Lynamicins A−E, Chlorinated Bisindole Pyrrole Antibiotics from a Novel Marine Actinomycete†

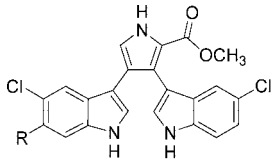
Katherine A. McArthur,‡ Scott S. Mitchell,‡ Ginger Tsueng,‡ Arnold Rheingold,§ Donald J. White,‡ Jennifer Grodberg,‡ Kin S. Lam,‡ and Barbara C. M. Potts*,‡

Nereus Pharmaceuticals, Inc., 10480 Wateridge Circle, San Diego, California 92121, and University of California, San Diego, 9500 Gilman Drive, La Jolla, California 92093

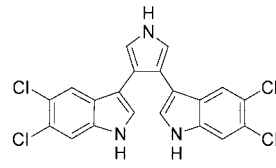
Received May 9, 2008

A series of chlorinated bisindole pyrroles, lynamicins A−E (**1**−**5**), was discovered from a novel marine actinomycete, NPS12745, which was isolated from a marine sediment collected off the coast of San Diego, California. Close to full length 16S rRNA sequence analysis indicated that NPS12745 is a novel strain of a recently described marine actinomycete with the proposed genus name *Marinispora.* The antimicrobial spectrum of these compounds was evaluated against a panel of 11 pathogens, which demonstrated that these substances possess broad-spectrum activity against both Gram-positive and Gram-negative organisms. Significantly, compounds **1**−**5** were active against drug-resistant pathogens such as methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant *Enterococcus faecium*.

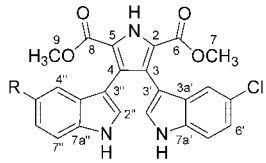
Concerns over the availability of antibiotic treatment options for both early- and late-stage infections from bacteria have recently increased in response to the evolutionary development of resistance to existing antibiotics.[1] As such, new antimicrobials and new sources of antimicrobials are increasingly valuable. Marine microorganisms, which have developed unique metabolic and physiological capabilities to ensure survival in extreme ocean habitats, represent such a source by offering the potential to produce new antibiotics that would not be observed from terrestrial microorganisms.[2,3] In fact, we and others[4,5] have recently reported novel antibiotic scaffolds derived from a new group of marine actinomycetes with the proposed genus name of *Marinispora*.[4] We describe herein an entirely new chemical series from this same genus.



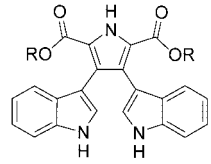
1 R = H
2 R = Cl



3



4 R = Cl
5 R = H



6 R = H
7 R = CH_3

Results and Discussion

Sediment samples were collected from Mission Bay in San Diego, California, and subsequently processed for the cultivation of marine actinomycetes, which led to the isolation of NPS12745, a novel marine organism. Close to full length 16S rRNA sequence analysis indicated that NPS12745 belongs to the proposed genus *Marinispora.* Crude extracts obtained from fermentation broths of this organism were subjected to bioassay-guided fractionation, in which a strong correlation was observed between a series of natural products (**1**−**5**) present in the fractions and biological activity against methicillin-sensitive *Staphylococcus aureus* (MSSA) (Figure 1). LC-MS analysis of these fractions indicated that the biological activity was associated with a family of related compounds with one to four chlorine atoms per molecule. The UV spectra of compounds **4** and **5** were in good agreement with chromopyrrolic acid (lycogalic acid A; **6**) and its dimethyl ester (**7**), which are bisindole pyrroles bearing carboxylic acid or methyl ester substituents at the C-2 and C-5 positions of the pyrrole ring (see structures **4** and **5** for atom numbering).[6,7] The collective body of preliminary spectroscopic and antimicrobial data indicated that we had encountered a family of antibiotics unprecedented in nature, which were thus targeted for scale-up purification, structure elucidation, and biological evaluation. Collectively, antibiotic compounds **1**−**5** were characterized as bisindole pyrroles in which the pyrrole ring bears indole substituents at positions C-3 and C-4. In each compound in the series, at least one indole is substituted with chlorine at positions C-5′ and/or C-6′. The pyrrole may be further substituted with a methyl ester at the C-2 and/or C-5 positions. The compounds have one to four chlorine atoms, and two of the molecules are symmetrical (**3** and **4**). Evaluation against a panel of pathogenic bacteria demonstrated broad-spectrum antibiotic activity for the series. The detailed characterization of the structures of **1**−**5** and their biological activities are described below.

The ethyl acetate extract of the NPS12745 fermentation culture broth was processed for the recovery of **1**−**5** using reversed-phase preparative HPLC. The compounds were eluted using a solvent gradient of 60% MeOH/40% H_2O to 100% MeOH in the order **5**, **1**, **4**, **2**, and **3** and further purified by HPLC to obtain pure compounds. The structures were elucidated by analysis of spectroscopic data, including HRMS, from which the isotope distribution patterns were used to determine the number of chlorine atoms. Values for the ^{13}C NMR chemical shifts were generated on the basis of analysis of both one- and two-dimensional NMR spectra, especially multiplicity-edited HSQC data and HMBC data. Comparison of the ^{13}C NMR chemical shifts reported for 5- and 6-chloroindole[8,9] (Table 1) and 5-chlorotryptophan[10] provided strong evidence for the 5-chloroindole substitutions found throughout the bisindole pyrrole series (*vide infra*). The structure elucidation for each unique compound follows.

