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Nitropyrrolins A–E, Cytotoxic Farnesyl-α-Nitropyrroles from a Marine-Derived Bacterium Within the Actinomycete Family *Streptomycetaceae*

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Abstract

Five new farnesyl-α-nitropyrroles, nitropyrrolins A–E (**1**–**5**), were isolated from the saline culture of the marine actinomycete strain CNQ-509. This strain belongs to the "MAR4" group of marine actinomycetes, which have been demonstrated to be a rich source of hybrid isoprenoid secondary metabolites. The structures of the nitropyrrolins are composed of α-nitropyrroles with functionalized farnesyl groups at the C-4 position. These compounds are the first examples of naturally-occurring terpenyl-α-nitropyrroles. Chemical modifications, including one-step acetonide formation from an epoxide, and application of the modified Mosher method, provided the full stereostructures and absolute configurations of these compounds. Several of the nitropyrrolins, nitropyrrolin D in particular, are cytotoxic toward HCT-116 human colon carcinoma cells, but show weak to little antibacterial activity against methicillin-resistant *Staphylococcus aureus* (MRSA).

As part as our continuing interest in the chemistry and biomedical potential of sediment-derived marine actinomycetes, we have isolated a variety of new actinomycete taxa, which show the capacity to produce unique secondary metabolites.1 These taxa include the chemically prolific genera *Salinispora*2 and "*Marinispora*",3 members of which produce new structural classes of secondary metabolites including salinosporamide A,4 a potent proteasome inhibitor that recently completed phase I clinical trial for the treatment of cancer.5 Another group of marine-derived actinomycetes, designated as MAR 4, is proving to be of significant chemical interest.1 To date, at least 15 strains belonging to this group have been isolated from diverse marine sediments. Previous chemical studies of cultured MAR4 strains led to the discovery of a broad range of polyketide-terpenoid secondary metabolites including marinone,6 compounds in the napyradiomycin series7 and azamerone.8 Further chemical analysis of the cultured MAR4 strain CNQ-509, isolated from a marine sediment sample collected off La Jolla, CA, has led to the discovery of a new set of hybrid

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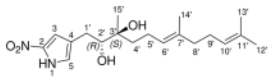
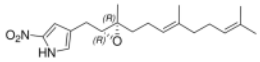
Supporting Information Available: Spectroscopic data sets (1D and 2D NMR, ESI MS, UV/VIS, HRMS, etc) for **1**–**5**, ^{1}H NMR spectra for **6** and (S)-Mosher ester of **11**, ^{1}H NMR and ^{1}H-^{1}H COSY spectra for **9a**, **9b**, **12a**, **12b**, **13a**, **13b**, **14a** and **14b**, ^{1}H NMR and 2D NOESY spectra for **10** and **11**, and ^{1}H and ^{13}C NMR spectra for **7** and **8**. This material is available free of charge via the Internet at http://pubs.acs.org

polyketide-terpenoid metabolites composed of sesquiterpenoid and α-nitropyrrole components. These compounds, nitropyrrolins A–E (**1**–**5**), are unusual examples illustrating rare pyrrole nitration in the α position with linear sesquiterpenoid substitution. The structures of these new compounds, including their absolute configurations, were established by combined spectroscopic and chemical methods. The absolute configuration of the epoxide functionality in compound **2** was determined via a one step acetonide formation from the epoxide, a method which when coupled with Mosher ester analysis leads to the assignment of the absolute configuration of trisubstituted epoxides. In this paper we describe this new method as well as the structure elucidation of five new sesquiterpenoid α-nitropyrroles.



nitropyrrolin A (**1**)



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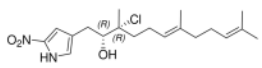
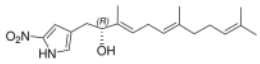
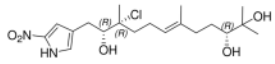


nitropyrrolin C (**3**)



nitropyrrolin D (**4**)



nitropyrrolin E (**5**)

Results and Discussion

The MAR 4 strain CNQ-509 was isolated from a marine sediment collected at a depth of 44 m offshore of La Jolla, California. The strain was cultured in 8 × 4 L volumes for seven days and then extracted using Amberlite XAD-7 resin. The resin was filtered, eluted with acetone, and the extraction solvent concentrated under reduced pressure. The extract was subjected to a diversity of HPLC-based purification steps to afford pure samples of nitropyrrolins A–E (**1–5**).

Nitropyrrolin A (**1**) was obtained as a light yellow oil that analyzed for the molecular formula $C_{19}H_{30}N_2O_4$ by interpretation of HR FAB-MS (obsd $[M–H]^-$ at *m/z* 349.2124) and NMR data. The UV spectrum of **1** displayed absorption bands at 350 nm, indicating the presence of a significant chromophore in the molecule. Two substructures, a linear sesquiterpenoid chain and a pyrrole moiety, were assigned by analyses of ^1H, ^{13}C, ^1H-^1H COSY, HSQC and HMBC NMR spectroscopic data recorded in $CDCl_3$ (Tables 1, 2 and Supporting Information). The linear sesquiterpenoid chain was defined by analysis of NMR data starting from the terminal olefinic gem dimethyl protons (H_3-12′ and H_3-13′) at δ_H 1.69 and 1.61 that showed HMBC correlations to one another, to C-10′ (δ_C 124.1), and to C-11′ (δ_C 131.6). The proton signal (δ_H 5.10) derived from H-10′ showed a COSY correlation to an aliphatic methylene proton signal at δ_H 2.09 (H_2-9′), which in turn showed a COSY correlation to another aliphatic methylene proton signal at δ_H 2.01 (H_2-8′). These NMR data established a terminal isoprene unit, which was expanded to a sequential isoprene unit by the observation of HMBC correlations from the methyl singlet of H_3-14′ (δ_H 1.65) to C-8′ (δ_C 39.7), and from an olefinic proton H-6′ (δ_H 5.18) to C-14′ (δ_C 16.1), and by a series of COSY correlations between the H-6′ signal and two aliphatic methylene protons signals at δ_H 2.20, 2.14, 1.73 and 1.49 (H_2-5′ and H_2-4′). A remaining isoprenoid fragment was constructed starting from the last methyl proton at δ_H 1.27 (H_3-15′), which showed HMBC correlations to a carbinol carbon (C-2′), an oxygenated quaternary carbon (C-3′) and a methylene carbon (C-4′) at δ_C 78.3, 74.6 and 36.2, respectively. In addition, the H-2′ proton

signal (δ_H 3.58) showed a COSY correlation to the diastereotopic methylene proton signals H_2-1′ observed at δ_H 2.73 and 2.53. Because the ^{13}C NMR chemical shifts of C-2′ (δ_C 78.3) and C-3′ (δ_C 74.6) in **1** ($CDCl_3$) were very similar to those of an *anti*-α,β-dihydroxylated terpenoid structural unit (δ_C 78.4 and 74.8, respectively), previously reported in the literature,9 the hydroxy groups were positioned at C-2′ and C-3′. The geometry of C-6′-C-7′ double bond was assigned an *E* configuration on the basis of a NOESY correlation between H-6′ and H_2-8′. This is supported by the ^{13}C chemical shifts of C-14′ and C-8′ (δ_C 16.1 and 39.7), while their chemical shifts would be approximately δ_C 23.5 and 40.0, in a *Z* double bond.9 In addition, the assignment of two terminal methyl groups, C-12′ and C-13′, was confirmed by a NOESY correlation between H-10′ and H_3-12′, coupled with a lack of NOESY correlation between H-10′ and H_3-13′.

