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Amaranzoles B−F, Imidazole-2-carboxy Steroids from the Marine Sponge *Phorbas amaranthus*. C24-*N*- and C24-*O*-Analogues from a Divergent Oxidative Biosynthesis

Brandon I. Morinaka,[†] Joseph R. Pawlik,[§] and Tadeusz F. Molinski*[,†,‡]

[†]*Department of Chemistry and Biochemistry, and* [‡]*Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California, San Diego, La Jolla, California 92093-0358, and* [§]*Department of Biology and Marine Biology, Center for Marine Science, University of North Carolina Wilmington, 5600 Marvin K. Moss Lane, North Carolina 28403-3297*

tmolinski@ucsd.edu

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Five new steroidal imidazoles, amaranzoles B−F, were isolated from extracts of the marine sponge *Phorbas amaranthus* along with the known amaranzole A. The C24-*N*-(4-*p*-hydroxyphenyl)imidazol-5-yl constitution found in amaranzoles A, C, and D is replaced by a C24-*O*-(4-*p*-hydroxyphenyl)imidazole-2-carboxylate motif in amaranzoles B, E, and F. The structures were elucidated by interpretation of spectroscopic data. The C24 side chain configuration was assigned by synthesis of a model ester followed by chiroptical comparisons of its CD spectrum with that of an amaranzole B derivative.

Introduction

Extracts of the marine sponge *Phorbas amaranthus* were highly deterrent in feeding assays using the bluehead wrasse, *Thalassoma bifasciatum*.[1] In the course of investigations to identify the antifeedant constituents from *P. amaranthus*, we previously reported the ring-A contracted steroids, phorbasterones A−D,[2] and an unusual *N*-imidazolyl sterol, amaranzole A (**1**).[3] Partial fractionation of extracts of *P. amaranthus* and interpretation of results of bioassay-guided separations, using laboratory and field fish feeding assays, pointed to the aqueous MeOH extracts of *P. amaranthus* as the location of the feeding deterrent components. Scarcity of material necessitated recollection of larger amounts of the sponge that, after more extensive fractionation of the polar extracts, led to pure compounds and characterization of five minor steroidal alkaloids, amaranzoles B−F (**2**−**6**) in addition to **1**. Amaranzoles C (**3**) and D (**4**) were simple double bond isomers of dehydro-**1**, but to our surprise, amaranzoles B (**2**), E (**5**), and F (**6**) differed from **1** by replacement of the unique C24-*N* imidazolyl structure with 24-*O*-steroidal 2-carboxyimidazolecarboxylate esters. Because nonfused imidazole rings, excluding simple histamines, are less common among natural products,[4] the discovery of the latter two constitutional variants in the same organism is intriguing from the perspective of biogenesis. A biosynthetic hypothesis is proposed that sheds light

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(2) Masuno, M. N.; Pawlik, J. R.; Molinski, T. F. *J. Nat. Prod.* **2004**, *67*, 731–733.
(3) Morinaka, B. I.; Masuno, M. N.; Pawlik, J. R.; Molinski, T. F. *Org. Lett.* **2007**, *9*, 5219–5222.
(4) For example, isobromotopsentin: (a) Murray, L. M.; Lim, T. K.; Hooper, J. N. A.; Capon, R. J. *Aust. J. Chem.* **1995**, *48*, 2053. For comprehensive reviews of imidazole-containing natural products, see: (b) Jin, Z. *Nat. Prod. Rep.* **2009**, *26*, 382−445 and earlier reviews in the series, including (c) Lewis, J. R. *Nat. Prod. Rep.* **1996**, *13*, 435−467 and references cited within.

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TABLE 1. ^{1}H NMR Data for Amaranzoles B−F (2−5) [CD_3OD, 600 MHz δ (mult, J (Hz))]