† Dedicated to the memory of Lyn Sethna.

* To whom correspondence should be addressed. Tel: (858) 200-8324. Fax: (858) 587-4088. E-mail: bpotts@nereuspharm.com.
‡ Nereus Pharmaceuticals, Inc.
§ University of California, San Diego.

10.1021/np800286d CCC: $40.75
Published on Web 10/09/2008

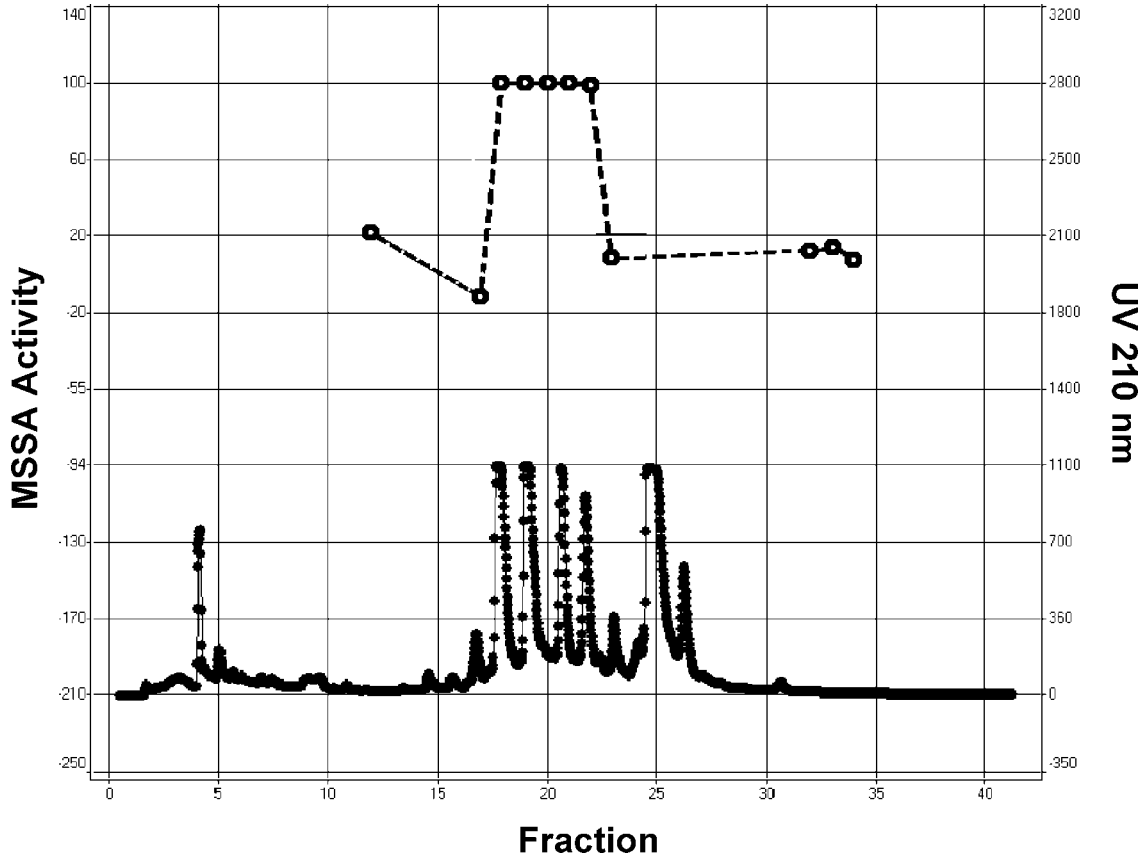


Figure 1. Initial detection of bisindole pyrroles in fractions derived from the crude extract of NPS12745. Chromatography was monitored by UV absorbance at 210 nm (solid circles). A strong correlation was observed between fractions containing compounds in this series and growth inhibition of MSSA (open circles).

Table 1. ^{13}C NMR Assignments[a] for Compounds **1**−**7** and 5- and 6-Chloroindole (δ_c, mult.)