Other characteristic features of the ^{1}H NMR spectrum of **1** were the presence of two aromatic protons at δ_H 7.06 (1H, br s) and 6.90 (1H, br s), and an exchangeable proton at δ_H 9.49 (1H, br s). The ^{1}H-^{1}H COSY spectrum showed the cross correlations between these three proton signals, indicating the presence of the NH group in the aromatic ring. HSQC NMR data showed that the aromatic proton signals at δ_H 7.06 and 6.90 (H-3 and H-5) correlated with ^{13}C NMR bands at δ_C 111.2 and 122.2, respectively. In the HMBC spectrum of **1**, proton H-5′ correlated to two unassigned aromatic quaternary carbons C-2 (δ_C 137.5) and C-4 (δ_C 124.3), and the H-3 signal showed an HMBC correlation with C-2. Furthermore, the chemical shift of C-2′, the UV absorption features of **1**, and the remaining unaccounted for elements (NO_2), suggested that the aromatic ring of **1** was a disubstituted nitropyrrole. Strong IR absorption at 1504 cm^{-1} also supported the presence of a nitro group in **1**. Confirming this assignment, the UV spectrum of **1** showed a maximum absorption at 350 nm characteristic for an α-nitropyrrole, while the UV absorption band for β-nitropyrroles has been recorded at 315 nm.10 HMBC correlations from the diastereotopic methylene protons at δ_H 2.73 and 2.53 (H_2-1′) to the C-3, C-4 and C-5 signals allowed the diol-substituted farnesyl chain of **1** to be connected with the α-nitropyrrole group through C-1′ and C-4. Finally, the γ-sesquiterpenyl-α-nitropyrrole structure of **1** was confirmed by the comparison of NMR and UV data of **1** with those of synthetic γ-farnesyl-α-nitropyrrole and β-farnesyl-α-nitropyrrole. These two isomers were synthesized for comparison to **1**. A mixture of pyrrole (7.5 mmol), farnesyl bromide (2.8 mmol), and zinc powder (650 mg) in THF (10 mL) was stirred at room temperature for 10 h to afford 3-farnesyl-pyrrole (**6**) (270 mg). Nitration of **6** (0.08 mmol) in acetic anhydride (1.0 mL) with a solution of HNO_3 (0.08 mmol) in acetic anhydride (0.5 mL), followed by chromatographic purification, yielded γ-farnesyl-α-nitropyrrole (**7**) (5.0 mg) and β-farnesyl-α-nitropyrrole (**8**) (8.7 mg) (Scheme 1). The ^{1}H and ^{13}C NMR signals for the nitropyrrole moiety in **1**, as well as UV spectroscopic data, were identical to those of synthetic γ-farnesyl-α-nitropyrrole.

The absolute configurations of the two asymmetric centers of **1** were first assigned by application of the modified Mosher method11 and by interpretation of the results of a 2D-NOESY NMR experiment. Treatment of **1**, in separate experiments, with *(R)*-(−)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (*R*-MTPA-Cl) and (*S*)-(+)-MTPA-Cl, yielded the *S*-Mosher ester **9a** and *R*-Mosher ester **9b**, respectively. Interpretation of the ^{1}H NMR chemical shift differences ($\Delta\delta_{S-R}$) between **9a** and **9b,** applying classic Mosher ester analysis, revealed that the absolute configuration of C-2′ is *R* (Figure 1). The absolute configuration of the tertiary alcohol at C-3′ in **1** was then assigned by defining the relative configuration of C-2′ and C-3′. In the 2D NOESY spectrum, measured in $CDCl_3$, the H-2′ and H_2-4′ signals showed consistent NOE correlations with H_3-15′ and H_2-1′. In addition, the H_3-15′ signal showed a NOESY correlation with H_2-1′. However, there was no apparent NOE correlation between H-2′ and H_2-4′ (Figure 2). These NOE data indicated that the 1,2-diol at C-2′ and C-3′ in **1** was in the *erythro* configuration. This configuration was further supported by the ^{13}C chemical shifts of C-2′ and C-3′ (δ_C 78.3 and 74.6).9

Nitropyrrolin B (**2**) was isolated as a light yellow oil that analyzed for the molecular formula $C_{19}H_{28}N_2O_3$ by HR-FAB MS data ($[M-H]^-$ at *m/z* 331.2029) and interpretation of combined NMR data (Tables 1, 2 and Supporting Information). The UV absorption spectrum of **2** showed an absorption band at 351 nm, which was identical with the UV data obtained from nitropyrrolin A (**1**). The IR spectrum of **2** was also identical to that of **1**. Comprehensive analysis of 1D and 2D NMR data for **2**, obtained from ^{1}H, ^{13}C, ^{1}H-^{1}H COSY, HSQC and HMBC NMR experiments**,** allowed complete assignment of the proton and carbon signals, leading to the confident construction of a planar structure of nitropyrrolin B. Unlike **1**, the ^{13}C NMR chemical shifts of C-2′ and C-3′ in **2** were observed at δ_C 63.1 and 61.5. The H-2′ proton signal was also observed at higher field (δ_H 2.94) than that of **1** (δ_H 3.58). These data, and considering the unsaturation of **2**, indicated an epoxide functionality at C-2′ – C-3′ instead of the diol group as in **1**.9

The relative configuration of H-2′ and H_2-4′ in nitropyrrolin B (**2**) was determined as *syn* by analysis of 1D NOE data. Irradiation of proton H-2′ (δ 2.94) enhanced proton H_2-4′ (δ 1.49 and 1.70) signals. The relative configuration of H_2-1′ and H_3-15′ of **2** was also assigned to be *syn* on the basis of the NOE correlation between H_2-1′ proton signal and H_3-15′ methyl signal. Based on these NOE analyses, the relative configuration at C-2′ and C-3′ was assigned as 2′*R** 3′*R** Next, the absolute configuration of the epoxide in **2** was defined by conversion of the epoxide to the diol via the intermediate acetonide followed by application of the modified Mosher method.11 Treatment of **2** in acetone with BF_3-Et_2O resulted in a smooth conversion of the epoxide to the acetonide **10**.12 In the 2D NOESY spectrum of **10**, the H-2′ proton (δ_H 3.91) showed a strong correlation with H_3-15′ (δ_H 1.23) indicating that the configuration at C-3′ had been inverted to 3′*S** after formation of the acetonide. Furthermore, a NOESY correlation between H-2′ and H_2-4′ was not observed, supporting the configurations of H-2′ and H_3-15′ in **10** (Figure 2). Treatment of **10** in methanol with excess pyridinium *p*-toluenesulfonate (PPTS) yielded a 2′,3′-diol, whose ^{1}H NMR and NOESY spectra were identical with those of **1**. The ^{1}H NMR spectrum of the (*S*)-Mosher ester of this product was also identical with those of (*S*)-Mosher ester of **1** (**9a**). Thus, the absolute configurations at C-2′ and C-3′ in **2** were assigned as *R* and *R*, respectively.

