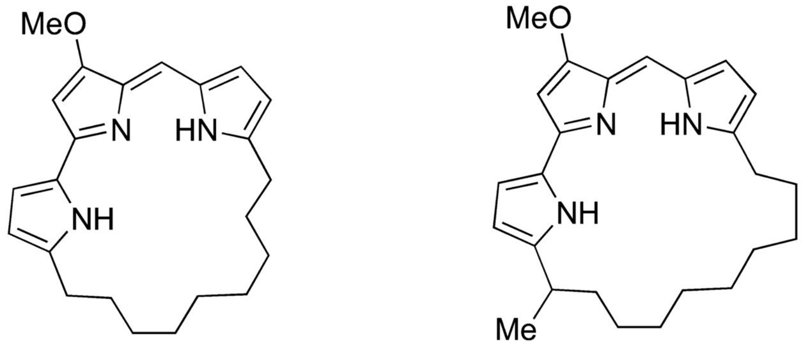
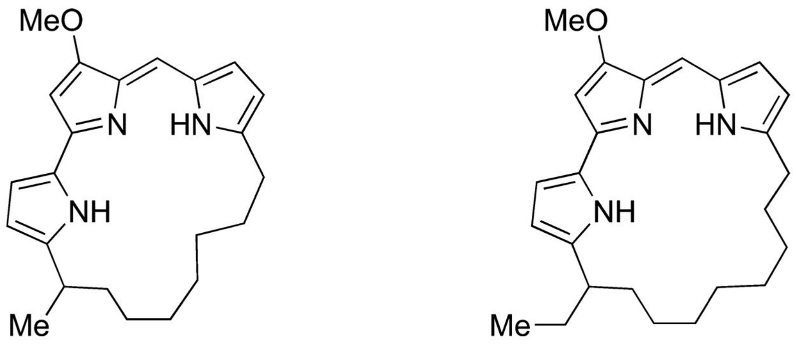
Figure 19.
Structure of nonylprodigiosin isolated from *A. madurae*.



31 **32**

Figure 20.

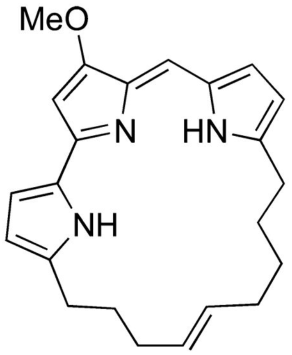
Structures of carbocyclic derivatives **31** and **32** of nonylprodigiosin and undecylprodigiosin isolated from *A. madurae* and *A. pelletieri*, respectively.



33 **34**

Figure 21.

Structures of the minor prodiginine congeners **33** and **34**, produced by *A. madurae* and *A. pelletieri*, respectively.



35

predominant tautomer

vs.

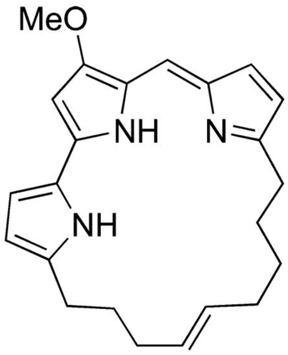


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Figure 22.

Two possible tautomers of a key intermediate in cyclononylprodigiosin synthesis, which were discriminated by use of X-ray crystallographic analysis.

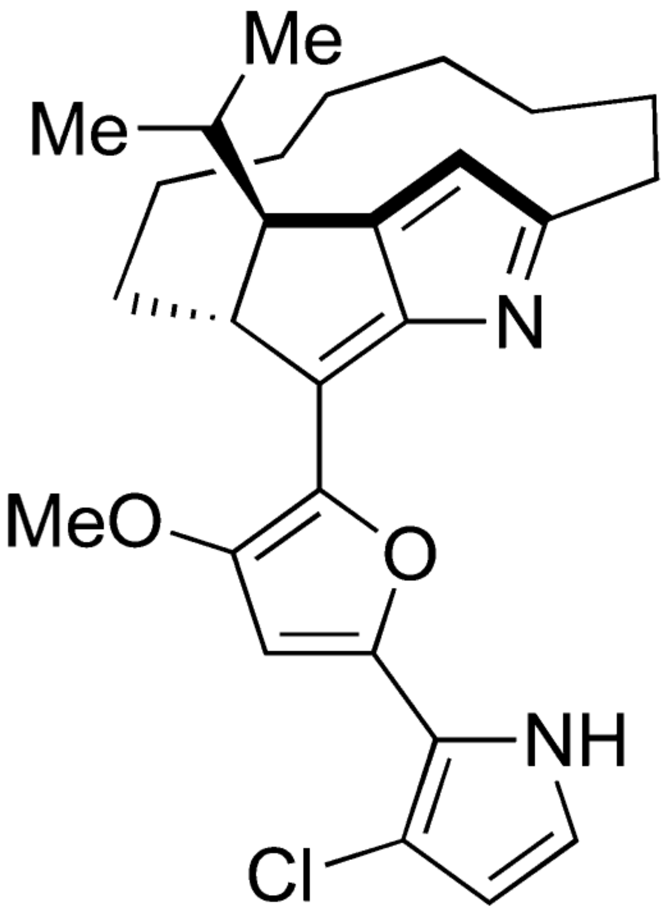

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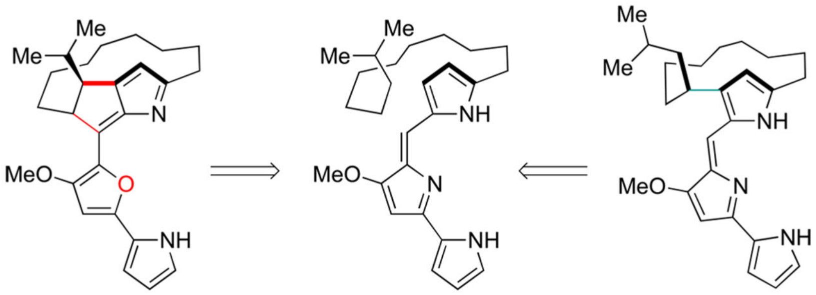
Figure 23.

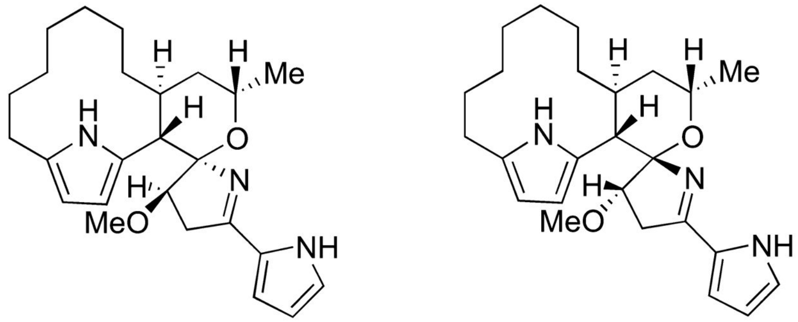
Structure of roseophilin (only the relative stereochemistry was reported at the time of isolation).



38 **39** **40**

Figure 24.

Structures of 11′-dimethylundecylprodigiosin (**39**), dechlororoseophilin (**38**), and prodigiosin R1 (**40**) and their proposed biosynthetic relationship.



41

42

Figure 25.
Structures of marineosins A and B.

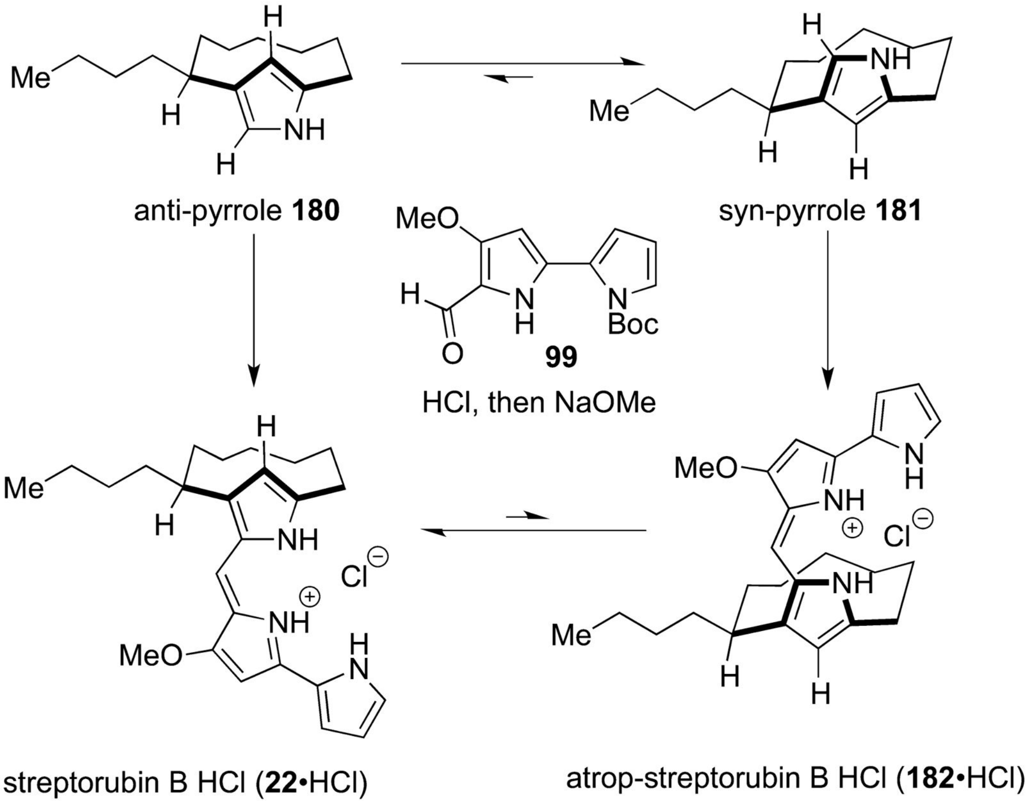


Figure 26.

Atropisomerism phenomenon in streptorubin B and its synthetic pyrrole intermediate, discovered in the course of total synthesis by Thomson and co-workers.[66]

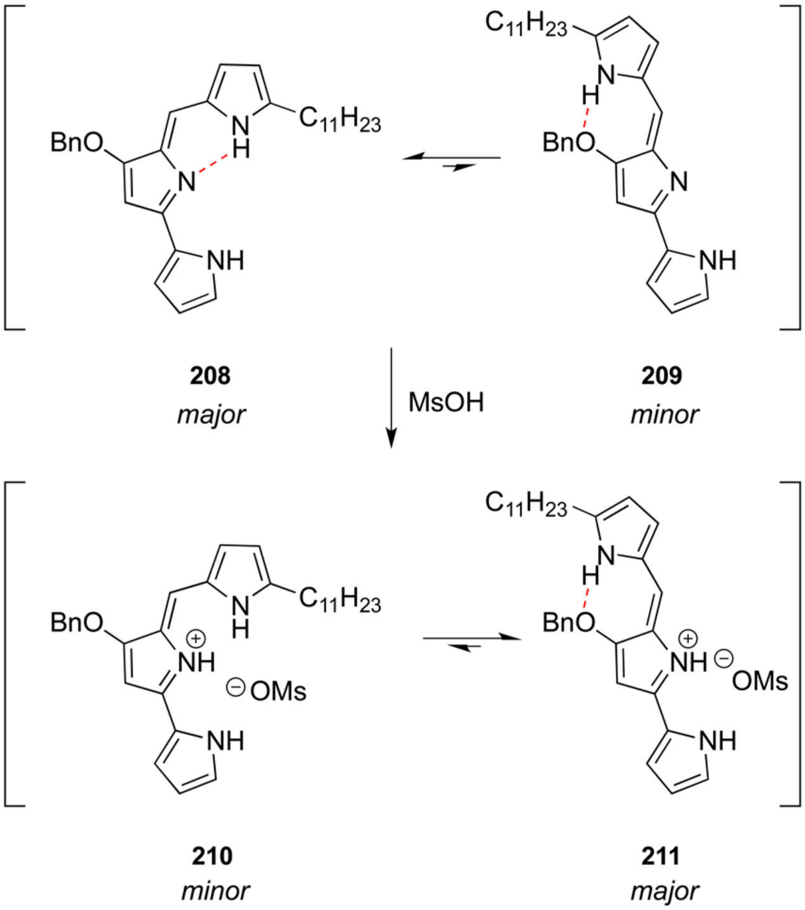
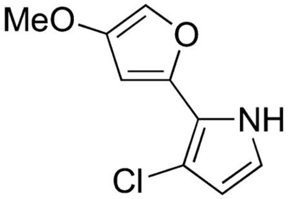


Figure 27.

Rotomer interconversion phenomenon.



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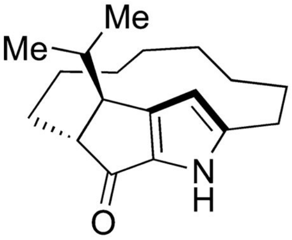
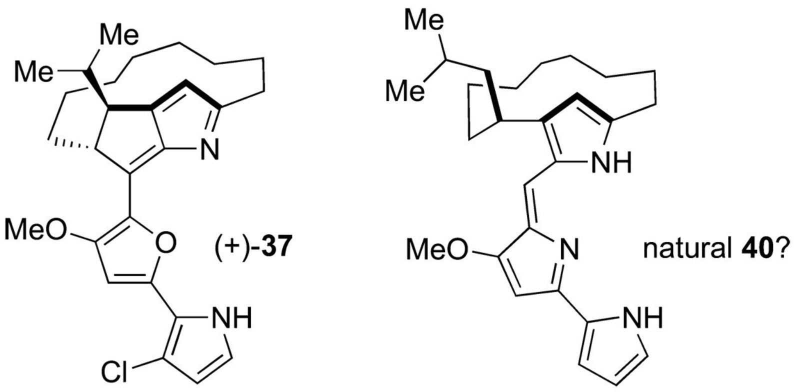


217

Figure 28.

Commonly targeted synthetic roseophilin fragments.



(+)-**37**

natural **40**?

Figure 29.

Absolute configuration of natural roseophilin, (+)-**37**, and suspected configuration of natural prodigiosin R1, **40**.