position	**1**	**2**	**3**[b]	**4**[b]	**5**	**7**[7c]	5-Cl-In[8d]	6-Cl-In[9d]
2	120.9, qC	120.97, qC	117.7, CH	123.0, qC	122.9, qC[e]	124.2		
3	121.6, qC	121.6, qC	115.7, qC	124.5, qC	124.6, qC[f]	126.9		
4	120.3, qC	119.9, qC			125.2, qC[f]			
5	121.0, CH	120.95, CH			123.0, qC[e]			
6	161.4, qC	161.3, qC		160.8, qC	161.0, qC[g]	163.0		
7	51.4, CH_3	51.5, CH_3		52.0, CH_3	51.91, CH_3[h]	51.9		
8					160.9, qC[g]			
9					51.90, CH_3[h]			
2′	126.2, CH	126.3, CH	124.8, CH	126.5, CH	126.4, CH	125.9	125.6	NR[i]
3′	110.0, qC	109.9, qC	112.0, qC	109.0, qC	109.2, qC	109.6	102.3	NR[i]
3a′	129.2, qC	129.1, qC	127.5, qC	129.0, qC	129.1, qC	129.2	128.9	NR[i]
4′	120.1, CH	120.0, CH	121.6, CH	119.9, CH	119.8, CH	120.7	120.0	121.6
5′	125.4, qC	125.5, qC	125.8, qC	125.5, qC	125.4, qC	121.8	125.3	120.6
6′	122.2, CH	122.3, CH	123.8, qC	122.2, CH	122.1, CH	119.6	122.2	127.9
7′	112.47, CH	112.5, CH	112.9, CH	112.4, CH	112.4, CH	111.9	112.0	111.0
7a′	134.5, qC	134.5, qC	135.3, qC	134.2, qC	134.2, qC	137.4	134.1	NR[i]
2″	124.2, CH	124.7, CH			125.0, CH			
3″	110.5, qC	110.7, qC			109.1, qC			
3a″	128.2, qC	127.0, qC			127.9, qC			
4″	119.4, CH	121.1, CH			119.9, CH			
5″	125.6, qC	125.7, qC			120.2, CH			
6″	122.4, CH	123.8, qC			122.0, CH			
7″	112.48, CH	112.8, CH			111.3, CH			
7a″	134.6, qC	135.0, qC			135.9, qC			

[a] δ ^{13}C values referenced to internal solvent for CD_2Cl_2 at 53.8 ppm, unless otherwise noted. [b] Due to symmetry, assignments for carbons 2″−7″ are identical to those of analogous carbons 2′−7′. Similarly, assignments for C-4 and C-5 are identical to those of C-3 and C-2, respectively. For compound **4**, the methyl esters are also chemically equivalent. [c] Chemical shifts in CD_3OD. [d] Chemical shifts in $CDCl_3$. [e−h] Chemical shifts may be interchangeable. [i] Not reported.

Lynamicin A (**1**) was obtained as an off-white, optically inactive solid. The molecular formula, $C_{22}H_{15}N_3O_2Cl_2$, was derived from HRESIMS (*m*/*z* 424.0612 $[M + H]^+$) and indicated 16 degrees of unsaturation, while the isotope pattern was consistent with the presence of two chlorines. The proton and carbon spectra largely comprised aromatic resonances, with the exception of one methyl ester [δ 161.4 (C-6); 51.4 (C-7), δ_H 3.69 (3H, s)]. In fact, two sets of eight nearly degenerate carbon resonances were observed. Comparison of the chemical shifts against those of 5-chlorotryptophan (data not shown)[10] and 5- and 6-chloroindole[7,8] (Table 1)

Table 2. ^{1}H NMR Assignments for Compounds **1**−**5** and 5- and 6-Chloroindole [δ_H, multi (*J* in Hz)[a]]

position	**1**	**2**	**3**[b]	**4**[b]	**5**	5-Cl-In[8c]	6-Cl-In[9c]
1NH	9.44, br	9.40, br	8.54, br	10.01, br	9.99, br		
2			7.08, d (3.0)				
5	7.309, d (3.1)	7.28, d (3.5)					
7	3.69, s	3.69, s		3.73, s	3.73[d], s		
9					3.71[d], s		
1′NH	8.36, br	8.35, br	8.18, br	8.24, br	8.15, br	NR[f]	NR[f]
2′	7.18, d (2.5)	7.17, d (2.5)	7.00, d (2.5)	7.04, d (2.7)	6.95, d (2.5)	7.11	NR[f]
3′						6.45	NR[f]
4′	7.19, br d (2.0)	7.19, dd (2.0, 0.5)	7.57, s	7.15, d (1.9)	7.18[e]	7.59	7.54
5′							7.09
6′	7.07, dd (8.5, 2.0)	7.07, ddd (8.5, 2.0, 0.5)		7.03, dd (8.6, 1.9)	7.02, dd (8.5, 2.2)	7.11	
7′	7.312, d (8.5)	7.31, dd (8.5, 0.5)	7.49, s	7.24, d (8.6)	7.18, d (8.8)	7.19	7.37
1″NH	8.13, br	8.13, br			8.15, br		
2″	6.79, d (2.5)	6.83, d (2.5)			6.97, d (2.5)		
4″	7.47, br d (1.9)	7.51, d (0.6)			7.17, d (8.2)		
5″					6.91, ddd (7.6, 7.6, 1.0)		
6″	7.08, dd (8.5, 1.9)				7.07, ddd (7.6, 7.6, 1.0)		
7″	7.25, br d (8.5)	7.43, d (0.6)			7.28, d (8.2)		

[a] All chemical shifts are referenced to internal solvent for CD_2Cl_2 at 5.32 ppm unless otherwise noted. [b] Due to symmetry, NH-1″ and C-2″−C-7″ assignments are identical to analogous protons NH-1′ and C-2′−C-7′. For compound **3**, H-2 is chemically equivalent to H-5. For compound **4**, the methyl esters are chemically equivalent. [c] Chemical shifts in $CDCl_3$. [d] Chemical shifts may be interchanged. [e] Due to spectral overlap, multiplet type could not be determined. [f] Not reported.