It can be hypothesized that nitropyrrolin A (**1**) is biosynthesized by an epoxide hydrolase - induced ring opening of **2**. Epoxide hydrolases (EHs) are ubiquitous enzymes that catalyze the hydrolysis of an epoxide to furnish the corresponding vicinal diol. In particular, EHs isolated from actinomycetes such as *Rhodococcus* and *Nocardia* sp, were reported as stereoselective biocatalysts for highly substituted epoxides.13 It thus appears that the isolation of compounds **1** and **2** from strain CNQ-509 is suggestive of the presence of an EH, which stereoselectively converts (2*R*,3*R*)-epoxides to their corresponding (2*R*,3*S*)-diols (Scheme 2). This hypothesis remains to be tested, however, in other epoxide-diol conversions.

Nitropyrrolin C (**3**) was isolated as a dark yellow oil, which was assigned the molecular composition $C_{19}H_{29}ClN_2O_3$ based on analysis of HR-FAB MS data (obsd $[M-H]^-$ at *m/z* 367.1791). The isotope ratio (3:1) between the $[M-H]^-$ and $[M-H+2]^-$ pseudomolecular ion peaks in the mass spectrum clearly indicated that **3** contained one chlorine atom. The UV spectrum of **3** was also identical with those from **1** and **2**, indicating the presence of the α-nitropyrrole moiety. The chemical shifts of C-2′, C-3′ and C-4′ in the ^{13}C NMR spectrum of **1** were 78.3, 74.6 and 36.2, while those of **3** were 78.4, 78.0 and 39.6, respectively. The downfield shifts of C-3′ and C-4′ signals in ^{13}C NMR spectrum of **3** were suggestive of the chlorination at C-3′ in **3**.14

The absolute configurations of the asymmetric centers in **3** were determined by using the modified Mosher's method combined with the interpretation of data from a 1D NOE

experiment. Treatment of **3** with the *R* and *S*-MTPA chlorides as before gave the (*S*)-MTPA ester **12a** and (*R*)-MTPA ester (**12b**) via esterification of the 2′ hydroxy group in **3**. Analysis of ^{1}H NMR chemical shift differences ($\Delta\delta_{S-R}$) between **12a** and **12b** led to the assignment of the *R*-configuration at C-2′ of **3** (Figure 1). The 1D NOE spectrum of **3** in $CDCl_3$ showed inconclusive NOE correlations (Figure 2), possibly because of the high flexibility of the sesquiterpenoid chain or the eclipsed conformation of chlorine at C-3′ and hydroxy group at C-2′ of **3** in $CDCl_3$. However, the analysis of NOE spectroscopic data using DMSO-d_6 instead of $CDCl_3$ clearly revealed a *threo* configuration of the C-2′ hydroxy and the C-3′ chlorine. Irradiation of the H-2′ signal showed enhancement of the H_2-4′ signals and the H_3-15′ signal, while irradiation of H_2-1′ resulted in only in an observable NOE correlation with H_3-15′. These data established *threo* configurations between the hydroxy and chlorine groups, and between H-2′ and H_3-15′. The absolute configurations at C-2′ and C-3′ in **3** were thus assigned as *R* and *R*.

Nitropyrrolin D (**4**) was isolated as a light yellow oil that analyzed for the molecular formula $C_{19}H_{28}N_2O_3$ by interpretation of HR-FAB MS (obsd $[M-H]^-$ at *m/z* 331.2023) and NMR data. The UV and IR spectra of **4** were virtually identical to those of **1**. Compound **4** was also composed of a linear sesquiterpenoid chain and a nitropyrrole moiety, features that were confidently assigned by analyses of IR, UV and 1D and 2D NMR spectroscopic data. The pyrrole moiety of **4** was identical to those of compounds **1–3**, while the sesquiterpenoid chain of **4** contained one additional double bond. In the ^{1}H-^{1}H COSY spectrum of **4**, the H-6′ olefinic proton signal at δ_H 5.08 showed a correlation to the H_2-7′ methylene protons signal at δ_H 2.73, which in turn showed a COSY correlation to another olefinic proton signal at δ_H 5.38 (H-4′). HMBC NMR correlations from H-4′ to H_3-15′ and H_2-2′ indicated the presence of the additional double bond in the C-3′ - C-4′ position in **4**. The geometry of this olefinic bond, and that of the C-6′ - C-7′ double bond, were assigned as *E* on the basis of NOESY correlations between H-6′ and H_2-8′, and between H-2′ and H-4′. The absolute configuration of C-2′ of **4** was assigned as *R* based on Mosher analysis, which showed diagnostic ^{1}H NMR chemical shift differences ($\Delta\delta_{S-R}$) between the MTPA esters of **4** (**13a** and **13b)** (Figure 1).

Nitropyrrolin E (**5**) was obtained as a light yellow oil that was assigned the molecular formula $C_{19}H_{31}ClN_2O_5$, by analysis of HR-FAB MS (obsd $[M-H]^-$ at *m/z* 401.1837) and NMR data. The isotope ratio (3:1) between the $[M-H]^-$ and $[M-H+2]^-$ pseudomolecular ion peaks in the mass spectrum clearly indicated that **5** also contained one chlorine atom. The ^{13}C NMR chemical shifts of the pyrrole carbons and C-1′ thru C-8′ of **5** were virtually identical with those of **3**, while the C-10′ and C-11′ olefinic carbon signals of **1–4** were not observed in the ^{13}C NMR spectrum of **5**. The HMBC NMR spectrum of **5** showed correlations from the terminal gem-dimethyl group at δ_H 1.22 and 1.18 (H_3-12′ and H_3-13′) to C-10′ (δ_C 78.2), and to C-11′ (δ_C 73.2). The H-10′ carbinol proton signal (δ_H 3.36) showed a COSY correlation to the H_2-9′ methylene proton signals at δ_H 1.60 and 1.42. These 2D NMR spectroscopic data revealed the presence of a diol at C-10′ and C-11′ instead of the terminal olefin found in all of the other nitropyrrolins.