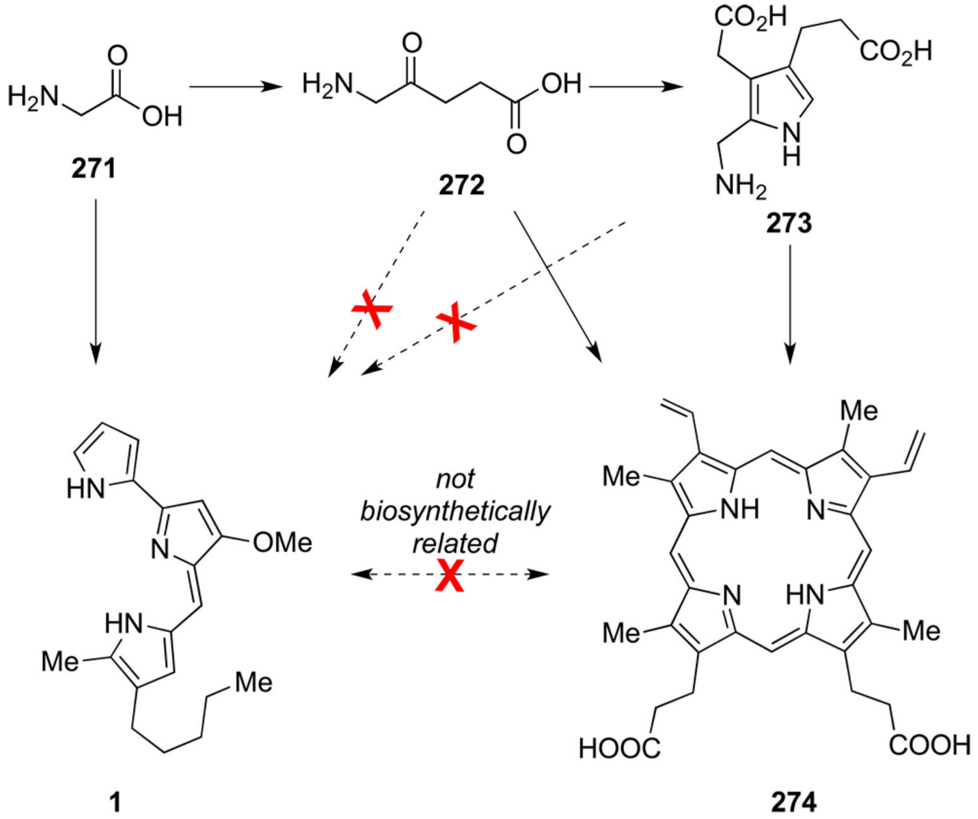


Figure 30.

Before 1960, scientists speculated that a biosynthetic relationship might exist between prodiginines and porphyrins.

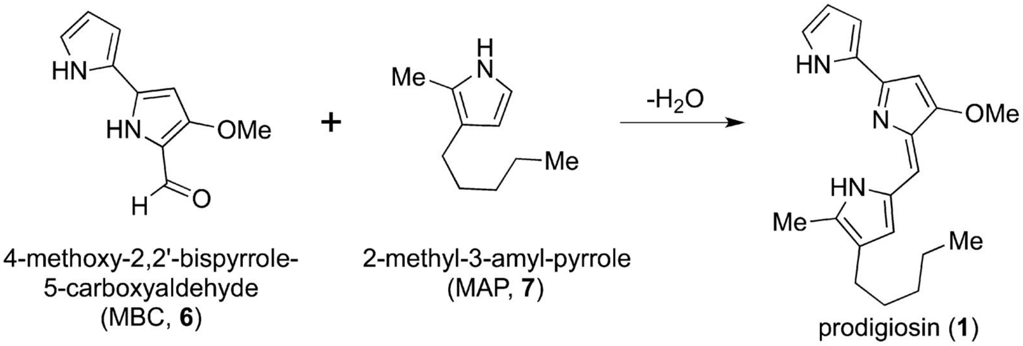


Figure 31.

Structures of MBC (**6**) and MAP (**7**), key intermediates in prodigiosin (**1**) biosynthesis.

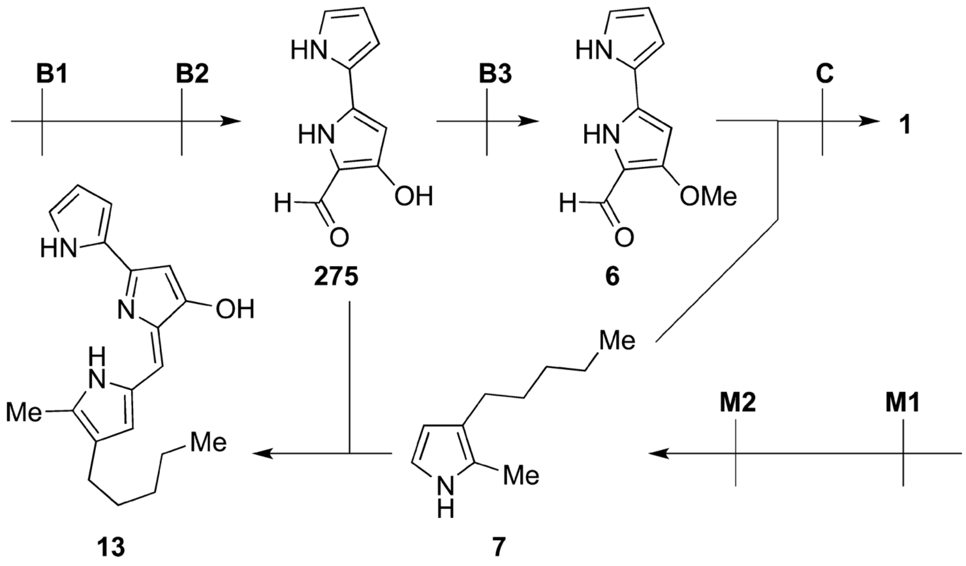


Figure 32.

Morrison's map of prodigiosin biosynthesis.[126] Mutants blocked at different stages of the pathway are indicated by the vertical lines through the arrows.

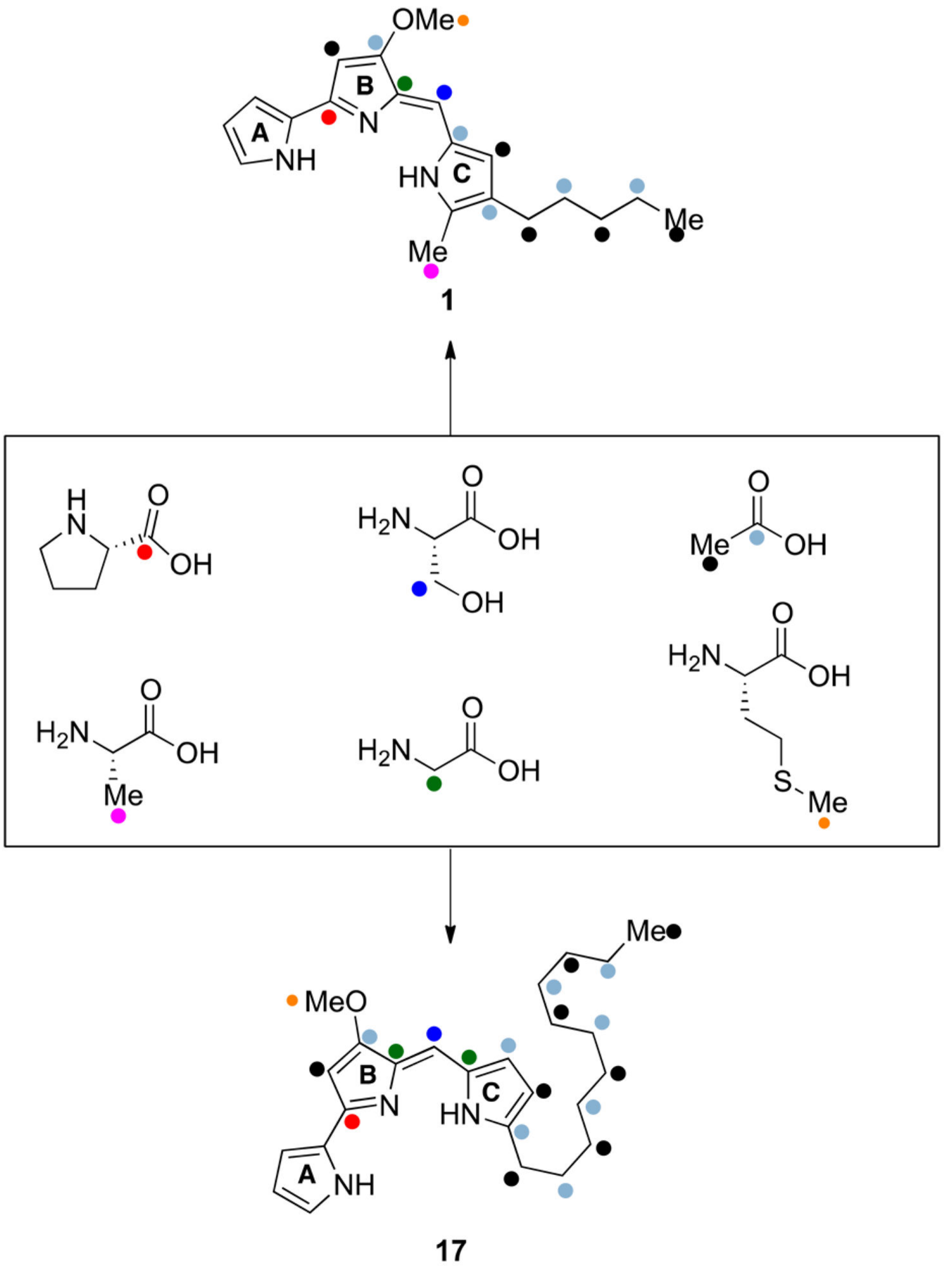


Figure 33.

Precursor incorporation pattern determined by Wasserman et al.[127,128] for prodigiosin (**1**) and undecylprodigiosin (**17**). Colored dots indicate ^{13}C or ^{14}C labels.

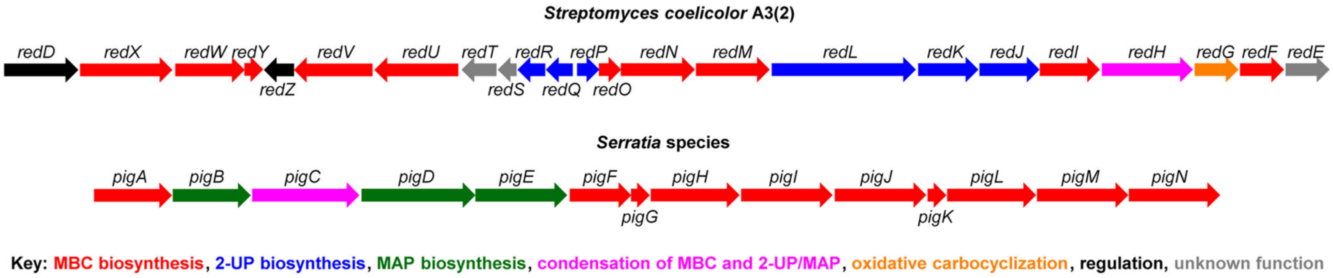
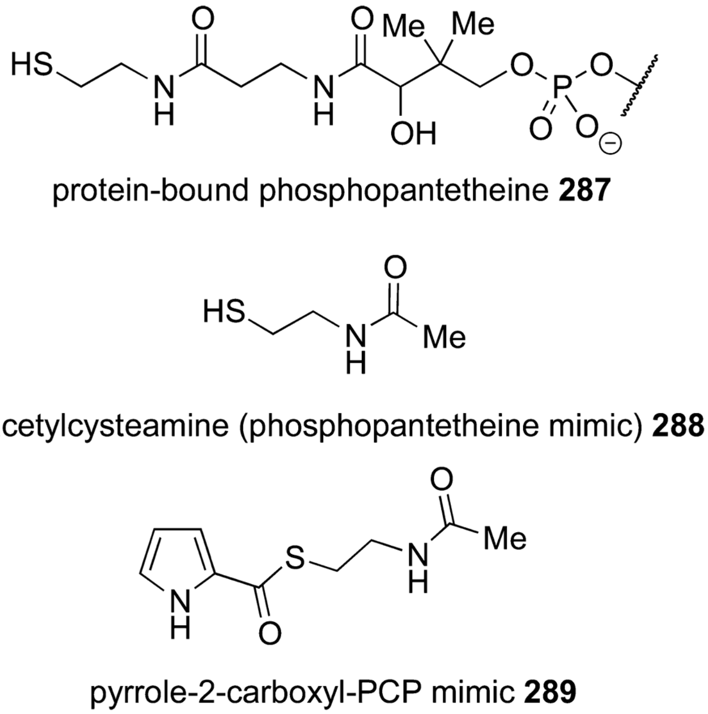


Figure 34.

Organization of the *red* gene cluster in *S. coelicolor* A3(2), responsible for undecylprodigiosin (**17**)/streptorubin B (**22**) biosynthesis, and the *pig* gene cluster in *Serratia* species, responsible for prodigiosin (**1**) biosynthesis.



protein-bound phosphopantetheine **287**

N-acetylcysteamine (phosphopantetheine mimic) **288**

pyrrole-2-carboxyl-PCP mimic **289**

Figure 35.

Phosphopanteteine is a prosthetic group essential for the function of PCPs and ACPs in metabolic pathways. *N*-Acetylcysteamine (NAC) **288** mimics the phosphopantetheine prosthetic group, and NAC thioesters are thus able to replace acyl-ACPs and PCPs in vitro and in vivo. Condensation of pyrrole 2-carboxylic acid with NAC generates thioester **289**, which mimics the pyrrole 2-carboxyl-PCP intermediate **279** in MBC biosynthesis.