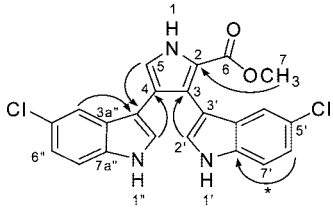


Figure 2. Key HMBC correlations (arrows) used to assign the structure of **1**. *May be assigned to C-6′ to C-7a′ and/or C-6″ to C-7a″.

strongly supported the presence of two 5-chlorindole moieties with further substitution at the C-3 positions. For the first indole moiety, the proton NMR resonances and coupling constants [H-6′ (δ_H 7.07, dd 8.5, 2.0), H-7′ (δ_H 7.312, d, 8.5), and H-4′ (δ_H 7.19, br d, 2.0)] were in agreement with this assignment (Table 2); additional support was provided by HMBC correlations, including from H-6′ to C-7a′ (and/or H-6″ to C-7a″), which further established the chlorine at C-5′. Similar spectroscopic patterns were observed for the second chloroindole moiety, which also gave rise to a well-resolved correlation from H-4″ to C-3″, independently establishing the chlorine at C-5″ on this chloroindole ring system. The remaining atoms in the molecule, C_4H_2N, could be reasonably assigned to a trisubstituted pyrrole. The methine doublet at δ_H 7.31 (H-5, 1H, d, 3.1) gave rise to a COSY correlation with the broad signal at δ_H 9.44 (NH-1), indicating that both the nitrogen of the pyrrole ring and one adjacent carbon were unsubstituted. The substitution pattern about the pyrrole ring was initially delineated from the HMBC spectrum. Key long-range correlations were observed from H-5 (δ 7.309, 1H, d, J = 3.1 Hz) and H_3-7 (δ 3.69, 3H, s) to the quaternary carbon assigned as C-2 (δ120.9) and to carbonyl C-6. Indole protons H-2′ and H-2″ were correlated to carbons at δ 121.6 and 120.3 assigned to C-3 and C-4, respectively, while pyrrole H-5 was correlated to indole C-3″, which together established the relative positions of the two indole ring systems about the pyrrole ring (Figure 2). The collective data were in agreement with a dichlorobisindole pyrrole for structure **1** but were also consistent with substitution of the carbomethoxy group at C-3 of the pyrrole ring, with the second indole substituent at C-2. The X-ray crystal structure of **2** and the structure of symmetrical compound **3** each supported bisindole substitution at C-3 and C-4 of the pyrrole ring system (*vide infra*). Confirmation of this substitution pattern for these two compounds led us to extend this assignment to the remaining compounds in the series, including **1**. We note that the molecular asymmetry induced by the single methyl ester substituent gives rise to diagnostic chemical shifts that are useful in distinguishing between the two indole substituents. For example, the proton resonances for H-2″ and H-4″ were shifted by 0.39 ppm upfield and 0.28 ppm downfield, respectively, for the indole ring that is anti to the methyl ester substituent of the pyrrole. The differences in the H-2′/H-2″ chemical shifts may be rationalized by unique resonance forms, since H-2′ is in the δ-position of an $\alpha,\beta,\gamma,\delta$-unsaturated ester. A search for published reports of related compounds led to a small series of unhalogenated secondary metabolites, including the naturally occurring chromopyrrolic acid (**6**)[7] and chlorinated indole analogues that were produced through combinatorial biosynthesis.[11] The ^{13}CNMR data for **1** were in reasonable agreement with that of **7**, the dimethyl ester derivative of **6** (Table 1).

Lynamicin B (**2**) was obtained as an off-white, optically inactive solid. The molecular formula, $C_{22}H_{14}N_3O_2Cl_3$, was derived from HRESIMS (*m*/*z* 480.0059 $[M + Na]^+$) and indicated 16 degrees of unsaturation, while the isotope pattern was consistent with the presence of three chlorines. The remaining spectroscopic data were similar to those of compound **1**. The most obvious exceptions were the replacement of the H-6″ proton with chlorine, which resulted in small chemical shift changes in neighboring protons and carbons, together with the simplification of the H-4″ and H-7″ multiplets to a pair of weakly coupled doublets (J = 0.6 Hz; Table 2). The structure of this moiety was further supported by ^{1}H−^{13}C HMBC correlations from both H-7″ and H-4″ to C-5″, C-6″, and C-7a″, while H-4″ was specifically correlated to C-3″. The substitution pattern about the pyrrole ring was defined by a series of weak but definitive HMBC correlations, including H-5/C-3″ and H-2″/C-4, which indicated that the 5,6-chloroindole represented the C-4 substituent of the pyrrole ring, while the H-2′/C-3 correlation helped assign the 5-chloroindole as the C-3 pyrrole substituent. A weak correlation was also observed from the methyl ester protons (H_3-7) to the pyrrole C-2. Moreover, the relative chemical shifts of H-2″ and H-4″ versus H-2′ and H-4′ followed the same pattern observed for compound **1**, further supporting the assigned substitution pattern about the pyrrole ring. The proposed structure was confirmed by X-ray crystallography, which further revealed a nonplanar 3-D structure for compound **2**. The monochlorinated indole ring adjacent to the methyl ester substituent of the pyrrole ring is rotated out of plane of the pyrrole ring. 5,6-Dichlorinated indoles have been rarely reported in nature.[12]