The absolute configurations of the three asymmetric centers in **5** were determined by application of the modified Mosher ester NMR method and interpretation of NOESY data. Treatment of **5** in pyridine with *(R)*-MTPA-Cl and *(S)*-MTPA-Cl, in the same manner as before, yielded the *bis*-(*S*)-Mosher ester **14a** and *bis*-(*R*)-Mosher ester **14b**, respectively (Figure 1). Analysis of ^{1}H NMR data comparing the shifts of **14a** and **14b** yielded positive $\Delta\delta_{S-R}$ values for H_2-1′ and negative values for H_2-4′ and H_2-5′ resulting from the anisotropic effects of the Mosher ester group at C-2′. In addition, a negative $\Delta\delta_{S-R}$ value for H_3-12′ was observed, while the H_2-8′ and H_2-9′ proton signals showed positive differences. These data allowed the absolute configurations at C-2′ and C-10′ to be assigned as *R* and *R*,

respectively. The 2D NOESY spectrum of **5** showed identical NOE correlations between H_2-1′, H-2′, H_2-4′ and H_3-15′ as observed in **3**, indicating the *threo* configuration between hydroxyl and chlorine groups, and the *threo* configuration of H-2′ and H_3-15′. The absolute configurations for all of the asymmetric centers in **5** were thus assigned as *R*.

We evaluated the cancer cell cytotoxicities and the antibacterial activities of the nitropyrrolins A–E (**1–5**), as well as several synthetic derivatives, against the human colon carcinoma cell line HCT-116 and against methicillin-resistant *Staphylococcus aureus* (MRSA). Against HCT-116 colon carcinoma, nitropyrrolin D (**4**) was the most active showing IC_{50} = 5.7 μM. The other nitropyrrolins were far less active, while some of the synthetic derivatives showed activities between 10–30 μM (see Table 3). Curiously, the synthetic analogs 3-farnesylpyrrole and γ-farnesyl-α-nitropyrrole showed modest, but significant cytotoxicities. This trend was also observed for activity against MRSA. The nitropyrrolins showed little to no activity, but the synthetic compound 3-farnesylpyrrole was active with a modest MIC of 2.8 μg/mL.

The nitropyrrolins are hybrid isoprenoids composed of two distinct biosynthetic units, an α-nitropyrrole and a linear sesquiterpenoid chain. Secondary metabolites possessing this specific structural composition have not been previously reported. Although there are many natural products composed of terpenoid substructures joined to aromatic ring systems,14 farnesyl pyrroles are rare.15 Only two examples of pyrroloterpene natural products, the glaciapyrroles16 and pyrrolostatin,17 are known to possess a linear terpenoid chain attached to a pyrrole ring or a pyrrole-2-carboxylic acid.

In summary, the structures of the nitropyrrolins were elucidated based on the analysis of spectroscopic data, application of the modified Mosher method, and direct acetonide formation from an epoxide. The rigidity of the acetonide ring allowed the relative configuration of the trisubstituted epoxide to be easily defined by NOE measurements. Hydrolysis to the diol then allowed the absolute configurations at both carbons to be defined by the classical Mosher ester NMR method. The nitropyrrolins appear to be derived from a mixed biosynthesis involving terpenoid precursors and either amino acids or polyketides. In addition, this strain produces terpenoid compounds incorporating phenazine and naphterpin scaffolds (unpublished data) thus making it a remarkable source of diverse hybrid isoprenoid secondary metabolites. The discovery of the nitropyrrolins from a MAR 4 strain provides additional evidence that this subgroup of actinomycetes in the family *Streptomycetaceae* is a rich source of secondary metabolites produced by mixed terpenoid biosynthetic pathways.

Experimental Section

General Experimental Procedures

Optical rotations were measured on PerkinElmer model 343 polarimeter. UV and IR spectra were recorded using PerkinElmer Lambda 35 UV/Vis spectrophotometer and Thermo Scientific Nicolet iS10 spectrometer, respectively. ^{1}H, ^{13}C and 2D NMR spectral data were obtained in $CDCl_3$ or DMSO-d_6 on a Varian Unity 500 MHz NMR spectrometer. Low resolution ESIMS were measured on a Waters Alliance 2695/Quattro micro API system. High-resolution mass spectral data were acquired on JEOL/JMS-AX505WA instrument. Lichroprep RP-18 (Merch, 40–63 um) was used for column chromatography. Semi-preparative HPLC separations were performed using a Gilson 321 HPLC system with a Phenomenex Luna C18(2) 10 μm column (10 × 250 mm) at a flow rate 4 mL/min. A Waters 1525 HPLC-PDA system with a Phenomenex Luna C18(2) 5 μm column (4.6 × 100 mm) was used for the analysis of extracts and fractions.

Isolation of Strain CNQ-509, Cultivation, Extraction

Strain CNQ-509 was isolated on A1 agar medium (10 g of starch, 4 g of peptone, 2 g of yeast extract and 18 g of agar in 1L of seawater) from a marine sediment sample collected at a depth of 44.2 m one mile Northwest of the Scripps Institution of Oceanography Pier (La Jolla, California). The 16S rRNA gene sequence for this strain (1451 base pairs) has been deposited with GenBank (accession number EF581384). It shares 99.2% sequence identity with the type strain for *Streptomyces aculeolatus* (AB184624), which interestingly is the only non-marine strain reported to date that falls within the MAR4 group. Strain CNQ-509 was cultured in 8 replicate 7 L fermentors (LiFlus GR, BioTron Inc.) each containing 4 L of fermentation medium A1 without agar for 7 days at 27 °C while stirring at 400 rpm. At the end of the fermentation period, 20 g/L Amberlite XAD-7 adsorbent resin was added into each fermentor and stirring was continued for one additional hour at 100 rpm. The resin was then collected by filtration through cheesecloth, washed with deionized water, and eluted twice with acetone. The acetone solution was concentrated to afford 3.5 g of organic extract.

Isolation and Purification of Nitropyrrolins A-E (1–5)

The extract (3.5 g) was absorbed on Celite (5 g) and subjected to C-18 (50 g) reversed-phase column chromatography eluting with a step gradient of MeOH-H_2O solvent mixtures (increasing the MeOH by 20% per 500 mL from 10% to 90% MeOH-H_2O). The combined 70% and 90% MeOH fractions (360 mg) was then further fractionated by C-18 reversed-phase HPLC with a MeCN-H_2O gradient solvent system (Phenomenex Luna C-18(2) 21.2 × 250 mm, 10 mL/min, 50–100% MeCN-H_2O for 40 min) to obtain five subfractions containing the nitropyrrolins (A, 16 mg; B, 30 mg; C, 27 mg; D, 11 mg and E, 5 mg). Each fraction was subsequently purified by C-18 reversed-phase HPLC using Phenomenex Luna C18(2) column (10 × 250 mm, 10 μm) with 75 % MeCN-H_2O as an eluting solvent (flow rate: 4 mL/min) to yield four pure compounds, nitropyrrolin A (**1**, 6.0 mg), nitropyrrolin B (**2**, 16 mg), nitropyrrolin C (**3**, 12 mg), nitropyrrolin D (**4**, 4 mg) and nitropyrrolin E (**5**, 2 mg).