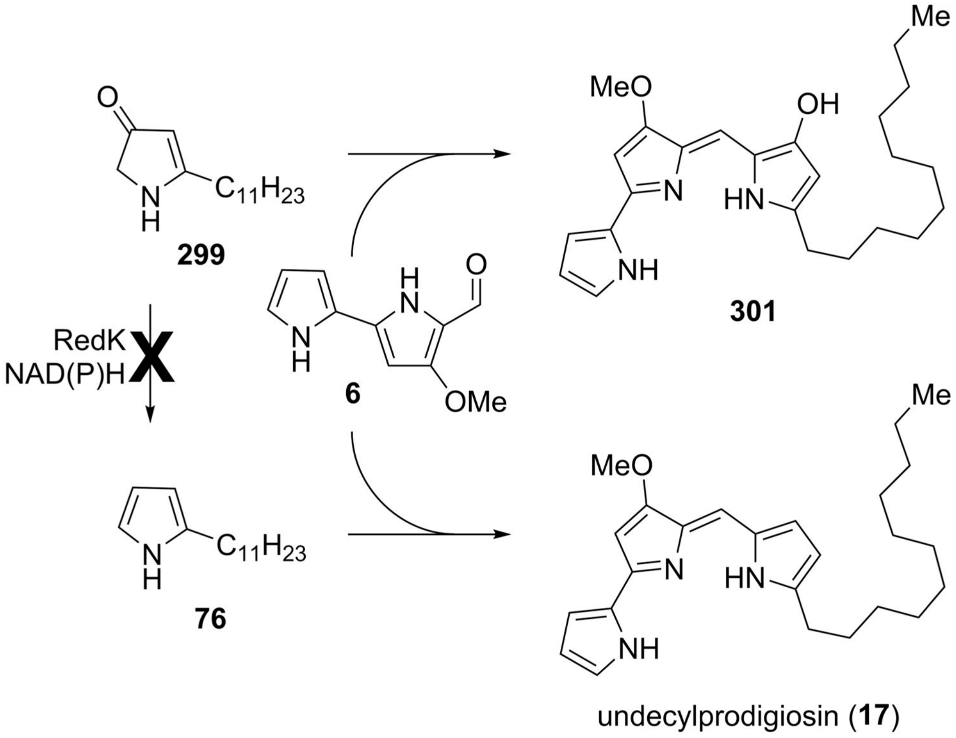
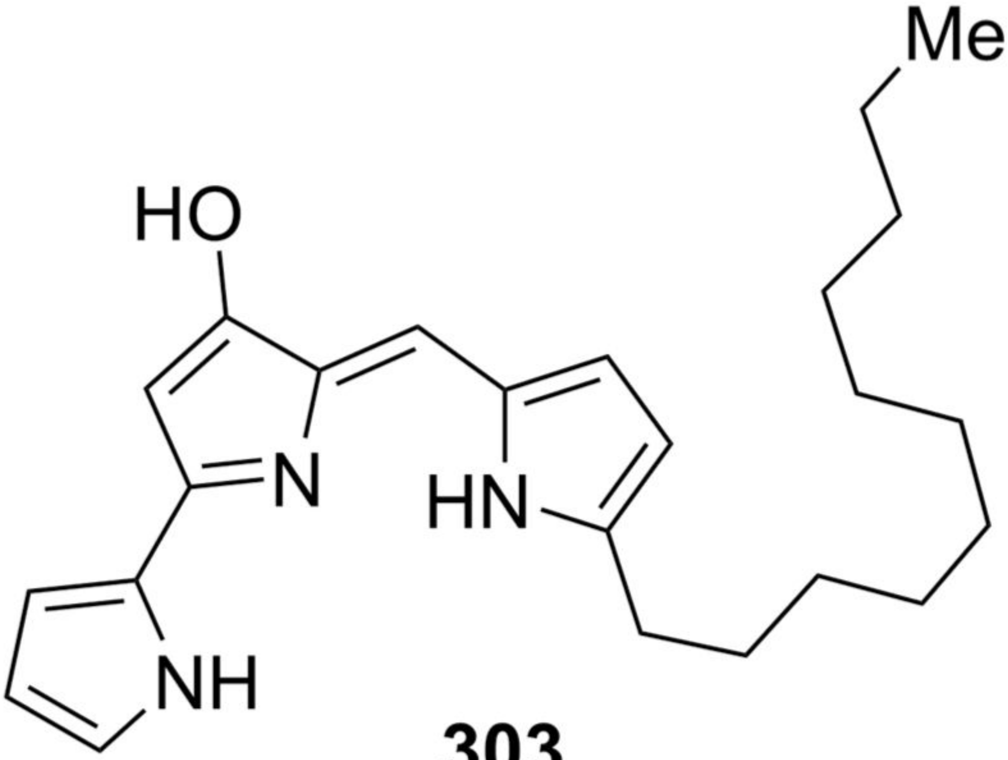


Figure 36.

Proposed origin of shunt metabolite **301** accumulated in a *redK* mutant of *S. coelicolor.*



303

Figure 37.

A *redO* mutant of *S. coelicolor* accumulates desmethylundecylprodigiosin (**303**) but not desmethylstreptorubin B, suggesting that undecylprodigiosin is the substrate of RedG.

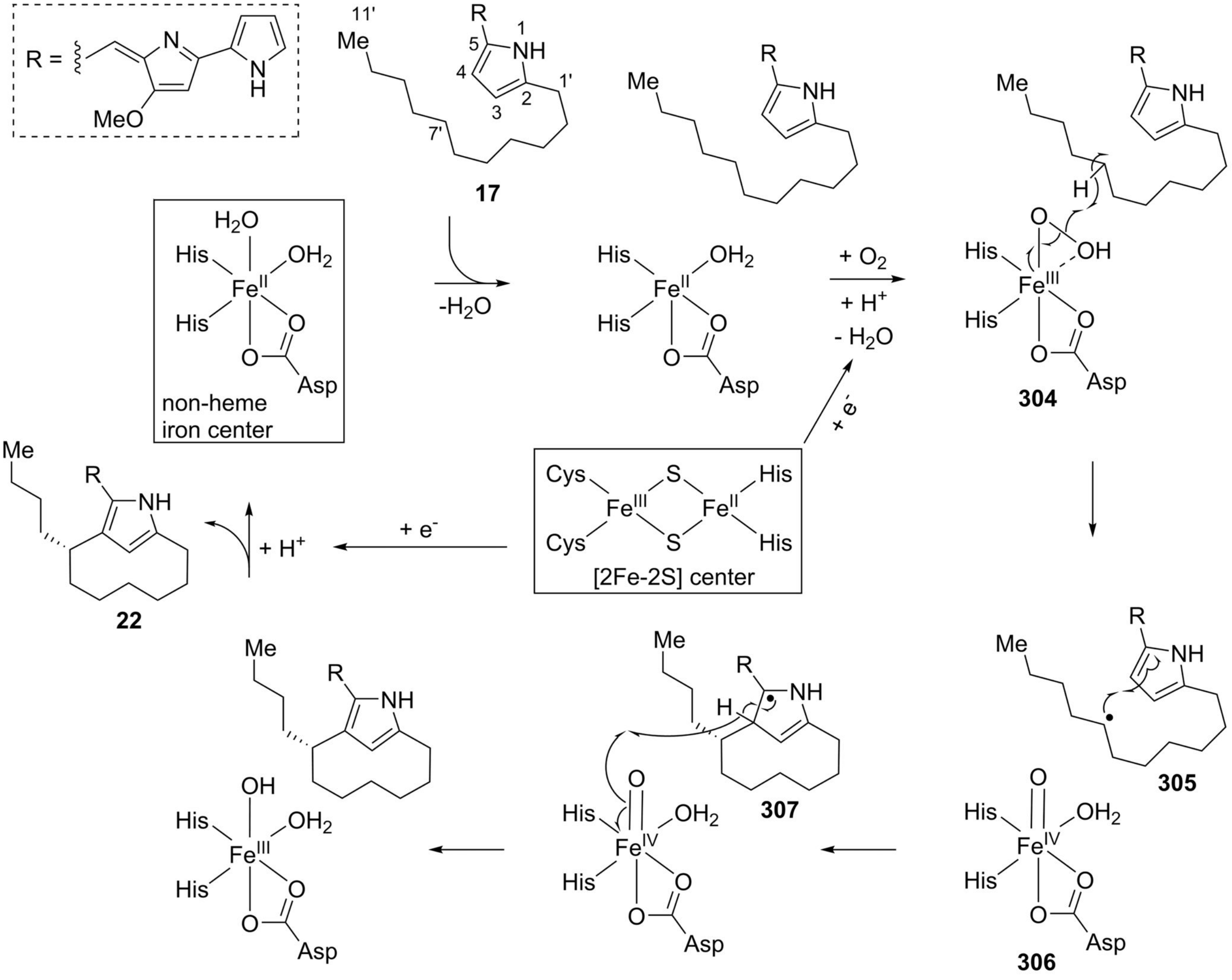


Figure 38.

Mechanism proposed by Challis and co-workers[151] for the conversion of undecylprodigiosin **17** to streptorubin B **22**, catalyzed by the Rieske oxygenase-like RedG enzyme. An Fe(V)O(OH) species and C-7′/C-5 cations are plausible alternatives to intermediates **304** and **305**/**307**, respectively.

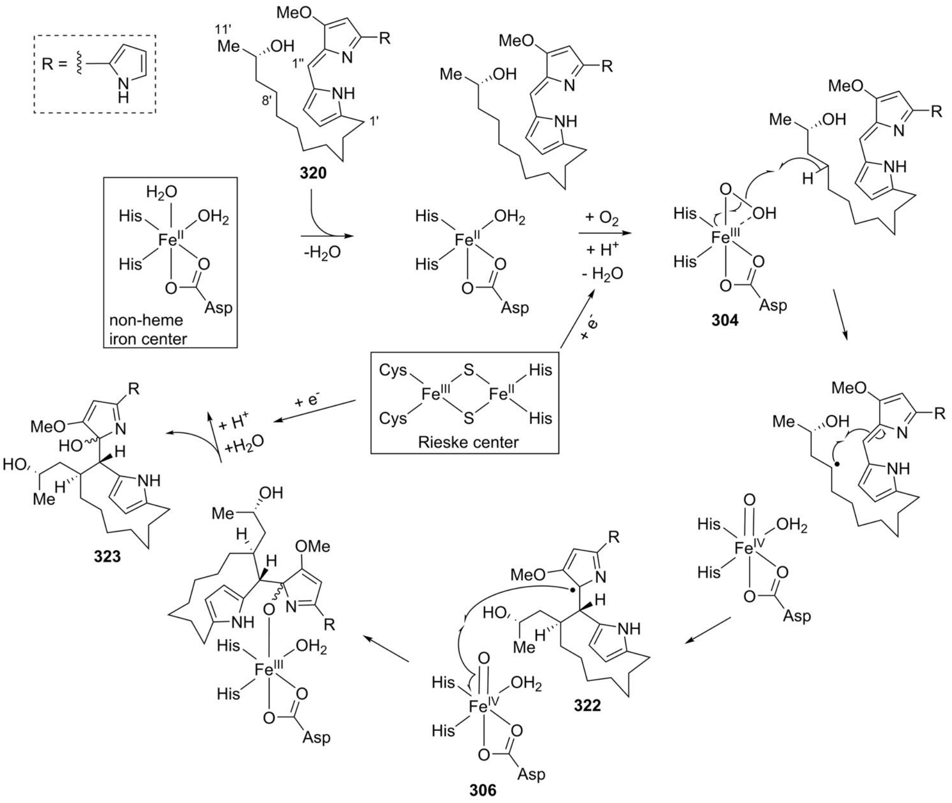
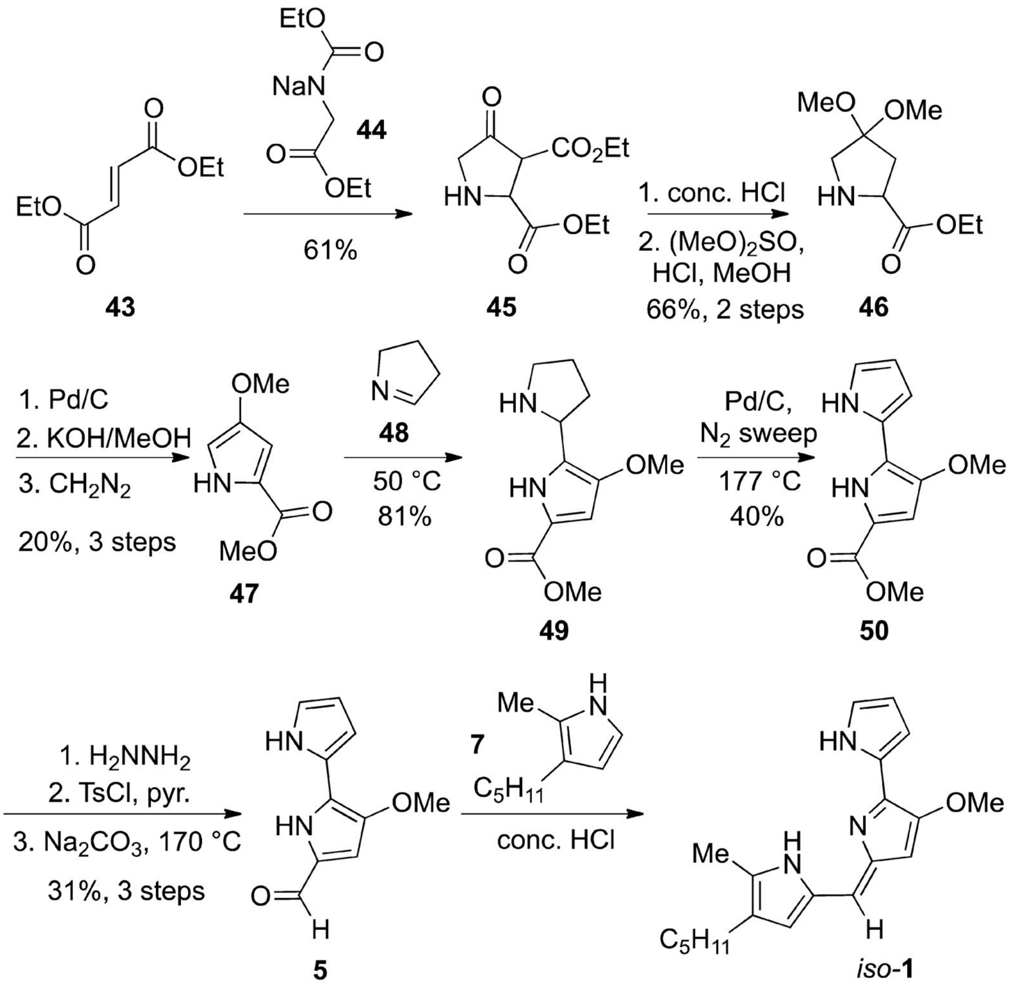


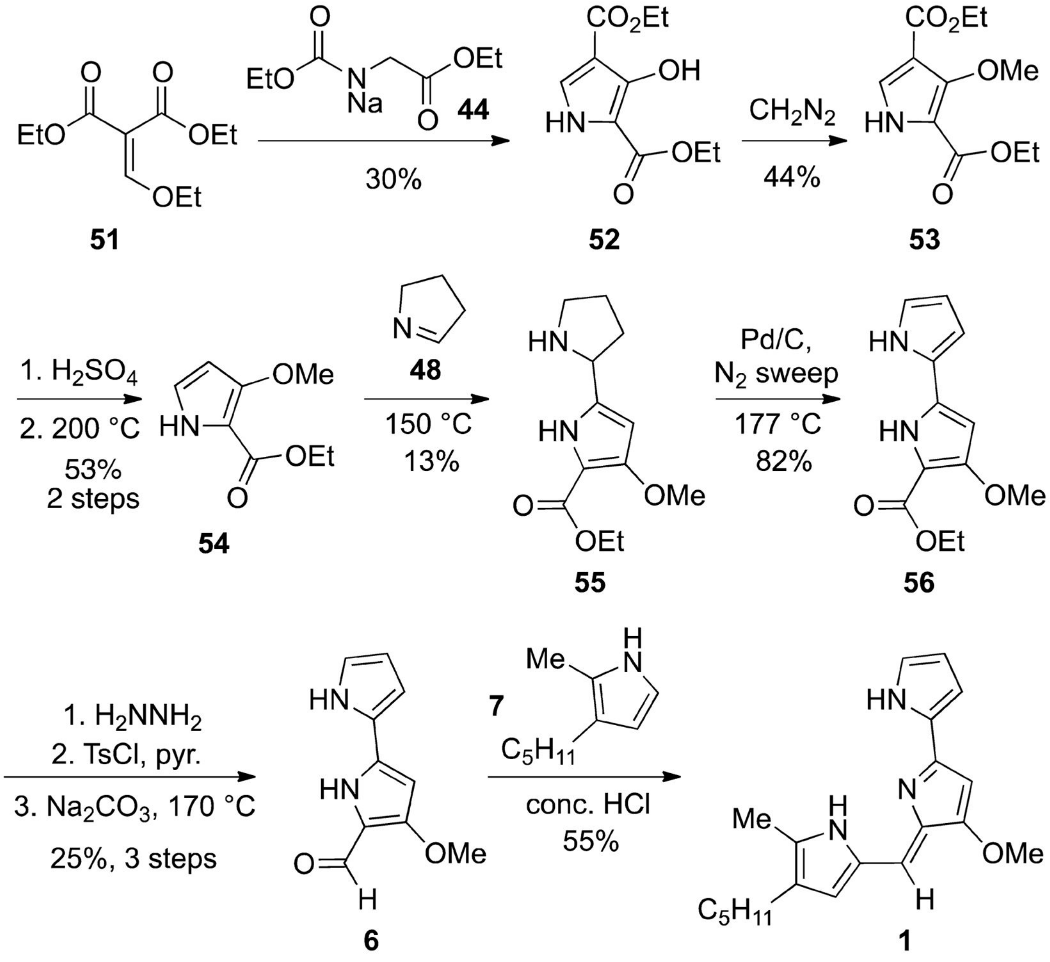
Figure 39.