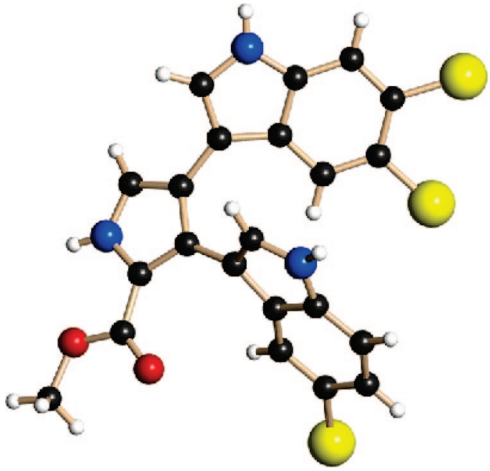


Figure 3. Structure of compound **2** as determined by X-ray crystallography.

Lynamicin C (**3**) was obtained as an off-white solid. The molecular formula, $C_{20}H_{11}N_3Cl_4$, was derived from HRESIMS (*m*/*z* 433.9771 $[M + H]^+$) and indicated 15 degrees of unsaturation. The isotope pattern was consistent with four chlorines. While **3** was clearly related to **1** and **2**, the methyl ester signals were conspicuously absent from the proton and carbon spectra. In fact, the carbon NMR spectrum comprised only eight unique indole signals and two unique pyrrole signals, indicating a symmetrical molecule. This was corroborated by the proton NMR spectrum, which contained a total of four aromatic signals, each of which integrated to twice that of a broad NH (δ 8.54, 1H) assigned to the pyrrole, while an additional NH signal integrated to 2H (δ 8.18), one for each indole ring system. Thus, the data were again consistent with a bisindole pyrrole. The NH protons gave rise to the two strongest correlations in the COSY spectrum: NH-1/H-2 and NH-1′/H-2′; the former indicated that the carbons flanking the pyrrole nitrogen were unsubstituted. The chemical shifts and singlet nature of the remaining two signals in the proton NMR spectrum (H-4′ and H-7′) were consistent with chlorine substitution at the 5- and 6-positions of the indole (Table 2). Key HMBC correlations that established unambiguously the substitution pattern and chemical shift assignments included H-2/C-3′, H-2′/C-3, and H-4′/C-3′. The collective data supported that of a symmetrically substituted pyrrole bearing 5,6-dichloroindole at C-3 and C-4.

Lynamicin D (**4**) was obtained as an off-white, optically inactive solid. The molecular formula, $C_{24}H_{17}N_3O_4Cl_2$, was derived from HRESIMS (*m*/*z* 482.0657 $[M + H]^+$) and indicated 17 degrees of unsaturation, with an isotope pattern that was consistent with two chlorines. As in the case of **3**, the carbon and proton NMR spectra were indicative of a symmetrical molecule; however, in this case, the data supported the presence of a bis-methyl ester. Furthermore, the indoles were each substituted with a single chlorine at the 5-position. HMBC correlations were observed from the methyl ester to C-2 of the pyrrole ring, establishing assignments within the ring. The collective data were consistent with the structure assigned to **4**.

Lynamicin E (**5**) was obtained as an off-white, optically inactive solid. The molecular formula, $C_{24}H_{18}N_3O_4Cl$, was derived from HRESIMS (*m*/*z* 448.1067 $[M + H]^+$) and indicated 17 degrees of unsaturation, with an isotope pattern that was consistent with only one chlorine. The molecular formula and the presence of two singlets [δ_H 3.73 (3H, s) and 3.71 (3H, s)] in the proton NMR spectrum indicated that **5** represented another bis-methyl ester analogue. COSY correlations established the spin system from H-4″ to H-7″ on the unchlorinated indole ring, and atom-specific resonances were assigned based on diagnostic carbon chemical shifts (e.g., C-7″, δ 111.3). For the chlorinated indole ring, a COSY correlation was noted between signals at δ_H 7.18 and 7.02 ppm, suggesting that the chlorine substitution was at either H-5′ or H-6′. Multiplicities could not be determined for H-4′ and H-7′ from the proton spectrum due to chemical shift overlap at 7.18 ppm. However, the two carbons were distinct in the HSQC spectrum, and the proton corresponding to the diagnostic C-7′ chemical shift at 112.4 was clearly a doublet, indicating that the chlorine was once again at C-5′. The remaining indole carbons were assigned on the basis of key correlations in the HMBC data. The other carbons were consistent with a bis-methyl ester pyrrole ring. An HMBC correlation was observed from the methyl ester protons to C-2 and/or C-5, and the remaining two aromatic carbons were thus assigned to C-3 and C-4.