Nitropyrrolin A (1)

a light yellow oil, $[\alpha]_D^{20}$ +8 (*c* 0.05, CH_2Cl_2); UV (MeOH) λ_{max} (log ε) : 350 nm (3.90) nm; IR (KBr) ν_{max}: 3360, 2967, 2926, 1504, 1452, 1384, 1367, 1293, 1272 cm^{-1}; See Table 1 for ^1H NMR data and Table 2 for ^{13}C NMR data. ESI MS: *m/z* 373 $[M+Na]^+$, 349 $[M-H]^-$; HR-FAB MS: *m/z* 349.2124 $[M-H]^-$ (calcd for $C_{19}H_{29}N_2O_4$,349.2127).

Nitropyrrolin B (2)

a light yellow oil, $[\alpha]_D^{20}$ +3 (*c* 0.05, CH_2Cl_2); UV (MeOH) λ_{max} (log ε): 351 nm (3.86); IR (KBr) ν_{max}: 3259, 3127, 2963, 2926, 2873, 1510, 1455, 1383, 1368, 1302, 1272, 1251 cm^{-1}; See Table 1 for ^1H NMR data and Table 2 for ^{13}C NMR data. ESI MS: *m/z* 355 $[M+Na]^+$, 331 $[M-H]^-$; HR-FAB MS: *m/z* 331.2029 $[M-H]^-$ (calcd for $C_{19}H_{27}N_2O_3$, 331.2022).

Nitropyrrolin C (3)

a light yellow oil, $[\alpha]_D^{20}$ -2 (*c* 0.07, CH_2Cl_2); UV (MeOH) λ_{max} (log ε): 348 nm (3.82); IR (KBr) ν_{max}: 3303, 3128, 2968, 2927, 2856, 2731, 1709, 1632, 1591, 1504, 1450, 1384, 1370, 1295, 1273 cm^{-1}; See Table 1 for ^1H NMR data and Table 2 for ^{13}C NMR data. ESI MS (rel. int.): *m/z* 391 $[M+Na]^+$, 369 (40) $[M-H+2]^-$, 367 (100) $[M-H]^-$; HR-FAB MS: *m/z* 367.1791 $[M-H]^-$ (calcd for $C_{19}H_{28}ClN_2O_3$, 367.1788).

Nitropyrrolin D (4)

a light yellow oil, $[\alpha]_D^{20}$ -9 (*c* 0.003, MeOH); UV (MeOH) λ_{max} (log ε): 351 nm (3.90); IR (KBr) ν_{max}: 3301, 2968, 2926, 1503, 1452, 1383, 1366, 1276 cm^{-1}; See Table 1 for ^1H

NMR data and Table 2 for ^{13}C NMR data. ESI MS: *m/z* 355 $[M+Na]^+$, 331 $[M-H]^-$; HR-FAB MS: *m/z* 331.2023 $[M-H]^-$ (calcd for $C_{19}H_{27}N_2O_3$, 331.2021).

Nitropyrrolin E (5)

a light yellow oil, $[\alpha]_D^{20}$ +15 (*c* 0.002, MeOH); UV (MeOH) λ_{max} (log ε): 350 nm (3.85); IR (KBr) ν_{max} 3403, 2973, 2931, 1503, 1451, 1384, 1366, 1293, 1271 cm^{-1}; See Table 1 for ^{1}H NMR data and Table 2 for ^{13}C NMR data. ESI MS (rel. int.): *m/z* 403 $[M-H+2]^-$ (40), 401 $[M-H]^-$ (100); HR-FAB MS: *m/z* 401.1837 $[M-H]^-$ (calcd for $C_{19}H_{30}ClN_2O_5$, 401.1843).

Preparation of the 3-farnesylpyrrole by zinc-mediated farnesylation of pyrrole

A mixture of pyrrole (7.5 mmol), farnesyl bromide (2.8 mmol), zinc powder (650 mg) in THF (10 mL) was stirred at room temperature for 10 hours. After completion of the reaction as indicated by TLC analysis, the reaction mixture was quenched with saturated NH_4Cl (20 mL) and diluted with water (10 mL), and extracted with ethyl acetate (2 × 40 mL). Evaporation of the solvent followed by purification on normal phase HPLC [Luna Silica (2), 250 × 21.2 mm, flow rate 10 mL/min] as an isocratic condition [hexane-EtOAc (20:1)] afforded pure 3-farnesyl-pyrrole (**6**) (270 mg). For **3-Farnesylpyrrole (6)**: UV (MeOH) λ_{max} (log ε): 227 nm (4.49); ESI MS: *m/z* 270 $[M-H]^-$. ^{1}H NMR (500 MHz, $CDCl_3$) δ ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.02 (1H, NH), 6.75 (1H, br m, H-5), 6.58 (1H, br m, H-2), 6.12 (1H, br m, H-4), 5.41 (1H, br tq, *J* = 7.5, 1.0 Hz, C-2′), 5.17 (1H, br tq, *J* = 7.0, 1.0 Hz, H-6′), 5.13 (1H, br tqq, *J* = 7.0, 1.0, 1.0 Hz, H-10′), 3.25 (2H, d, *J* = 7.5 Hz, H_2-2′), 2.15 (2H, m, H_2-5′), 2.07–2.09 (4H, m, H_2-4′ and H_2-9′), 2.01 (2H, dd, *J* = 8.5, 7.0 Hz, H_2-8′), 1.72 (3H, br d, *J* = 1.0 Hz, H_3-15′), 1.71 (3H, br d, *J* = 1.0 Hz, H_3-12′), 1.63 (6H, br s, H_3-13′ and H_3-14′).