Proposed mechanism for MarG-catalyzed oxidative cyclization and hydroxylation of (10′*S*)-10′-hydroxyundecylprodigiosin **320**. As for the RedG catalytic mechanism, alternative Fe(V)O(OH) and carbocation intermediates are also possible.



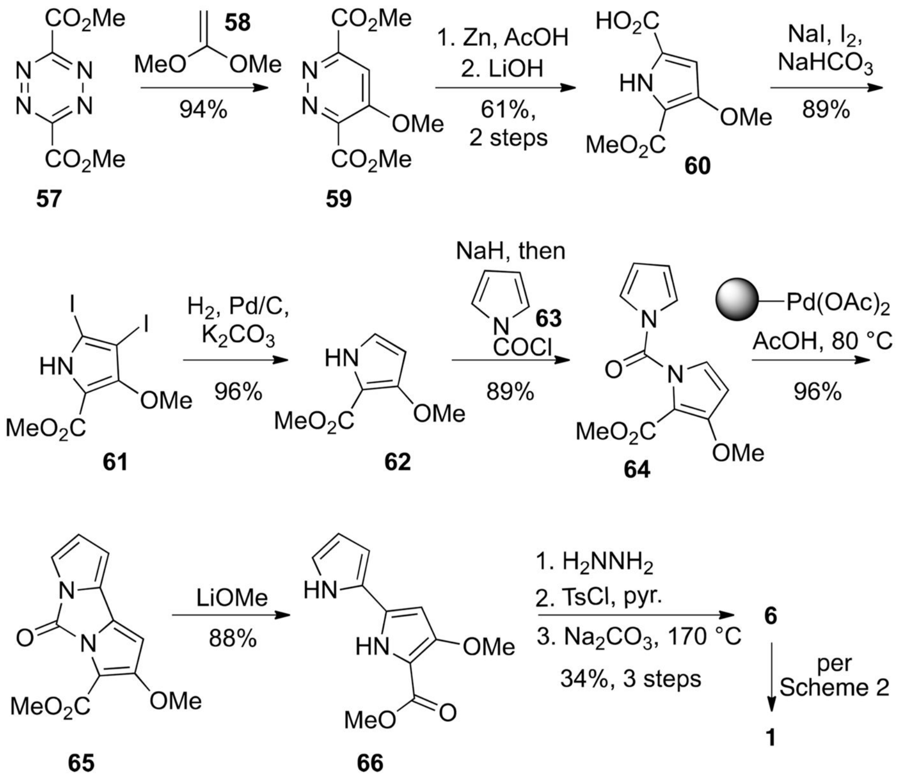
Scheme 1.

Synthesis of Prodigiosin Isomer *iso*-1 by Rapoport and Willson[43]



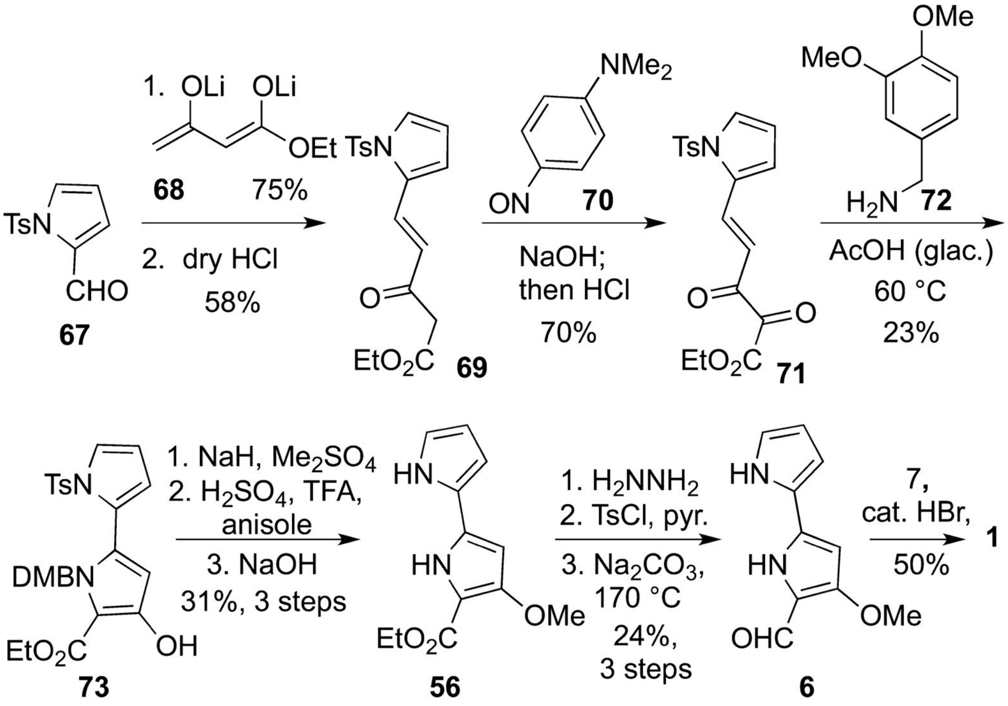
Scheme 2.

Synthesis of Prodigiosin (1) by Rapoport and Willson[43]



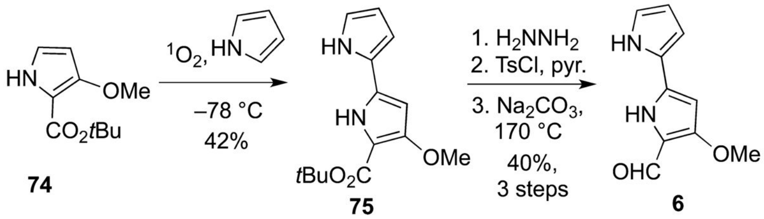
Scheme 3.

Synthesis of Prodigiosin (1) by Boger and Patel (1988)[94]



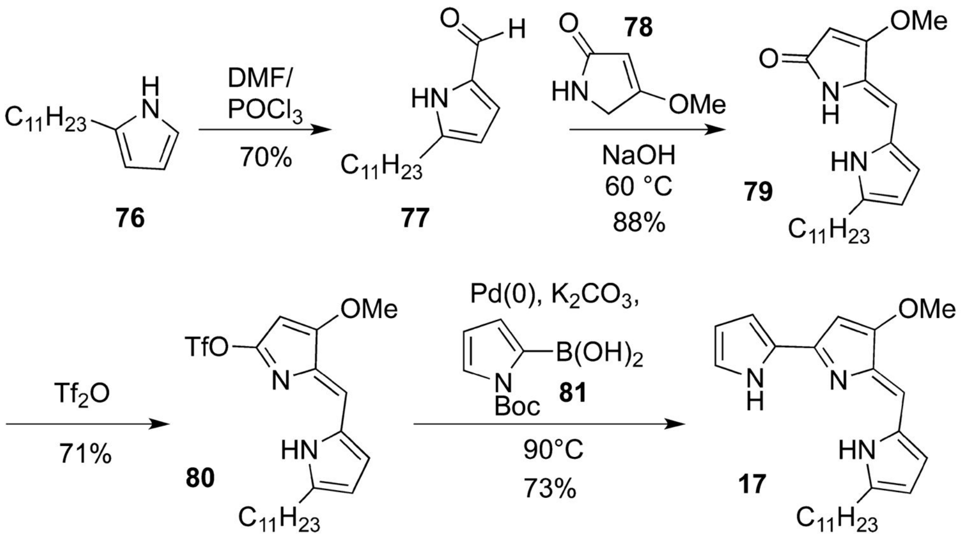
Scheme 4.

Synthesis of Prodigiosin (1) by Wasserman and Lombardo (1989)[95]



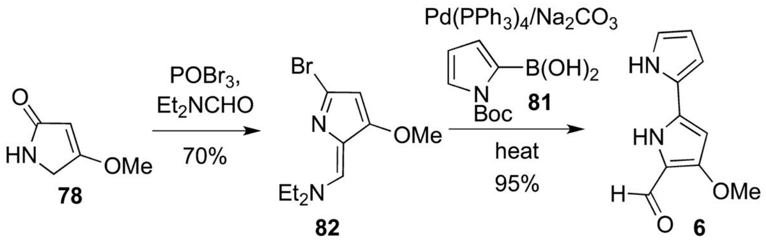
Scheme 5.

Synthesis of Key Bis(pyrrole) Aldehyde 6 by Wasserman et al. (1999)[96]



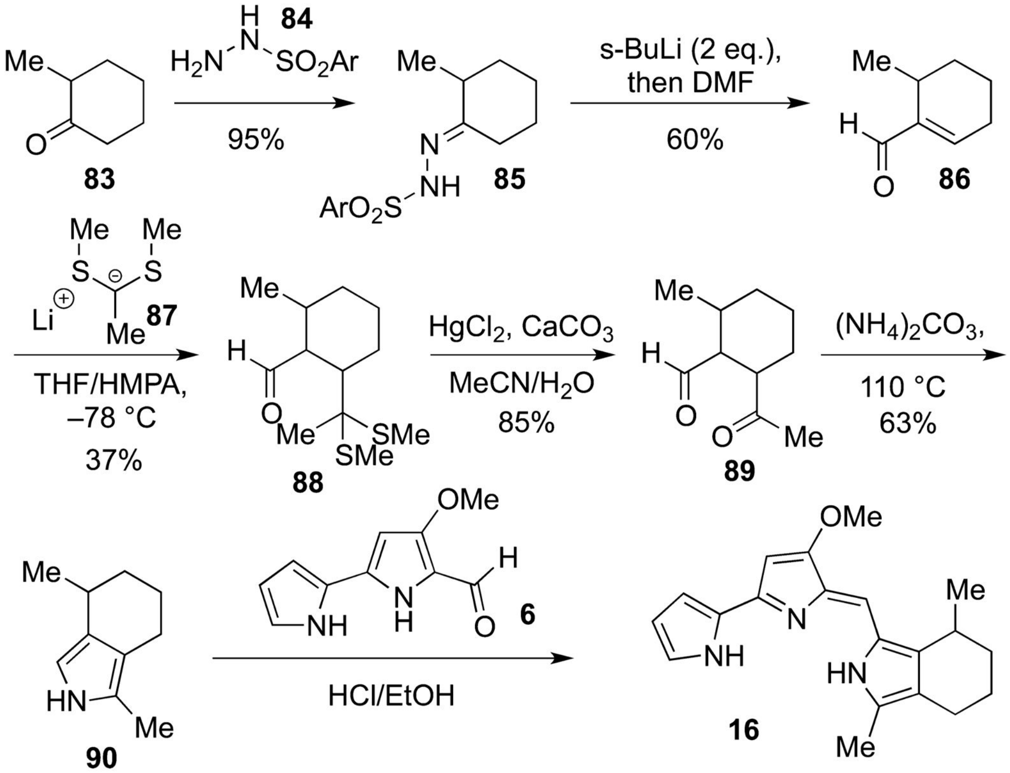
Scheme 6.

Synthesis of Undecylprodigiosin (17) by D’Alessio and Rossi[97]



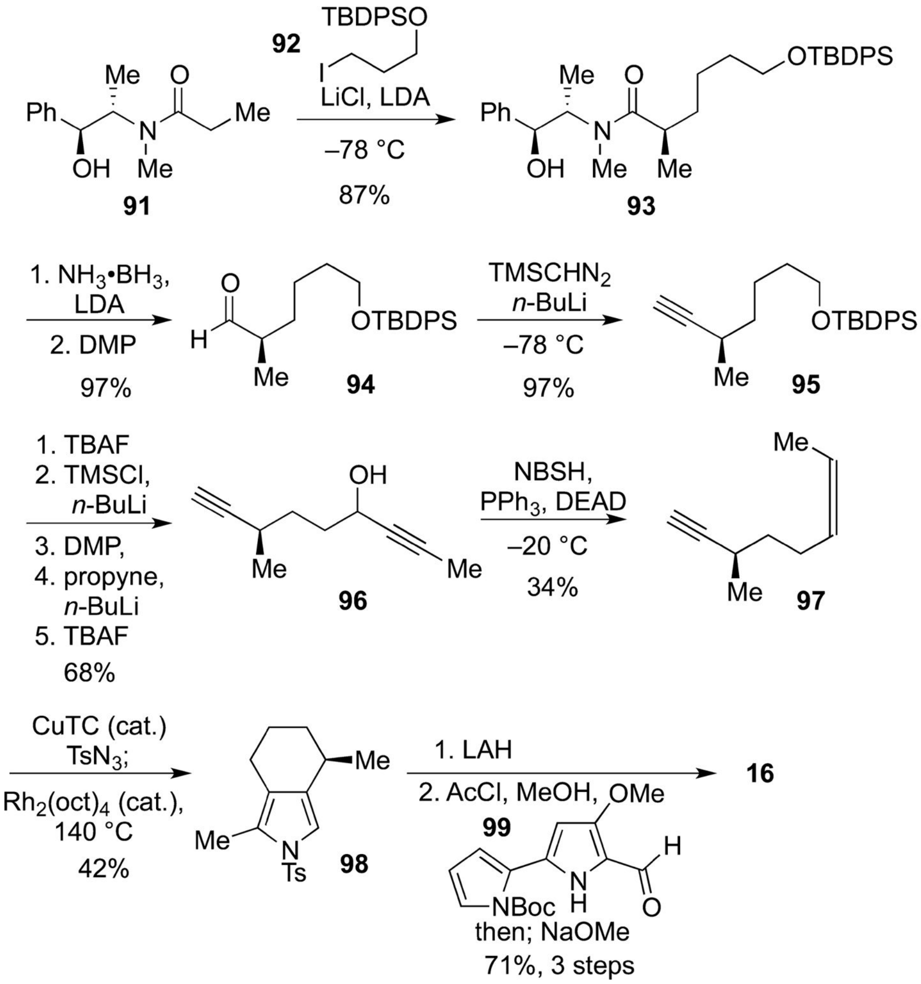
Scheme 7.