The structures of **1**−**5** suggest that they are derived from two units of tryptophan. The biosynthetic pathway of the related bisindole pyrrole, chromopyrrolic acid,[7] supports the hypothesis that the core bisindole pyrrole skeleton is tryptophan-derived. However, the precursor amino acids are modified in the final natural products in that the indole rings are substituted with chlorine. This series is exemplary of the unique chemotypes that may be obtained from the marine environment, which is well known for giving rise to chlorinated natural products that are not produced by terrestrial organisms.[13]

Bisindole alkaloids have attracted considerable attention, particularly those containing a bisindolylmaleimide core structure, such as the staurosporines and rebeccamycin, which are well known for their antiproliferative activity in cancer cells. The bisindole pyrroles represent a much smaller series of metabolites, including chromopyrrolic acid, produced by *Chromobacterium violaceum,*[7] and three bisindole pyrroles, lycogarubins A−C, produced by the myxomycete *Lycogala epidendrum*.[6,14] In these cases, no examples of halogenated derivatives were found, and only compounds bearing ester or carboxyl groups at both the C-2 and C-5 positions of the pyrrole ring were reported. This differs significantly from bisindole pyrroles **1** and **2**, which are exclusively substituted with an ester at the 2-position of the pyrrole ring. To our knowledge, the only biological activity cited previously for the bisindole pyrrole class is antiviral activity against HSV (IC_{50} 17.2 μg/mL, *in vitro*), as described by Hashimoto et al.[14]

Bisindole pyrroles **1**−**5** have been tested against a panel of Gram-positive and Gram-negative bacteria; complete data for several analogues are summarized in Table 3. In general, compounds in this series showed broad-spectrum antimicrobial activity, and MIC values were comparable across drug-sensitive and drug-resistant strains. This class of compounds showed good potency against *Staphylococci* and *Enterococci*, suggesting potential for treatment of nosocomial infections, while their moderate activity against *Streptococci* and *Haemophilus* may indicate potential in treating community-acquired infections.

Experimental Section

General Experimental Procedures. Melting points were acquired on a Mel-temp apparatus and are uncorrected. Optical rotations were measured on a Rudolph Autopol III polarimeter using a 4 × 100 mm sample cell. UV spectra were obtained with a Beckman Coulter DU 640 spectrophotometer. IR spectra were acquired on neat compound samples using a JASCO FTIR 480 Plus spectrometer. NMR spectra were collected using a 500 MHz Bruker Avance NMR spectrometer with an inverse probe equipped with *x*,*y*,*z*-gradients, except for the ^{13}C NMR spectra, which were acquired with a broad-band observe probe. All NMR data were acquired at 300 K in CD_2Cl_2, referenced to 5.32 ppm in the proton spectra and 53.8 ppm in carbon spectra. HRMS were acquired using a Micromass Q-Tof2 mass spectrometer in the ES+ mode. HRESIMS were referenced using a polyethylene glycol polymer mixture, which was co-injected during acquisition as an internal accurate mass standard. RP HPLC separations were performed on a Gilson HPLC equipped with a Gilson 215 fraction collector and an Agilent PDA detector. HPLC solvents were obtained from Fisher Scientific and VWR, and water used for HPLC was filtered through a NANOpure

Table 3. MIC Values (μM, [μg/mL]) for Compounds **1**−**5** against Bacterial Strains

organism	1	2	3	4	5
Staphylococcus aureus ATCC 29213−MSSA[a]	4.3 [1.8]	1.8 [0.8]	2.5 [1.1]	6.2 [3]	>45 [>20]
Staphylococcus aureus ATCC 43300−MRSA[b]	4.7 [2]	2.2 [1]	3.5 [1.5]	6.2 [3]	27 [12]
Staphylococcus epidermidis ATCC 700578[c]	9.5 [4]	2.2 [1]	2.3 [1]	8.3 [4]	45 [20]
Staphylococcus epidermidis ATCC 700582[c]	9.5 [4]	2.2 [1]	2.3 [1]	8.3 [4]	>72 [>32]
Streptococcus pneumoniae ATCC 49619−Pen S[d]	57 [24]	18 [8]	46 [20]	50 [24]	>72 [>32]
Streptococcus pneumoniae ATCC 51915−Pen R[e]	57 [24]	18 [8]	46 [20]	42 [20]	>72 [>32]
Enterococcus faecalis ATCC 29212−VSE[f]	19 [8]	3.3 [1.5]	5.8 [2.5]	17 [8]	>54 [>24]
Enterococcus faecium ATCC 700221−VRE[g]	19 [8]	4.4 [2]	4.6 [2]	17 [8]	>54 [>24]
Haemophilus influenzae ATCC 49247	38 [16]	4.4 [2]	9.2 [4]	10 [5]	>72 [>32]
Haemophilus influenzae ATCC 49766	28 [12]	13 [6]	15 [6]	17 [8]	>72 [>32]
Escherichia coli imp[h]	16 [16]	13 [6]	15 [6]	>67 [>32]	>72 [>32]

[a] Methicillin sensitive. [b] Methicillin resistant. [c] Multiple drug resistant. [d] Penicillin sensitive. [e] Penicillin resistant. [f] Vancomycin sensitive. [g] Vancomycin resistant. [h] Permeable mutant.

Infinity ultrafilter. Deuterated solvents were obtained from Cambridge Isotope Laboratories, Inc. (Andover, MA).

Microorganism. Strain NPS12745 was isolated from a marine sediment sample collected at Mission Bay, San Diego, CA. Close to full length 16S rRNA sequence analysis of NPS12745 indicated that it is a novel strain of a new group of marine actinomycetes with a proposed genus name of *Marinispora*.[4] The 16S rRNA sequence of NPS12745 was deposited in the GenBank database on November 3, 2006, and assigned the accession number EF551062. The culture was deposited on January 7, 2004, with the American Type Culture Collection (ATCC) in Rockville, MD, and assigned the ATCC patent deposition number PTA-5748.