Preparation of the γ-farnesyl-α-nitropyrrole (7) and β-farnesyl-α-nitropyrrole (8) by nitration of 3-farnesylpyrrole (6)

A solution of HNO_3 (0.08 mmol) in Ac_2O (0.5 mL) was added dropwise to a solution of 3-farnesylpyrrole (**6**) (0.08 mmol) in Ac_2O (1 mL), and the reaction was stirred at − 50°C for 2 h and then at room temperature for 1 h. After the reaction was complete (as indicated by TLC analysis), the reaction mixture was diluted with H_2O(5 mL), and extracted with Et_2O (2 × 10 mL). The organic layer was washed with aqueous $NaHCO_3$ solution (2%), concentrated under reduced pressure, then purified by normal phase HPLC [Luna silica (2), 250 × 10 mm, flow rate 4 mL/min] under isocratic conditions using hexane-EtOAc (92:8) to afford γ-farnesyl-α-nitropyrrole (**7**) (5.0 mg) and β-farnesyl-α-nitropyrrole (**8**) (8.7 mg). For **γ-farnesyl-α-nitropyrrole (7)** a light yellowish oil; UV (PDA detector using MeCN-H_2O solvent mixture) λ_{max}: 353 nm; ESI MS: *m/z* 315 $[M-H]^-$; ^{1}H NMR (500 MHz, $CDCl_3$); δ 9.24 (1H, br s, NH), 6.96 (1H, br s, H-3), 6.74 (1H, br s, H-1), 5.30 (1H, br tq, *J* = 7.5, 1.0 Hz, H-2′), 5.11 (1H, m, H-6′), 5.10 (1H, m, H-10′), 3.19 (2H, d, *J* = 7.5 Hz, H_2-1′), 2.13 (2H, m, H_2-5′), 2.06–2.08 (4H, m, H_2-4′ and H_2-9′), 1.99 (2H, m, H_2-8′), 1.69 (6H, s, H_3-12′ and H_3-15′), 1.61 (3H, s, H_3-13′), 1.60 (3H, s, H_3-14′); ^{13}C NMR (125 MHz, $CDCl_3$) δ 137.3[a] (C-2), 137.2 (C-3′), 135.3 (C-7′), 131.4 (C-11′), 127.1 (C-4), 124.3 (C-6′), 123.9 (C-10′), 121.5 (C-2′), 121.0 (C-5), 110.8 (C-3), 39.7 (C-8′), 39.6 (C-4′), 26.7 (C-9′), 26.4 (C-5′), 25.7 (C-12′), 25.3 (C-1′), 17.7 (C-13′), 16.1 (C-15′), 16.0 (C-14′). [a]This chemical shift was assigned by the analysis of HSQC and HMBC spectra because it was not observed in the ^{13}C NMR spectrum. For **β-farnesyl-α-nitropyrrole (8)**: a light yellowish oil; UV (PDA detector using MeCN-H_2O solvent mixture) λ_{max}: 340 nm; ESI MS: *m/z* 315 $[M-H]^-$; ^{1}H NMR (500 MHz, $CDCl_3$); δ 9.35 (1H, br s, NH). 6.86 (1H, br dd, *J*= 2.5, 2.0 Hz, H-5), 6.20 (1H, br dd, *J*= 2.5, 2.0 Hz, H-4), 5.35 (1H, br tq, *J* = 7.0, 1.0 Hz, H-2′), 5.12 (1H, m, H-6′), 5.10 (1H, m, H-10′), 3.62 (2H, d, *J*= 7.0 Hz, H_2-1′), 2.13 (2H, m, H_2-5′), 2.06–2.07 (4H, m, H_2-4′ and H_2-9′), 1.99 (2H, m, H_2-8′), 1.71 (3H, s, H_3-15′), 1.68 (3H, s, H_3-12′), 1.61 (6H, s,

H_3-13′ and H_3-14′); ^{13}C NMR (125 MHz, $CDCl_3$) δ 137.7 (C-3′), 135.2 (C-7′), 134.1[a] (C-2), 131.3 (C-11′), 129.3 (C-3), 124.3 (C-10′), 124.0 (C-6′), 121.9 (C-5), 120.2 (C-2′), 112.4 (C-4), 39.7 (C-8′), 39.6 (C-4′), 26.7 (C-9′), 26.5 (C-5′), 25.7 (C-1′ and C-12′), 17.7 (C-13′), 16.2 (C-15′), 16.0 (C-14′). [a]This chemical shift was assigned by the analysis of HSQC and HMBC spectra because it was not detected in the 1D ^{13}C NMR spectrum.

Preparation of MTPA Esters

Compounds **1**, **3**, **4**, **5** and **11** were divided into two portions, and each was dissolved in 600 μL of pyridine-d_5 in a 5 mm NMR tube. To each NMR tube were added a slight excess dimethylaminopyridine (DMAP). The samples were then treated with 5 μL of (*R*)-α-methoxy-a-(trifluoromethyl) phenylacetyl chloride (MTPA-Cl) and 5 μL of (*S*)-MTPA-Cl at room temperature. After 12 h, the reaction was complete, and ^{1}H NMR and ^{1}H-^{1}H gCOSY spectra for (*S*)-mosher esters (**9a**, **12a**, **13a**, **14a** and the (*S*)-mosher ester of **11**) and (*R*)-mosher esters (**9b**, **12b**, **13b** and **14b**) were recorded (see Supporting Information for NMR data). Because of the difficulty in interpreting some crude ester mixtures, compounds **13a**, **13b, 14a and 14b** were purified by RP-HPLC with a Luna C-8 column (250 × 10 mm) using 50% MeCN-H_2O to 100% MeCN as a linear gradient for 40 min (flow rate 4 mL/min),

Preparation of the Acetonide 10

Nitropyrrolin B (**2**) (2 mg) was dissolved in dry Me_2CO (1 mL), and $BF_3 \cdot Et_2O$ (20 μL) was added at 0 °C. The reaction was allowed to stir for 3 h at 0 °C, the reaction was then quenched with saturated aqueous $NaHCO_3$, and the aqueous phase was extracted twice with EtOAc. The combined EtOAc solution was concentrated under reduced pressure, and the residue was purified by RP HPLC with Luna C-8 column (250 × 10 mm) using a linear gradient solvent system of 50 % MeCN-H_2O to 100 % MeCN for 20 min to provide compound **10** (1.2 mg). For **acetonide 10**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.30 (1H, br s, NH), 7.07 (1H, br s, H-3), 6.90 (1H, br s, H-5), 5.16 (1H, dd, *J* = 7.0, 7.0 Hz, H-6′), 5.11 (1H, dd, *J* = 7.0, 7.0 Hz, H-10′), 3.91 (1H, dd, *J* = 10.0, 3.5 Hz, H-2′), 2.76 (1H, *J* = 15.0, 10.0 Hz, H_2-1′a), 2.58 (1H, *J* = 15.0, 3.5 Hz, H_2-1′b), 2.23 (1H, m, H_2-5′a), 2.08 (1H, m, H_2-5′b), 2.08 (2H, m, H_2-9′), 2.00 (2H, m, H_2-8′), 1.70 (3H, br s, H_3-12′), 1.65 (1H, m, H_2-4′a), 1.64 (3H, br s, H_3-14′), 1.62 (3H, br s, H_3-13′), 1.46 (3H, s, acetonide methyl), 1.37 (3H, s, acetonide methyl), 1.27 (1H, m, H_2-4′b), 1.23 (3H, s, H_3-15′); ESI MS: *m/z* 413 $[M+Na]^+$.