Synthesis of Bis(pyrrole) Aldehyde 6 by Lavallée and Co-workers[99]



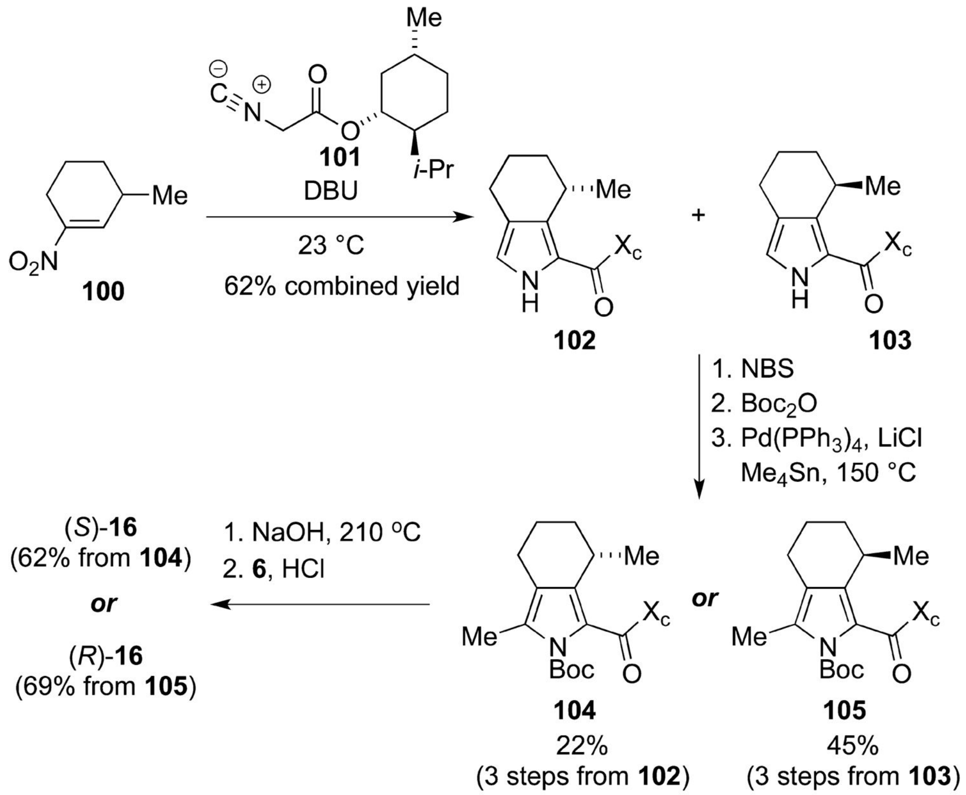
Scheme 8.

Synthesis of Racemic Cycloprodigiosin (16) by Wasserman and Fukuyama (1984)[54]



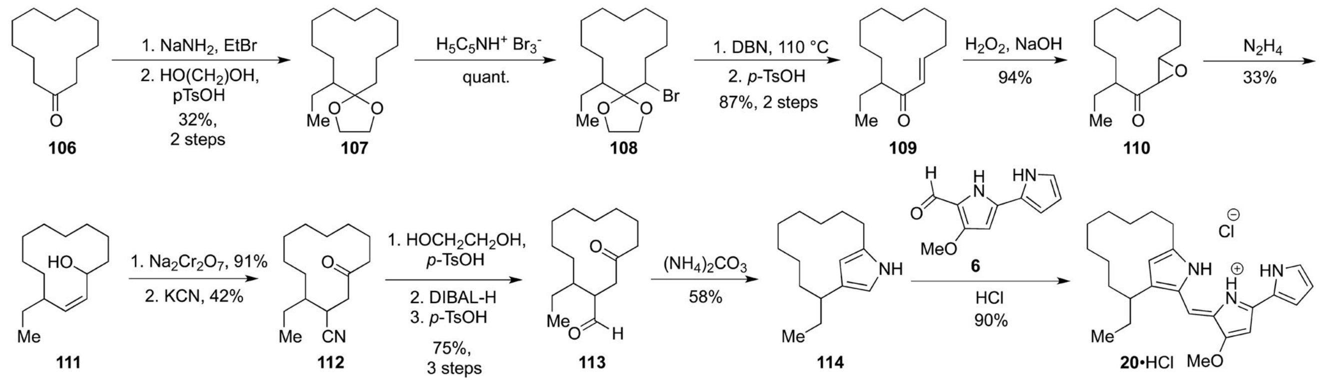
Scheme 9.

Enantioselective Synthesis of Cycloprodigiosin (16) by Schultz and Sarpong (2013)[100]



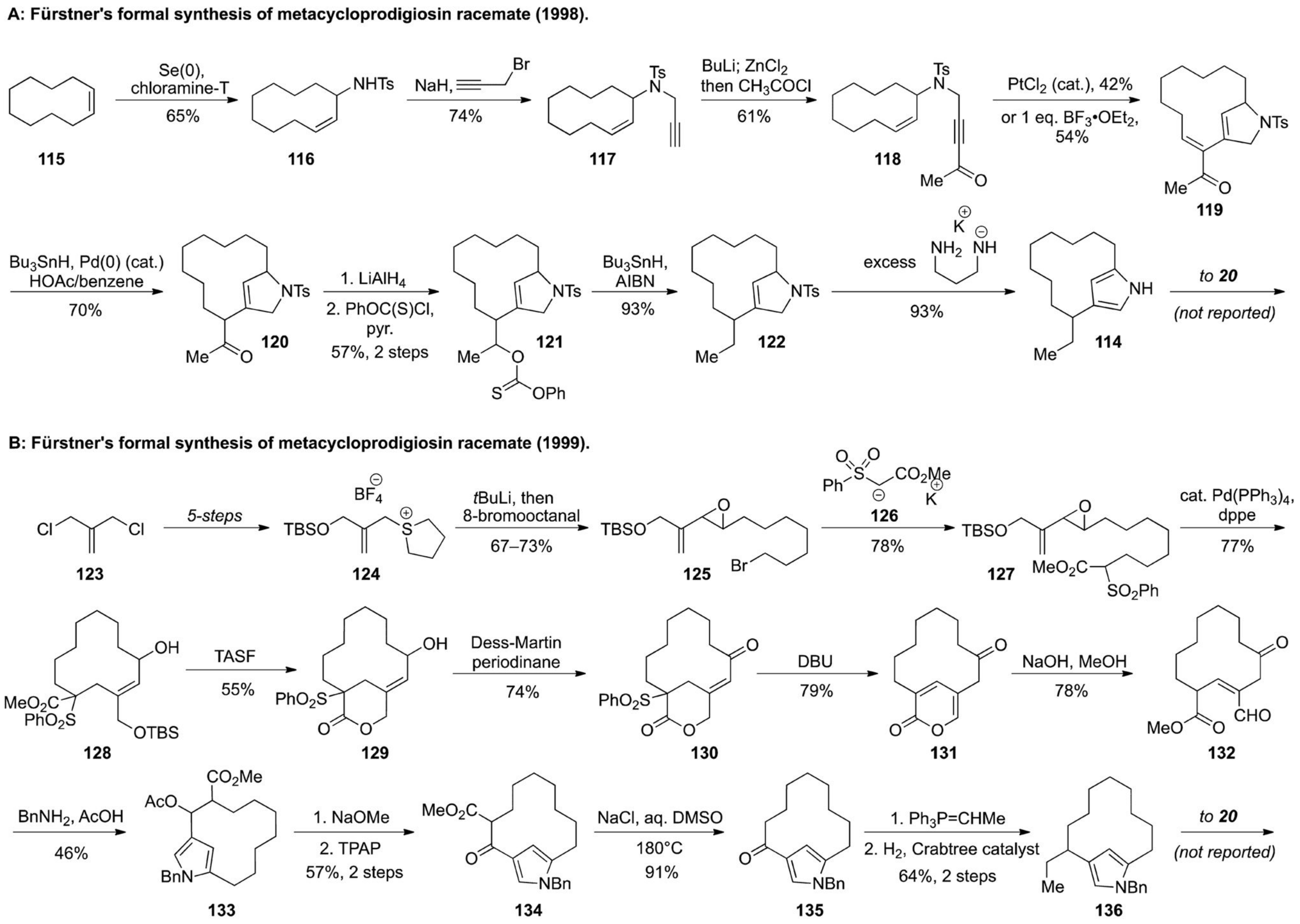
Scheme 10.

Enantioselective Synthesis of Both Enantiomers of Cycloprodigiosin (16) by Sarpong and Co-workers (2015)[55]



Scheme 11.

Total Synthesis of Metacycloprodigiosin by Wasserman et al. (1969)[61]

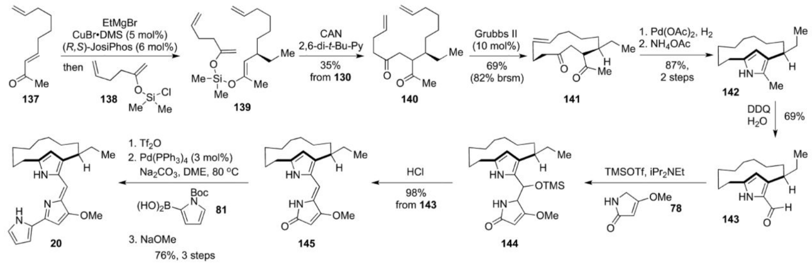


A: Fürstner's formal synthesis of metacycloprodigiosin racemate (1998).

B: Fürstner's formal synthesis of metacycloprodigiosin racemate (1999).

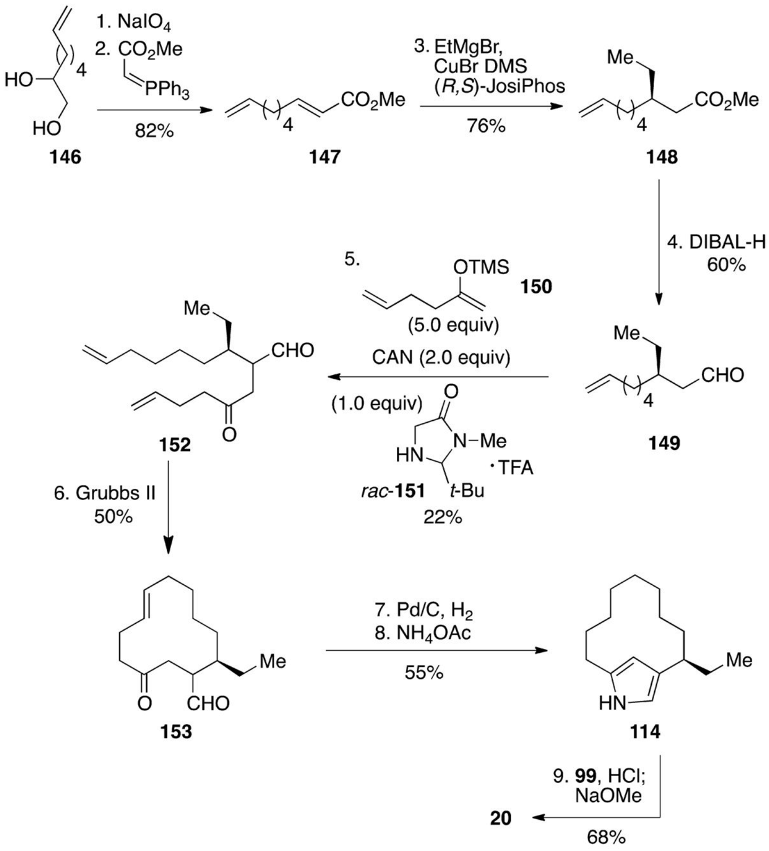
Scheme 12.

Formal Syntheses of Metacycloprodigiosin by Fürstner et al. (1998, 1999)[102,103]



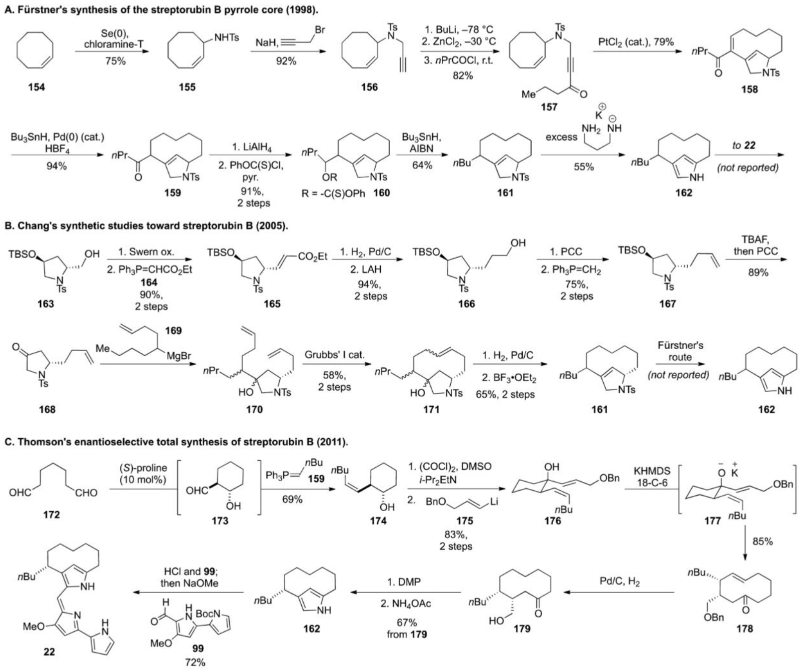
Scheme 13.

Enantioselective Synthesis of Metacycloprodigiosin (20) by Clift and Thomson (2009)[65]



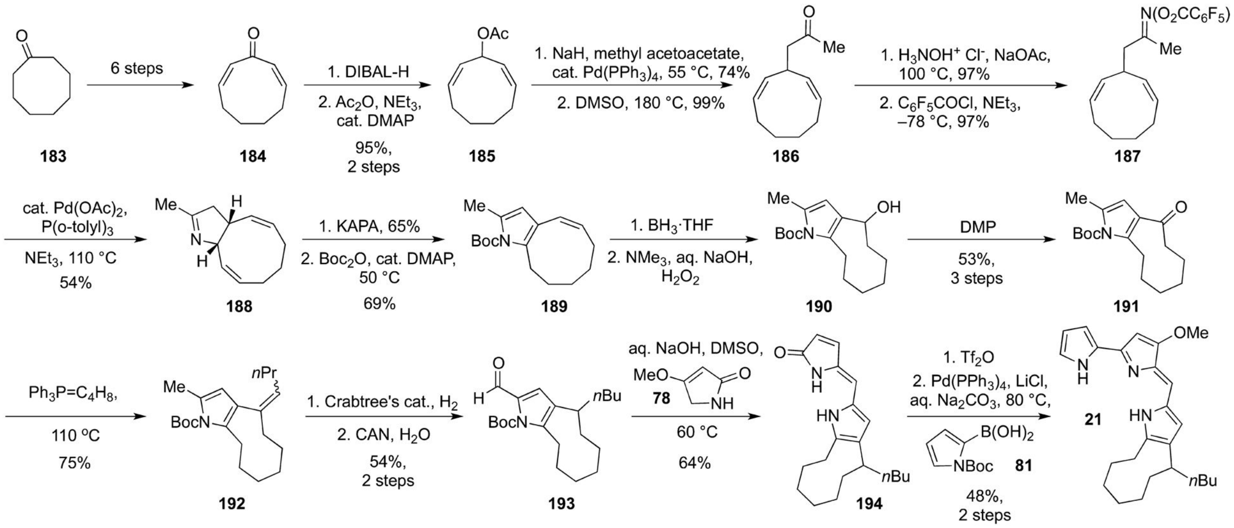
Scheme 14.