Fermentation. Seed culture of NPS12745 was inoculated into 100 mL of production medium consisting of the following per liter of seawater: starch, 10 g; yeast extract, 4 g; and peptone, 2 g. The production culture (100 flasks of 100 mL each) was incubated at 28 °C on a rotary shaker operating at 250 rpm for 7 days.

Extraction and Isolation. The combined culture broth was first shaken with 500 mL of acetone for 15 min and then extracted with 10 L of ethyl acetate. The extract was dried *in vacuo*. The dried extract (841 mg) was then processed for the recovery of **1**−**5**. The crude extract (50 mg) was dissolved in DMSO (900 μL), and this solution injected on an HPLC column (ACE 5 μm C_{18}-HL, 150 mm length by 21 mm i.d., flow rate 14.5 mL/min) and eluted using a solvent gradient of 60% MeOH/40% H_2O to 100% MeOH over 15 min. In total, 500 mg of crude extract was processed as described above. The compounds of interest eluted between 8.0 and 11.5 min in the following order: **5** (3.2 mg, 0.6%), **1** (24.8 mg, 5.0%), **4** (mixture of **1** and **4**), **2** (57 mg, 11.4%), and **3** (29.4 mg, 5.9%). Fractions containing a mixture of **1** and **4** were further purified by C18 preparative HPLC (ACE 5 μm C_{18}-HL, 150 mm length by 21 mm i.d., flow rate 14.5 mL/min) using an isocratic solvent elution of 65% MeOH/35% H_2O containing 0.1% ammonium acetate. Compounds **1** and **4** (12.6 mg, 2.5%) eluted at 14.5 and 17 min, respectively.

Lynamicin A (1): off-white solid; mp 127−130 °C; $[\alpha]^{22}_D$ 0 (*c* 0.51, CH_3OH); UV (CH_3OH) λ_{max} (log ε) 211 (4.57), 233 (4.70), 292 (4.22) nm; IR (neat) ν_{max} 3412, 1695, 1264 cm^{-1}; ^1H NMR (CD_2Cl_2), see Table 2; ^{13}C NMR (CD_2Cl_2), see Table 1; HREIMS *m/z* $[M + H]^+$ 424.0612 (calcd for $C_{22}H_{16}N_3O_2Cl_2$, 424.0620).

Lynamicin B (2): off-white solid; $[\alpha]^{22}_D$ 0 (*c* 0.51, CH_3OH); UV (CH_3OH) λ_{max} (log ε) 210 (4.42), 235 (4.59), 295 (4.26) nm; IR (neat) ν_{max} 3415, 1695, 1243 cm^{-1}; ^1H NMR (CD_2Cl_2), see Table 2; ^{13}C NMR (CD_2Cl_2), see Table 1; HREIMS *m/z* $[M + H]^+$ 458.0230 (calcd for $C_{22}H_{14}N_3O_2Cl_3$, 458.0244); $[M + Na]^+$ 480.0059 (calcd for $C_{22}H_{14}N_3O_2Cl_3Na$, 480.0049).

Lynamicin C (3): off-white solid; UV (CH_3OH) λ_{max} (log ε) 206 (4.24), 235 (4.06), 295 (3.25) nm; ^1H NMR (CD_2Cl_2), see Table 2; ^{13}C NMR (CD_2Cl_2), see Table 1; HREIMS *m/z* $[M + H]^+$ 433.9771 (calcd for $C_{20}H_{12}N_3Cl_4$, 433.9785).

Lynamicin D (4): off-white solid; mp 137−140 °C; $[\alpha]^{22}_D$ 0 (*c* 0.48, CH_3OH); UV (CH_3OH) λ_{max} (log ε) 209 (4.54), 229 (4.62), 258 (4.39), 300 (sh) (4.17) nm; IR (neat) ν_{max} 3411, 1699, 1236 cm^{-1}; ^1H NMR (CD_2Cl_2), see Table 2; ^{13}C NMR (CD_2Cl_2), see Table 1; HREIMS *m/z* $[M + H]^+$ 482.0657 (calcd for $C_{24}H_{18}N_3O_4Cl_2$, 482.0674).

Lynamicin C (5): off white solid; $[\alpha]^{22}_D$ 0 (*c* 0.17, CH_3OH); UV (CH_3OH) λ_{max} (log ε) 209 (4.45), 225 (4.55), 266 (4.27) nm; ^1H NMR (CD_2Cl_2), see Table 2; ^{13}C NMR (CD_2Cl_2), see Table 1; HREIMS *m/z* $[M + H]^+$ 448.1067 (calcd for $C_{24}H_{19}N_3O_4Cl$, 448.1064).