Preparation of the Diol 11 from 10

To a solution of **10** in anhydrous MeOH (2 mL) was added pyridinium-*p*-toluensulfonate (2 mg), and the reaction was stirred for 24 h. The reaction was then quenched with saturated aqueous $NaHCO_3$, and the aqueous phase was extracted twice with EtOAc. The EtOAc solution was concentrated under reduced pressure, and the residue was purified by RP HPLC [Luna C-8 column (250 × 10 mm), flow rate 4 mL/min, 45 % MeCN-H_2O to 95 % MeCN as a linear gradient for 40 min] to afford diol **11**. For **diol (11)**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.40 (1H, br s, NH), 7.06 (1H, br s, H-3), 6.90 (1H, br s, H-5), 5.18 (1H, br t, *J* = 7.0 Hz, H-6′), 5.10 (1H, tq, *J* = 7.0, 1.0 Hz, H-10′), 3.58 (1H, ddd, *J* = 11.0, 4.0, 2.0 Hz, H-2′), 2.73 (1H, dd, *J* = 15.0, 2.0 Hz, H_2-1′a), 2.53 (1H, dd, *J* = 15.0, 11.0 Hz, H_2-1′b), 2.20 (1H, m, H_2-5′a), 2.14 (1H, m, H_2-5′b), 2.14 (1H, d, *J* = 4.0 Hz, 2′-OH), 2.19 (2H, m, H_2-9′), 2.01 (2H, m, H_2-8′), 1.97 (1H, br s, 3′-OH), 1.73 (1H, m, H_2-4′a), 1.69 (3H, br s, H_3-12′), 1.66 (3H, br s, H_3-14′), 1.61 (3H, br s, H_3-13′), 1.49 (1H, m, H_2-4′b), 1.27 (3H, br s, H_3-15′); ESI MS: *m/z* 373 $[M+Na]^+$.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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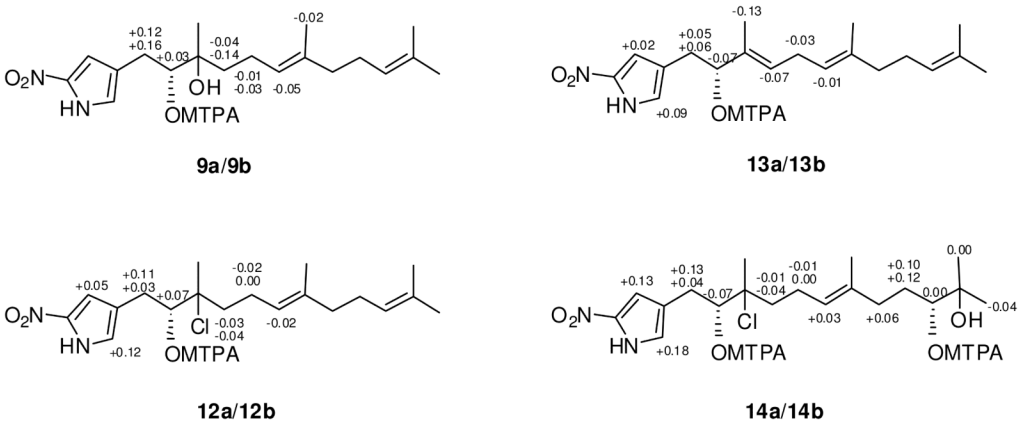


Figure 1.

$\Delta\delta_{S\text{-}R}$ ^{1}H NMR values in pyridine-d_5 for the Mosher esters **9a**/**9b,** the Mosher esters **12a**/**12b,** the Mosher esters **13a**/**13b, and** the *bis*-Mosher esters **14a**/**14b**.

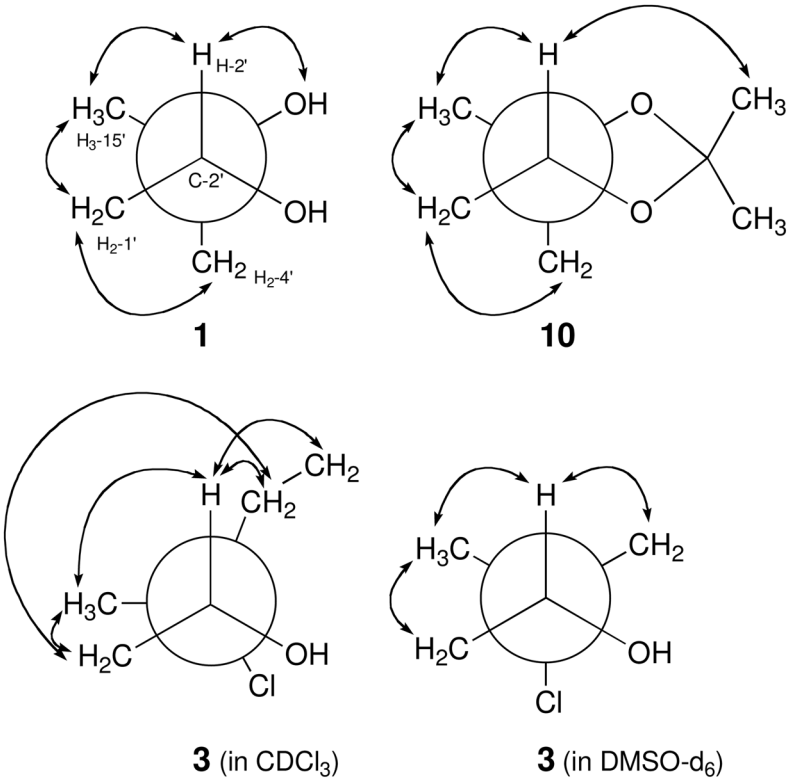
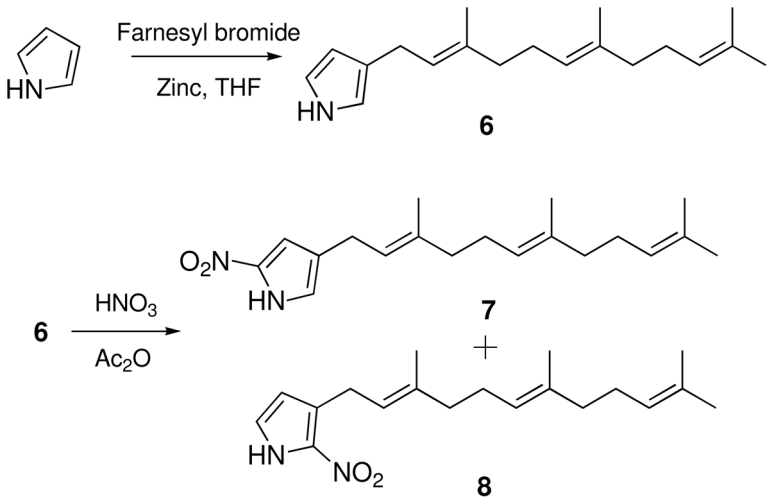
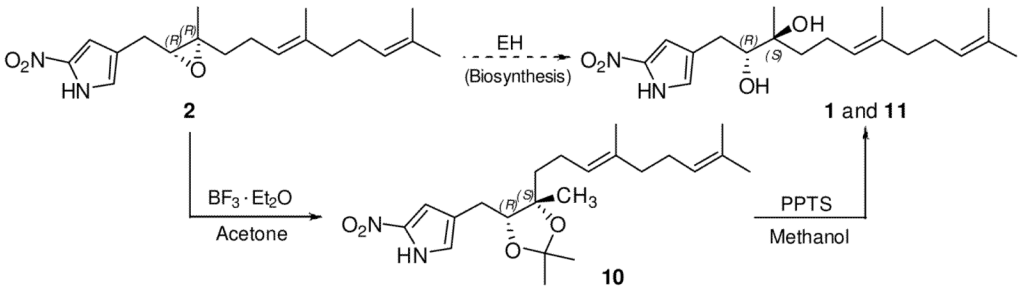