Enantioselective Synthesis of Metacycloprodigiosin (20) by Thomson and Co-workers (2015)



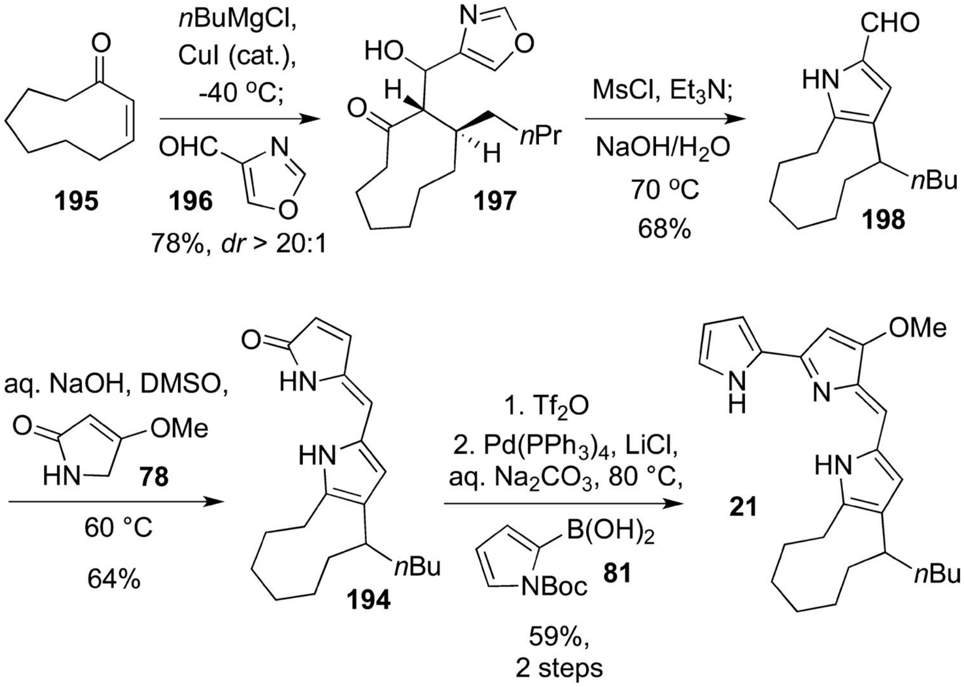
Scheme 15.

Synthetic Efforts toward Total Synthesis of Streptorubin B (22)



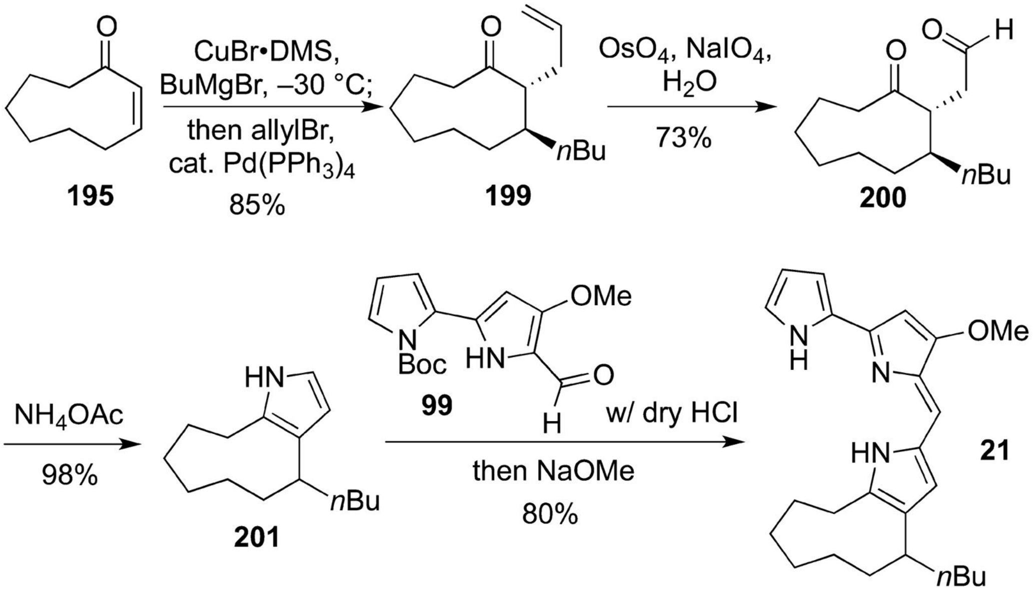
Scheme 16.

Synthesis of Butylcycloheptylprodigiosin (21) by Fürstner et al. (2005)[73]



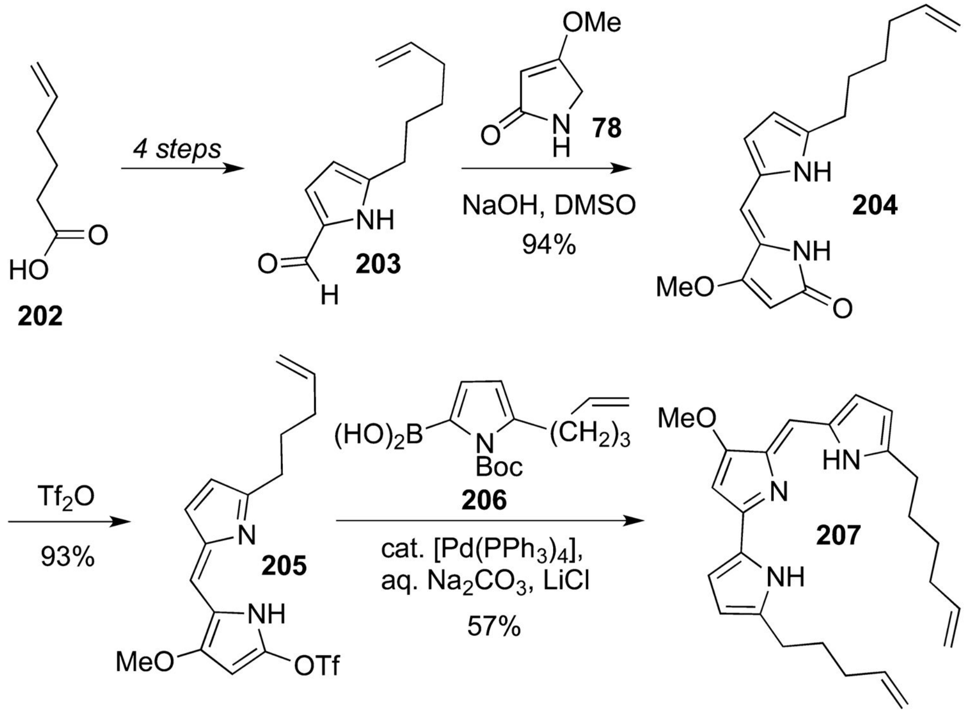
Scheme 17.

Synthesis of Butylcycloheptylprodigiosin (21) by Reeves (2007)[74]



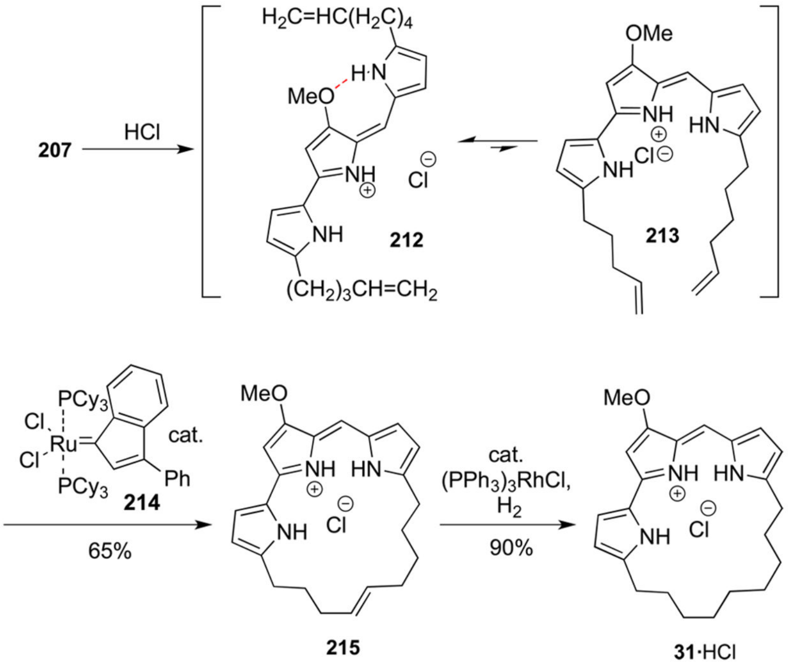
Scheme 18.

Synthesis of Butylcycloheptylprodigiosin (21) by Thomson and Co-workers (2013)[76]

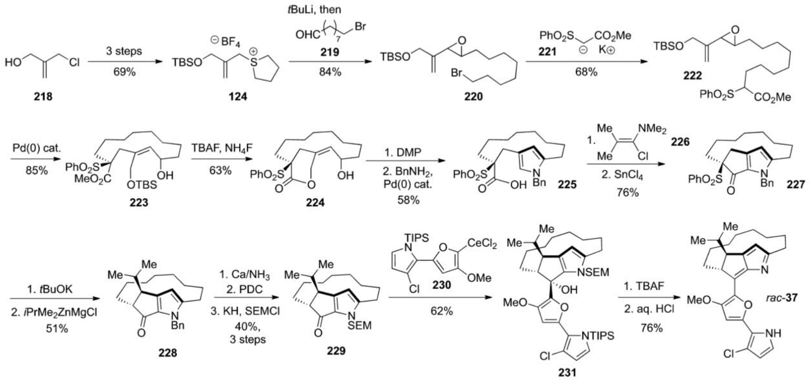


Scheme 19.

Synthesis of Cyclononylprodigiosin (31) by Fürstner et al. (1999),[84] Part I

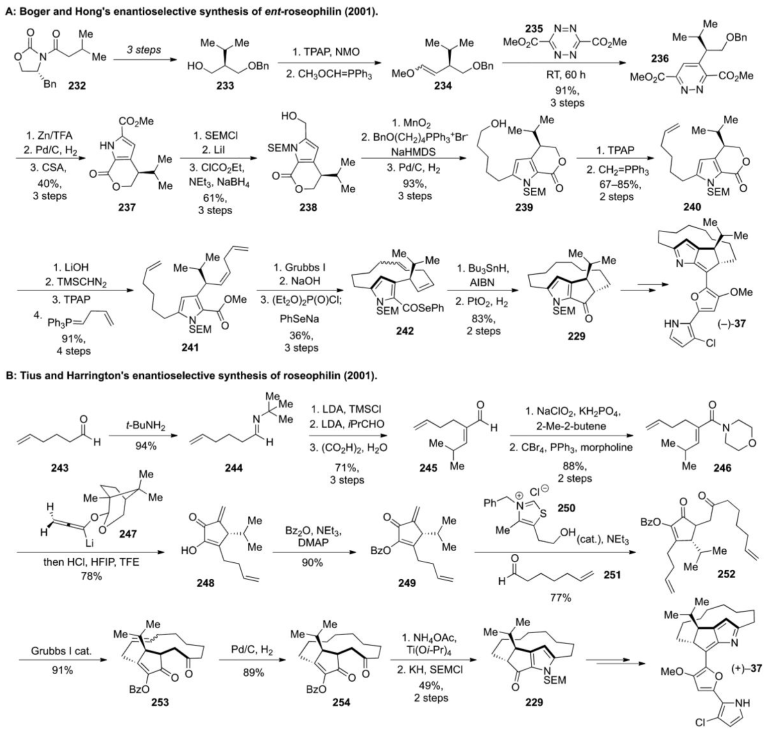


Scheme 20.
Synthesis of Cyclononylprodigiosin (31) by Fürstner et al.,[84] Part II



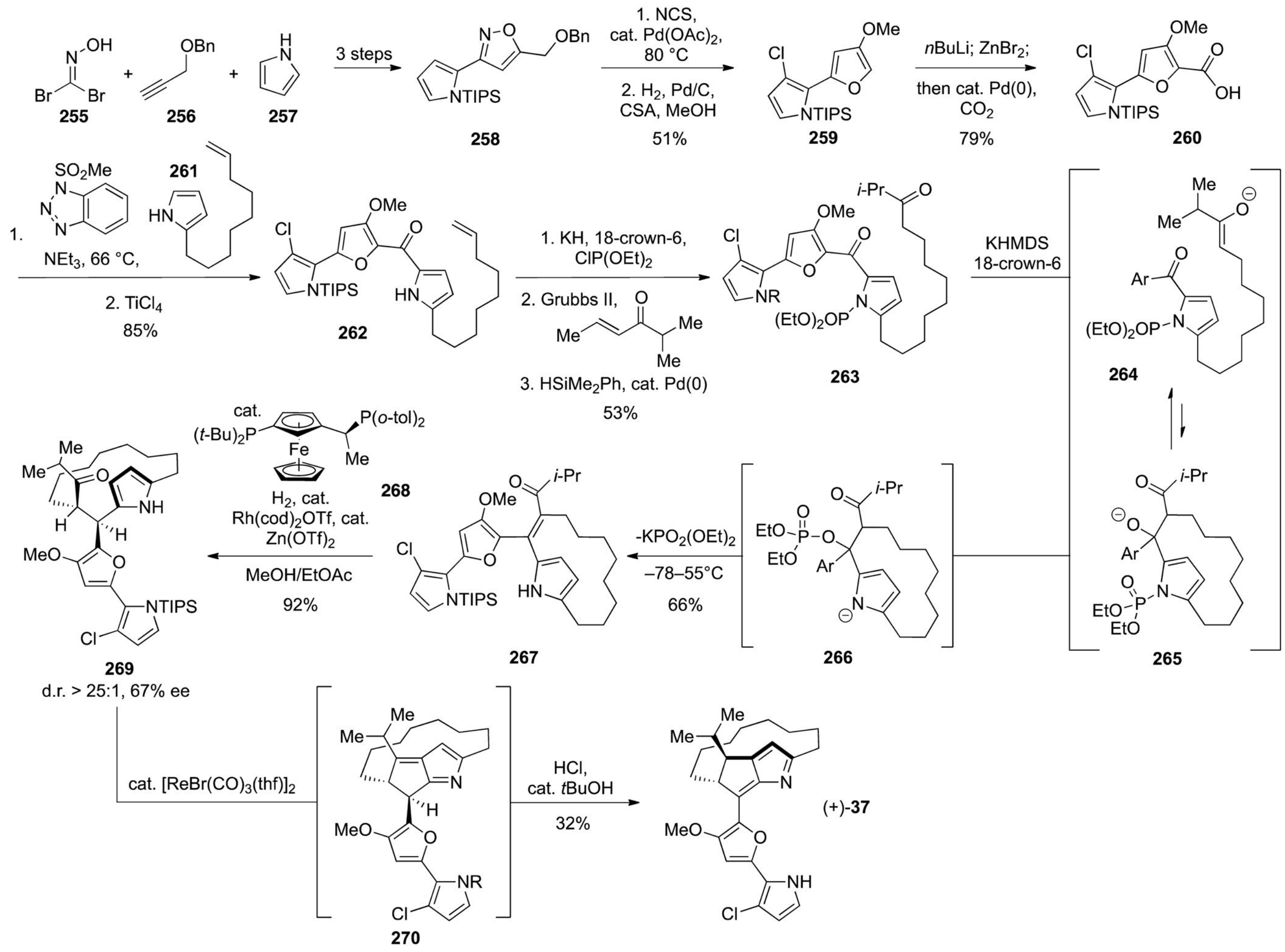
Scheme 21.