X-ray Crystallography of Compound 2.[15] Compound **2** was dissolved in a solvent system of 1:1:1 Ac_2O/EtOAc/hexanes at a concentration of 33 mg/mL. The solvent was slowly allowed to evaporate while the sample incubated at −20 °C, and the resulting crystals were observed on the sides of the vial after 48 h. Crystallographic data were collected on a Bruker SMART APEX CCD X-ray diffractometer ($F(000) = 1064$, Cu Kα radiation, $\lambda = 1.54178$ Å, $\mu = 3.915$ mm^{-1}, $T = 100$ K) at the University of California, San Diego Crystallography Laboratory. The software program used was SHELX-TL, 1997, and the refinement method was full-matrix least-squares on F^2. Crystal data for compound **2** cocrystallized with a molecule of acetone: $C_{25}H_{20}Cl_3N_3O_3$, MW = 516.79, monoclinic, space group *P*2(1)/*c*, $a = 12.4184(5)$ Å, $b = 17.5743(8)$ Å, $c = 10.6100(4)$ Å, $\alpha = 90°$, $\beta = 98.574(3)°$, $\gamma = 90°$, vol = 2289.70(16) $Å^3$, $Z = 4$, $\rho_{calcd} = 1.499$ g/cm^{-3}, crystal size, 0.40 × 0.22 × 0.14 mm^3, θ range, 3.60−68.03°, 14 579 reflections collected, 3996 independent reflections ($R_{int} = 0.0553$), *R* indices: $R_{1(all)} = 0.0652$, $wR_{2(all)} = 0.1300$, GOF = 1.018.

Antibiotic Activity Assay. The minimum inhibitory concentration (MIC) of compounds **1**−**5** was obtained against a variety of bacteria (Table 3) using a conventional broth dilution assay in accordance with standards recommended by the National Committee for Clinical Laboratory Standards (NCCLS).[16] The serial microbroth dilution method used BBL Mueller-Hinton broth (cation adjusted) except for the *Streptococcus pneumonia* and *Haemophilus influenza*. BBL Mueller-Hinton broth II (cation adjusted) with lysed horse blood and Haemophilus Test Medium were used for *S. pneumonia* and *H. influenza*, respectively. *S. pneumoniae* and *H. influenzae* were incubated in a CO_2 incubator, whereas the others were not (standard NCCLS). The final bacterial inoculum contained approximately 5×10^5 cfu/mL and was run on microtiter plates. The volume of each well was 100 μL, and the plates were incubated at 35 °C for 14−16 h. The MIC was defined as the lowest drug concentration that prevented visible growth of the bacterium.

Acknowledgment. We thank D. Tahmassebi and H. Citeau for allowing us to collect IR data at the University of San Diego on compounds **1**, **2**, and **4**.

References and Notes

(1) Walsh, C. *Nat. Rev. Microbiol.* **2003**, *1*, 65–70.
(2) Clardy, J.; Fischbach, M. A.; Walsh, C. T. *Nat. Biotechnol.* **2006**, *24*, 1541–1550.
(3) Fenical, W.; Jensen, P. R. *Nat. Chem. Biol.* **2006**, *2*, 666–673.
(4) Kwon, H. C.; Kauffman, C. A.; Jensen, P. R.; Fenical, W. *J. Am. Chem. Soc.* **2006**, *128*, 1622–1632.
(5) Macherla, V. R.; Liu, J.; Sunga, M.; White, D. J.; Grodberg, J.; Teisan, S.; Lam, K. S.; Potts, B. C. M. *J. Nat. Prod.* **2007**, *70*, 1454–1457.
(6) Fröde, R.; Hinze, C.; Josten, I.; Schmidt, B.; Steffan, B.; Steglich, W. *Tetrahedron Lett.* **1994**, *57*, 1689–1690.
(7) Hoshino, T.; Kojima, Y.; Hayshi, T.; Uchiyama, T.; Kaneko, K. *Biosci. Biotech., Biochem.* **1993**, *57*, 775–781.
(8) Morales-Rios, M. S.; del Rio, R. E.; Joseph-Nathan, P. *Magn. Reson. Chem.* **1989**, *27*, 1039–1047.
(9) Umezawa, K.; Ikeda, Y.; Uchihata, Y.; Naganawa, H.; Kondo, S. *J. Org. Chem.* **2000**, *65*, 459–463.
(10) Lee, M.; Phillips, R. S. *J. Heterocycl. Chem.* **1994**, *31*, 711–716.
(11) Sanchez, C.; Zhu, L.; Brana, A. F.; Salas, A. P.; Rohr, J.; Mendez, C.; Salas, J. A. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 461–466.

(12) Martinez-Luis, S.; Rodríguez, R.; Acevedo, L.; González, M. C.; Lira-Rocha, A.; Mata, R. *Tetrahedron* **2006**, *62*, 1817–1822.
(13) Faulkner, D. J. *Nat. Prod. Rep.* **2002**, *19*, 1–48.
(14) Hashimoto, T.; Yasuda, A.; Akazawa, K.; Takaoka, S.; Tori, M.; Asakawa, Y. *Tetrahedron Lett.* **1994**, *35*, 2559–2560.
(15) Crystallographic data for structure **2** reported in this paper have been deposited with the Cambridge Crystallographic Data Centre, deposition number 698305. Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44-(0)1223-336033 or e-mail: deposit@ccdc.cam.ac.uk).
(16) Ferraro, M. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically; Approved Standard (NCCLS)*; Villanova, PA, 2000; Vol. M7-A5, pp 1−33.

NP800286D