Figure 2.

Newman Projections along the C-2′-C-3′ bonds illustrating key NOE correlations defining the rotamers of **1** (in $CDCl_3$), **3** (in $CDCl_3$ and DMSO-d_6) and **10** (in $CDCl_3$).



Scheme 1.
Formation of 3-farnesyl-pyrrole (**6**) and the nitration of **6**



Scheme 2.
The formation of the acetonide **10** and the diol **11** from **2**.

Table 1

NMR Spectroscopic Data for Nitropyrrolins A-E (**1**–**5**) [500 MHz, $CDCl_3$ (δ 7.27), δ_H mult (*J* in Hz)]

	1	**2**	**3**	**4**	**5**
1	9.49, br s	9.51, br s	9.48, br s	9.38, br s	9.58, br s
3	7.06, br s	7.03, br s	7.07, br s	7.01, br s	7.08, br s
5	6.90, br s	6.87, br s	6.92, br s	6.84, br s	6.92, br
1′	2.73, br dd (15.0, 2.0)	2.71, dd (15.0, 6.0)	2.93, dd (15.0, 1.5)	2.72, m[a]	2.94, dd (15.0, 2.0)
	2.53, dd (15.0, 10.5)	2.68, dd (15.0, 6.0)	2.60, dd (15.0, 10.5)		2.60, dd (15.0, 10.5)
2′	3.58, br dd (10.5, 2.0)	2.94, dd (6.0, 6.0)	3.75, dd (10.5, 1.5)	4.18, dd (6.5, 6.5)	3.76, br d (10.5)
4′	1.73, br ddd (14.0, 10.5, 6.0)	1.70, ddd (13.5, 8.0, 6.0)	2.00, m	5.38, dd (7.0, 7.0)	1.98, ddd (14.0, 11.0, 5.0)
	1.49, br ddd (14.0, 10.5, 6.0)	1.49, ddd (13.5, 9.5, 6.5)	1.75, ddd (14.0, 11.0, 5.0)		1.72, ddd (14.0, 11.0, 5.0)
5′	2.20, m	2.11, m	2.28, m	2.73, m[a]	2.29, m[a]
	2.14, m		2.19, m		2.19, m[a]
6′	5.18, br t (7.0)	5.08, m[a]	5.15, td (7.0, 1.0)	5.08, m[a]	5.22, dd (7.0, 7.0)
8′	2.01, m	2.05, m	1.99, m	1.99, m	2.27, m[a]
9′	2.09, m	1.96, br dd (8.0, 7.0)	2.08, dd (7.5, 7.5)	2.07, m	2.09, ddd (15.0, 8.0, 8.0) 1.60, m[a] 1.42, dddd (14.0, 10.0. 8.0, 5.0)
10′	5.10, tq (7.0, 1.0)	5.08, m[a]	5.09, tq (7.0, 1.5)	5.09, m[a]	3.36, dd (10.0, 1.5)
12′	1.69, br d (1.0)	1.68, br d (1.0)	1.69, s	1.69, s	1.22, s
13′	1.61, s	1.60, s	1.61, s	1.61, s	1.18, s
14′	1.65, s	1.60, s	1.63, s	1.63, s	1.66, s
15′	1.27, s	1.38, s	1.64, s	1.71, s	1.62, s

[a]The multiplicity of this signal was unresolved due to peak overlapping and the chemical shift was assigned by interpretation of HSQC and HMBC spectroscopic data.

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Table 2

^{13}C NMR Spectroscopic Data for Nitropyrrolins A-E (**1**–**5**) [125 MHz, $CDCl_3$ (δ 77.0)]

C#	A (1)	B (2)	C (3)	D (4)	E (5)
2	137.5	137.4	137.5	137.5	137.4
3	111.2	110.9	111.3	111.5	111.5
4	124.3	123.0	124.0	123.7	123.9
5	122.2	121.6	122.2	122.1	122.2
1′	28.8	26.7	29.2	32.7	29.8
2′	78.3	63.1	78.4	77.6	78.3
3′	74.6	61.5	78.0	136.0	77.9
4′	36.2	38.6	39.6	126.3	39.9
5′	22.0	23.7	23.0	26.9	23.5
6′	124.1	123.3	123.0	121.9	123.9
7′	136.0	135.8	136.3	135.9	136.1
8′	39.7	26.7	39.7	39.6	36.9
9′	26.6	39.7	26.6	26.7	29.9
10′	124.1	124.2	124.1	124.2	78.2
11′	131.6	131.6	131.5	131.5	73.2
12′	25.7	25.8	25.7	25.7	26.2
13′	17.7	17.8	17.7	17.7	23.6
14′	16.1	16.1	16.1	16.1	16.1
15′	23.4	16.8	25.6	11.8	25.9

Assignments were made on the basis of DEPT and HMBC/HMQC experimental data.

Table 3

Cytotoxicities and Antibacterial Activities of the Nitropyrrolins A–E (**1–5**) and Some Synthetic Derivatives.

Compound	**HCT-116**	**MRSA Inhibition**
	IC_{50} (μM)	**MIC (μg/mL)**
nitropyrrolin A (**1**)	31.1	NSA
nitropyrrolin B (**2**)	31.0	NSA
nitropyrrolin C (**3**)	NSA	NSA
nitropyrrolin D (**4**)	5.7	NSA
nitropyrrolin E (**5**)	NSA	NSA
3-farnesylpyrrole	13.7	2.8
γ-farnesyl-α-nitropyrrole	9.2	NSA
α-nitropyrrole	NSA	NSA
β-nitropyrrole	NSA	NSA
2-farnesylpyrrole	24.4	NSA

NSA = Not Significantly Active (Inhibition observed only at >20 μg/mL)