Synthesis of Roseophilin (37) by Fürstner and Weintritt (1998)[86]



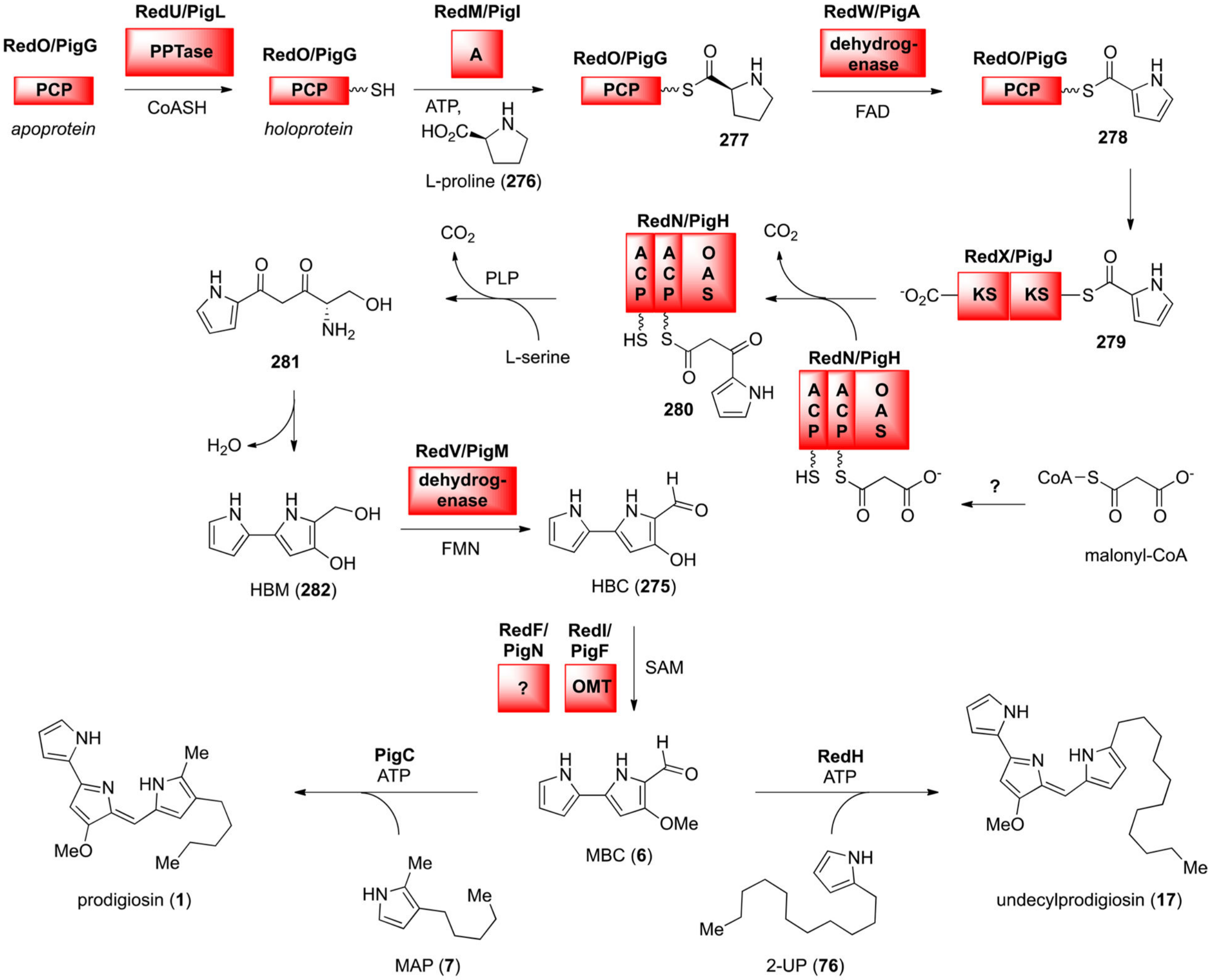
Scheme 22. (A) Synthesis of (22*S*,23*S*)-Roseophilin by Boger and Hong[87] and (B) Enantioselective Synthesis of (22*R*,23*R*)-Roseophilin (37) by Tius and Harrington[88,117] [a]

[a]HFIP = 1,1,1,3,3,3-hexafluoro-2-propanol; TFE = 2,2,2-trifluoroethanol.



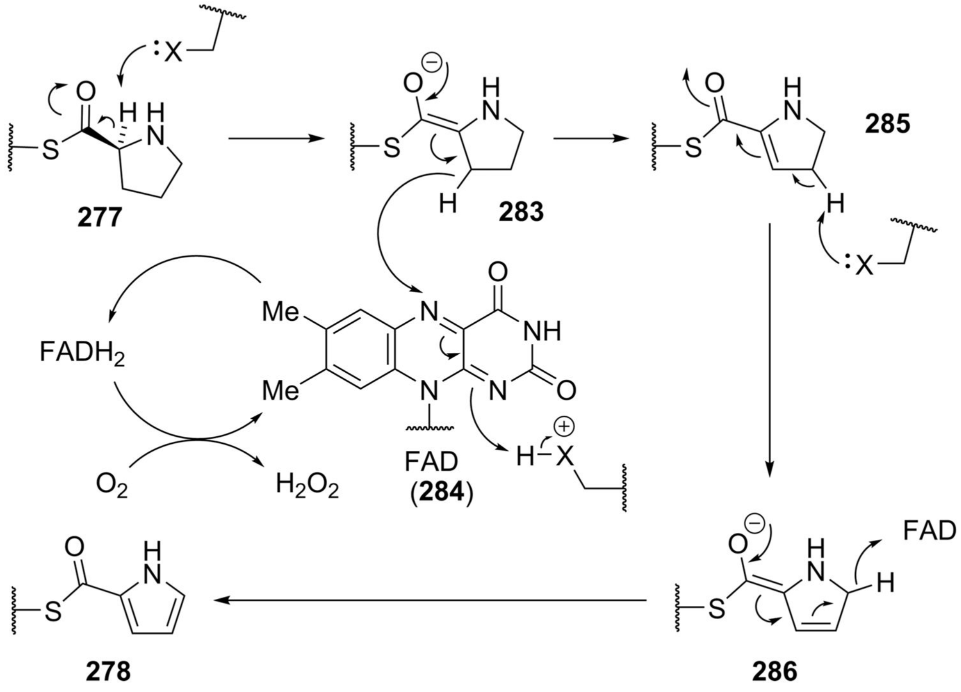
Scheme 23.

Enantioselective Synthesis of (+)-Roseophilin (37) by Frederich and Harran[119]



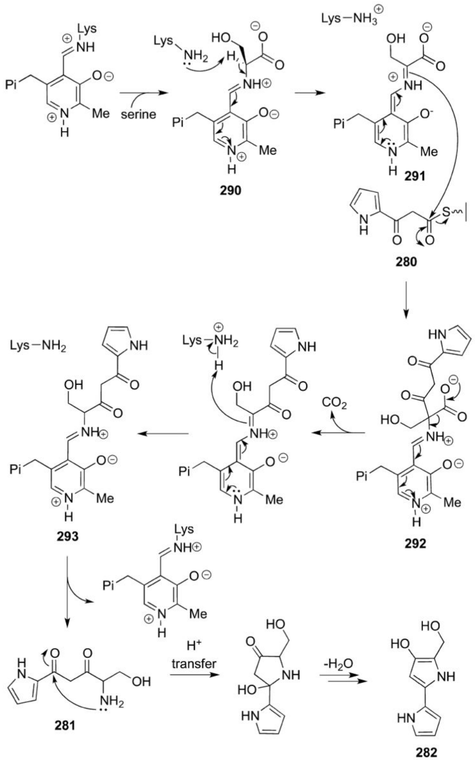
Scheme 24. Proposed Pathway for Assembly of MBC, a Common Intermediate in Biosynthesis of Prodigiosin in *Serratia* Species and Undecylprodigiosin in *S. coelicolor*[a]

[a] Abbreviations: A, adenylation; ACP, acyl carrier protein; KS, ketosynthase; OAS, α-oxamine synthase; OMT, O-methyltransferase; PCP, peptidyl carrier protein; PPTase, phosphopantetheinyl transferase.



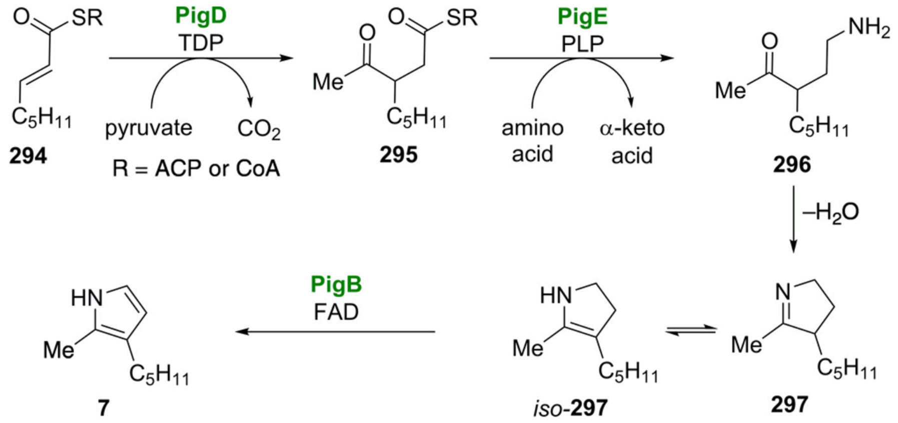
Scheme 25.

Possible Mechanism for Oxidation of L-Prolyl-PCP to Pyrrole-2-carboxyl-PCP by Flavin-dependent Dehydrogenases RedW, PigA, and PltE



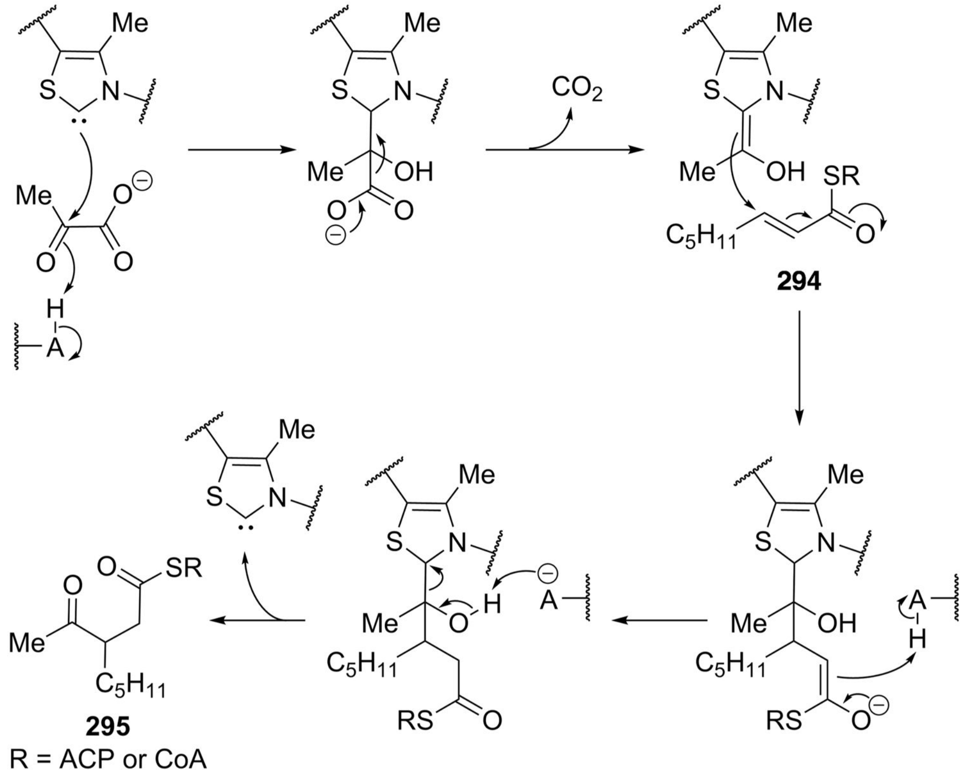
Scheme 26.

Proposed Mechanism for Release of *β*-Keto Thioester Intermediate 280 from RedN/PigH, Catalyzed by OAS Domain, and Subsequent Spontaneous Conversion of the Resulting *α*,*γ*-Dioxoamine 281 to HBM 282



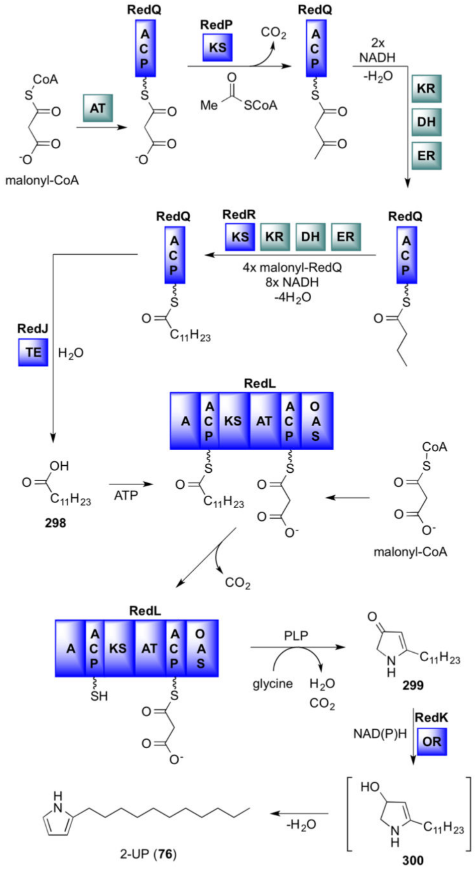
Scheme 27.

Proposed Pathway for MAP (7) Biosynthesis in *Serratia* Species



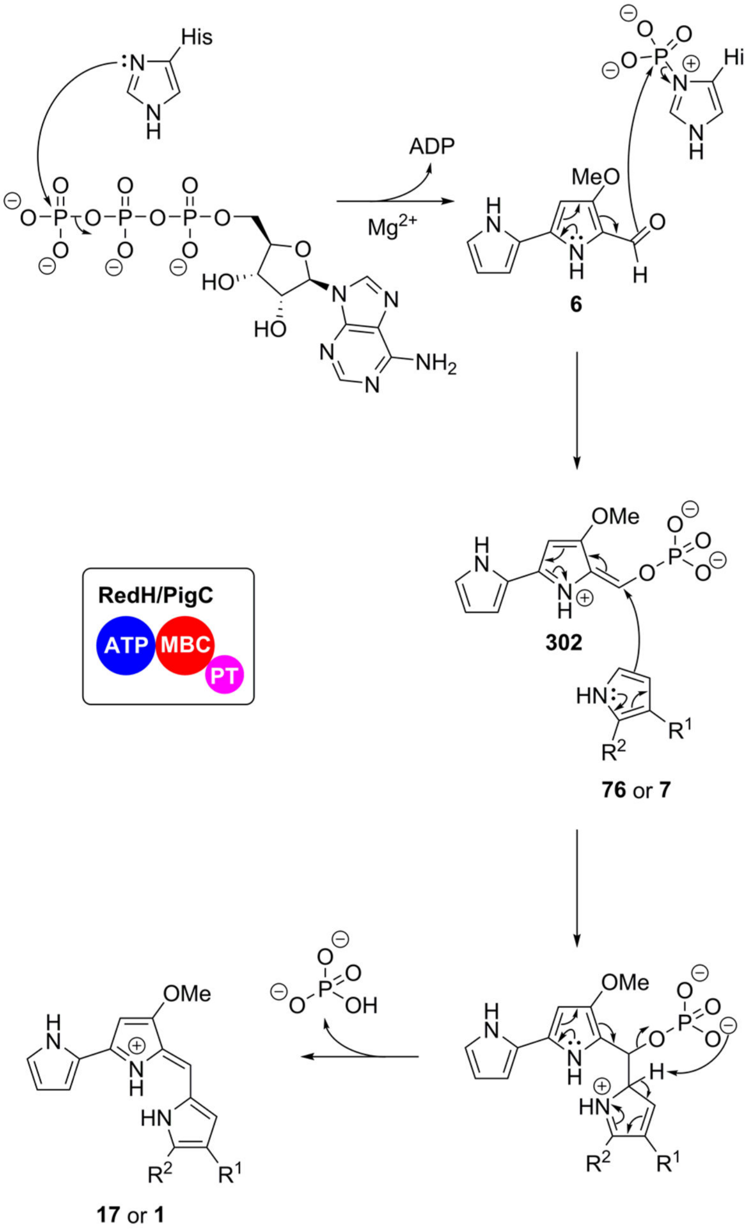
Scheme 28.

Proposed Catalytic Mechanism of TDP-dependent PigD Enzyme



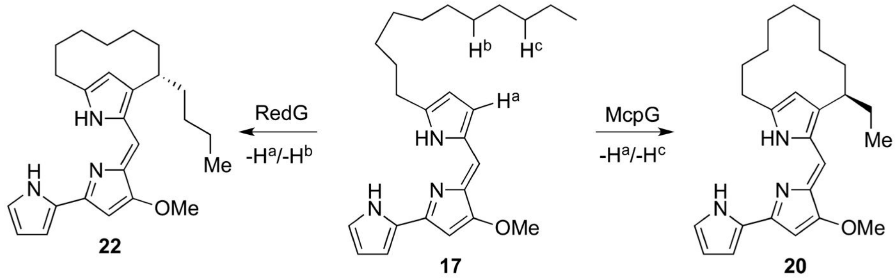
Scheme 29. Proposed Pathway for 2-UP Biosynthesis in *S. coelicolor*[a]

[a]Abbreviations: A, adenylation; ACP, acyl carrier protein; AT, acyltransferase; DH, dehydratase; ER, enoyl reductase; KS, ketosynthase; KR, ketoreductase; OAS, α-oxoamine synthase; OR, oxidoreductase; TE, thioesterase. Enzymes proposed to be "borrowed" from the core metabolic fatty acid synthase are shown in green.



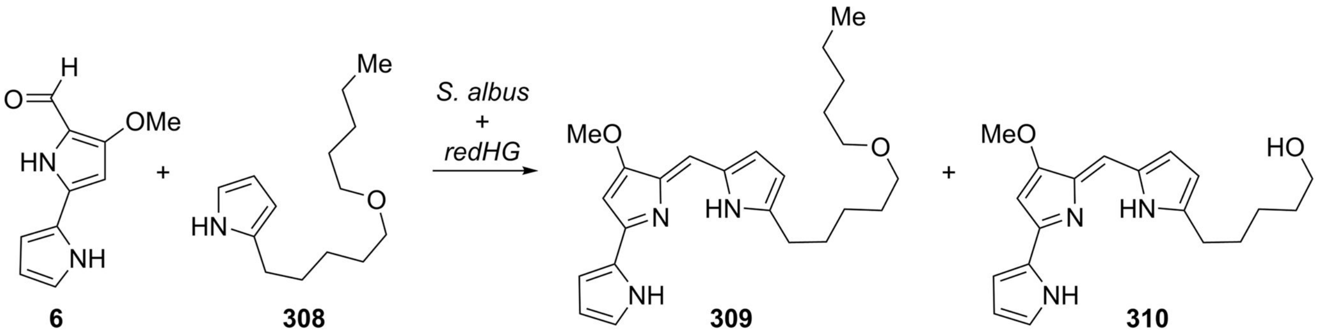
Scheme 30. Proposed Mechanism for RedH-catalyzed Condensation of MBC 6 with 2-UP 76[a] and for PigC-catalyzed Condensation of MBC 6 with MAP 7[b,c]

[a] $R^1 = H$, $R^2 = C_{11}H_{23}$. [b] $R^1 = C_5H_{11}$, $R^2 = Me$. [c] Organization of catalytic domains within RedH/PigC is shown in the inset. PT, phosphotransferase domain.



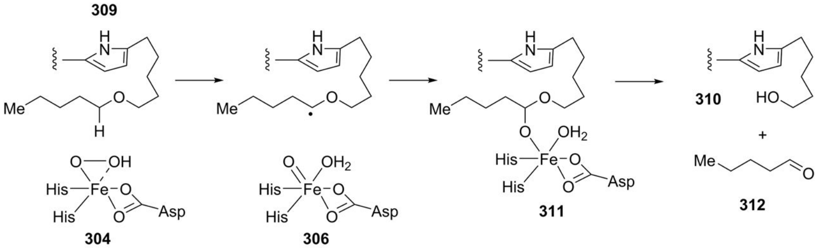
Scheme 31.

RedG and McpG Catalyze Regio- and Stereodivergent Oxidative Carbocyclizations of Undecylprodigiosin (17) To Form Streptorubin B (22) and Metacycloprodigiosin (20), Respectively



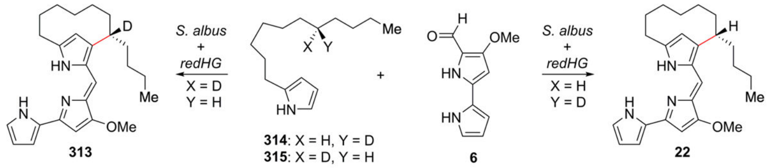
Scheme 32.

Fate of the 6′-Oxa Analog (308) of Undecylprodigiosin When Fed Along With MBC (6) to *S. Albus* Expressing *redHG*



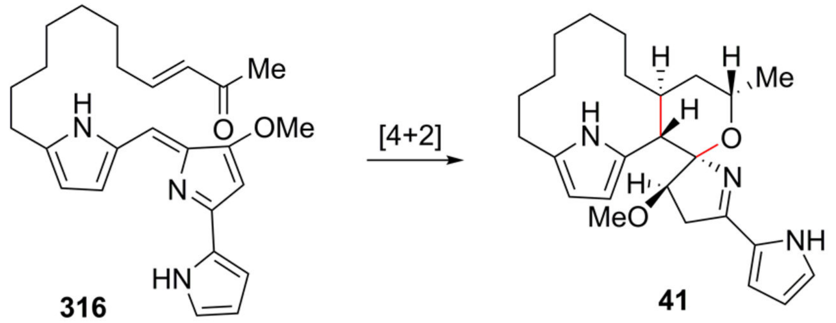
Scheme 33.

Proposed Mechanism for RedG-catalyzed Formation of 5-Hydroxypentylprodigiosin 310 from the 6′-Oxa Analogue 309 of Undecylprodigiosin



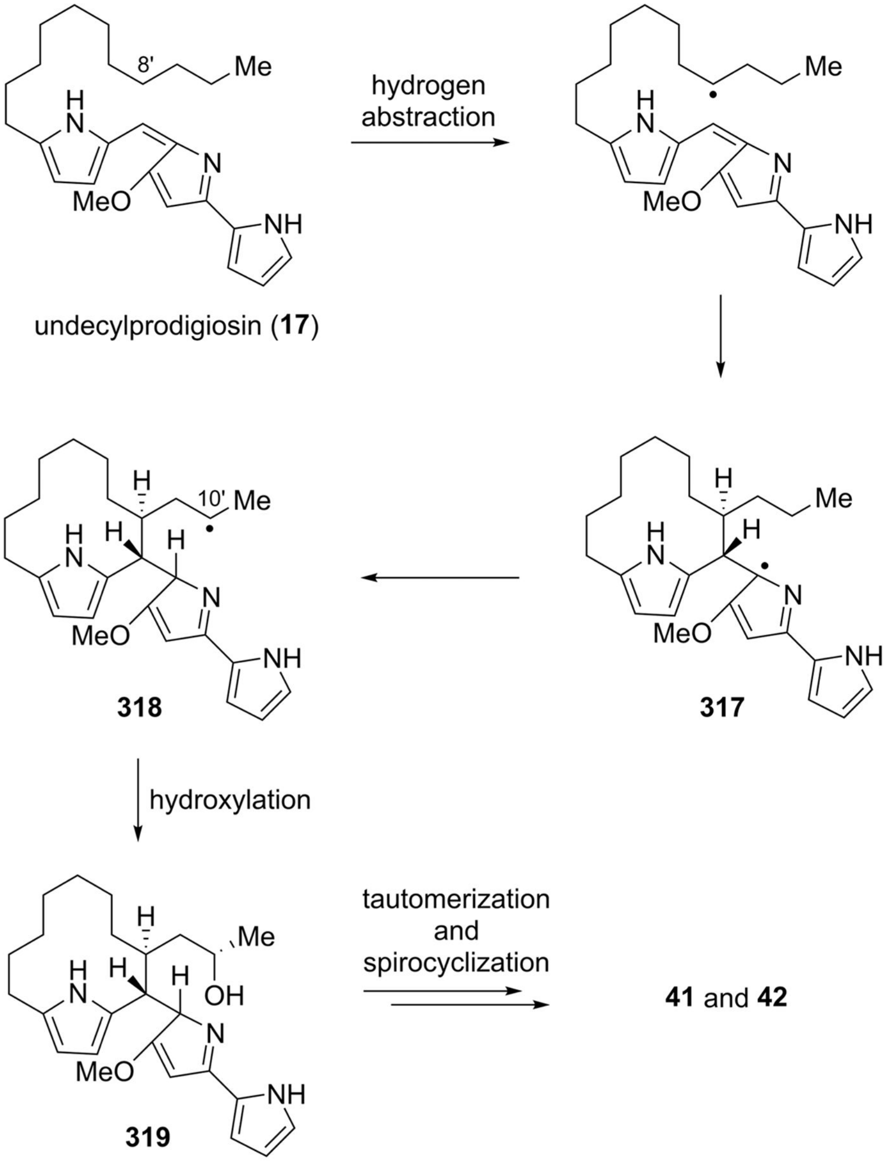
Scheme 34. Loss and Retention of Deuterium Label in Biosynthesis of Streptorubin B (22)[a]

[a]Biosynthesis of streptorubin B (**22**) via feeding of [7′-^{2}H]-(7′*R*)-2-undecylpyrrole **314** and MBC **6** to *S. albus* expressing *redHG* results in substantial loss of deuterium label, whereas the label is retained in streptorubin B produced by feeding of [7′-^{2}H]-(7′*S*)-2-undecylpyrrole **315** and MBC **6** to *S. albus* expressing *redHG*.



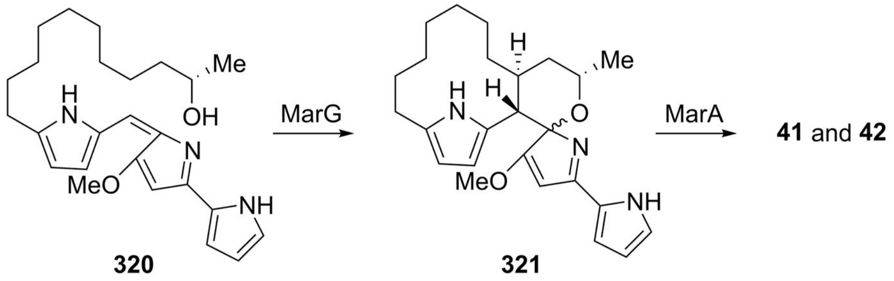
Scheme 35.

Pathway for Marineosin Biosynthesis Proposed by Fenical and Co-workers in 2008[91]

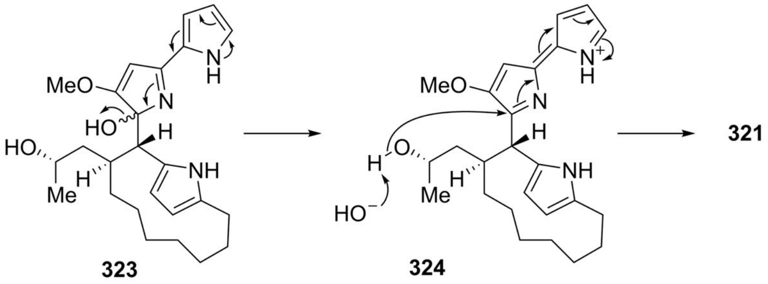


Scheme 36.

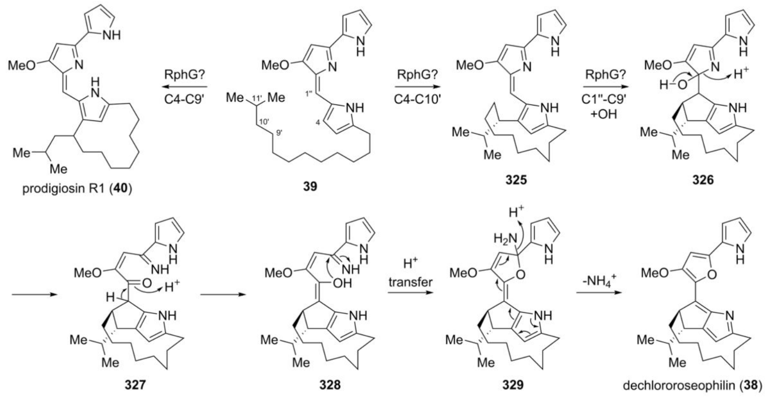
Alternative Proposal for Marineosin Biosynthesis Put Forward by Snider and Co-workers[156]



Scheme 37.
Pathway for Marineosin Biosynthesis Elucidated by Reynolds and Co-workers[157]



Scheme 38.
Mechanism for Conversion of Product 312 of the MarG-catalyzed Reaction to Premarineosins 311



Scheme 39.

Pathways for Biosynthesis of Dechlororoseophilin 38 and Prodigiosin R1 40 from 11′-Dimethylundecylprodigiosin 39, Proposed by Challis and Co-workers[152]