no.	2	3	4	5	6
1	1.17 (m) ax;	1.36 (m)	1.32 (dd, 14.6, 3.0)	1.31 (m)	1.32 (m)
	2.47 (dd, 14.8, 3.2) eq.	2.54 (dd, 14.6, 3.3)	2.47 (dd, 14.6, 3.0)	2.48 (14.6, 3.3)	2.56 (dd, 14.7, 3.2)
2	4.90 (brq, 2.8) eq.	4.91 (m)[a]	4.92 (brq, 3.0)	4.93 (brq, 3.1)	4.92 (brq, 2.9)
3	4.26 (dt, 12.3, 4.0) ax.	4.28 (dt, 12.1, 3.9)	4.30 (ddd, 12.5, 4.3, 3.6)	4.30 (ddd, 12.5, 4.5, 3.4)	4.30 (dt, 12.0, 4.0)
4	1.78 (q, 12.3) ax;	1.83 (q, 12.6)	1.80 (q, 12.5)	1.79 (brq, 12.5)	1.84 (q, 12.7)
	2.29 (brd, 13.4) eq.	2.36 (brd, 13.2)	2.32 (m)	2.31 (m)	2.37 (brd, 12.8)
5	1.31 (m) ax.	1.63 (m)	1.45 (ddd, 12.5, 10.9, 3.0)	1.46 (ddd, 12.5, 10.8, 3.0)	1.63 (m)
6	4.20 (td, 11.0, 4.5) ax.	4.51 (m)	4.12 (td, 10.9, 5.4)	4.12 (td, 10.8, 5.4)	4.51 (m)
7	1.00 (m) ax.	2.10 (m)	1.82 (brq, 12.5)	1.83 (brq, 12.6)	2.10 (m)
	2.34 (dt, 12.4, 4.5) eq.	2.73 (dd, 17.3, 6.5)	3.12 (dd, 13.8, 5.4)	3.13 (dd, 13.6, 5.4)	2.74 (dd, 17.8, 6.7)
8	1.53 (m)				
9	0.68 (m)		1.71 (m)	1.72 (m)	
11	1.34 (m) ax.	2.12 (m)	1.55 (m)	1.60 (m)	2.15 (m)
	1.53 (m) eq.		1.59 (m)		
12	1.13 (m) ax.	1.36 (m)	1.11 (m)	1.15 (m)	1.41 (m)
	2.00 (m) eq.	1.97 (m)	1.91 (brd, 12.6)	1.97 (brd, 12.6)	2.02 (m)
14	1.30 (m)	2.07 (m)			2.12 (m)
15	1.08 (m)	1.30 (m)	2.22 (m)	1.97 (m)	1.32 (m)
	1.59 (m)	1.62 (m)	2.29 (m)	2.27 (m)	1.62 (m)
16	1.22 (m)	1.06 (m)	1.22 (m)	1.38 (m)	1.33 (m)
	1.84 (m)	1.71 (m)	1.62 (m)	1.86 (m)	1.93 (m)
17	1.13 (m)	1.06 (m)	1.05 (m)	1.15 (m)	1.21 (m)
18	0.68 (s)	0.54 (s)	0.78 (s)	0.87 (s)	0.65 (s)
19	1.10 (s)	1.27 (s)	0.96 (s)	0.97 (s)	1.28 (s)
20	1.46 (m)	1.28 (m)	1.39 (m)	1.56 (m)	1.50 (m)
21	0.96 (d, 6.0)	0.90 (d, 6.2)	0.91 (d, 6.6)	1.00 (d, 6.6)	1.00 (d, 6.5)
22	1.15 (m)	0.86 (m)	0.90 (m)	1.24 (m)	1.16 (m)
	1.50 (m)	1.29 (m)	1.31 (m)	1.55 (m)	1.50 (m)
23	1.70 (m)	2.00 (m)	1.94 (m)	1.71 (m)	1.71 (m)
	1.92 (m)		2.00 (m)	1.92 (m)	1.93 (m)
24	5.39 (dd, 8.0, 5.6)	4.51 (m)	4.50 (m)	5.41 (dd, 7.8, 5.4)	5.41 (dd, 7.8, 5.4)
25					
26	4.94 (s)	4.67 (s)	4.69 (s)	4.95 (s)	4.95 (s)
	5.07 (s)	4.97 (s)	4.96 (s)	5.09 (s)	5.08 (s)
27	1.82 (s)	1.73 (s)	1.71 (s)	1.83 (s)	1.83 (s)
28		7.68 (brs)	7.79 (brs)		
29	7.41 (s)	6.83 (brs)	6.92 (brs	7.43 (brs)	7.46 (brs)
30					
31					
32	7.57 (d, 8.4)	7.14 (d, 8.4)	7.08 (d, 8.4)	7.59 (d, 8.4)	7.62 (d, 8.4)
33	6.80 (d, 8.4)	6.85 (d, 8.4)	6.70 (d, 8.4)	6.81 (d, 8.4)	6.83 (d, 8.4)

[a]Obscured by solvent peak.

on a possible origin of the two families of amaranzoles that unifies their C24-*N* and C24-*O* constitutional differences through a common intermediate.

Results and Discussion

Two specimens of *P. amaranthus* were collected in November 2006 from the Florida Keys. The first specimen (06-04-004a) was extracted sequentially with water, MeOH, and CH_2Cl_2. The aqueous MeOH partition was filtered through C_{18} reversed-phase column followed by repeated reversed-phase HPLC to give **1** (6.8×10^{-4} % dry wt) and a new analogue, amaranzole B (**2**, 2.0×10^{-4} % dry wt). Similar treatment of the second sponge specimen (06−04−004b) gave amaranzoles C (**3**, 1.4×10^{-4} % dry wt), D (**4**, 2.3×10^{-4} %), E (**5**, 6.3×10^{-5} %), and F (**6**, 3.3×10^{-5} %) in addition to **1** and **2**. Characterization of the new compounds on submilligram samples was carried out by FTIR, MS, and 600 MHz NMR facilitated by a state-of-the-art 1.7 mm microcryoprobe with exceptionally high mass sensitivity.[5] Amaranzole B (**2**) was assigned the molecular formula $C_{37}H_{49}N_2Na_3O_{15}S_3$ (m/z 903.2108, [M − Na]$^-$, Δ = +1.8 mmu) based on negative-ion HRESIMS that corresponded the addition of the elements of CO_2 to **1**, consistent with a carboxy derivative of amaranzole A. ^{1}H NMR, COSY, and HSQC data of **2** revealed ^{1}H and ^{13}C chemical shifts for the sterol core that were essentially identical with

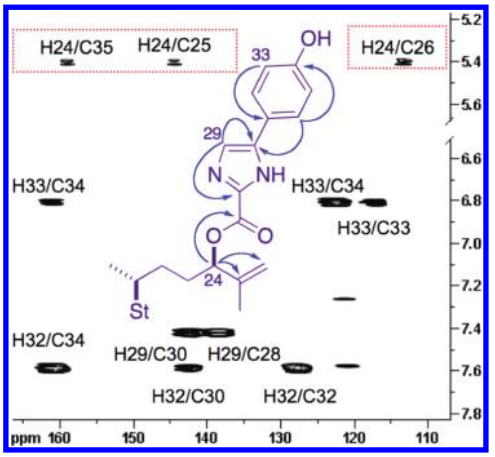
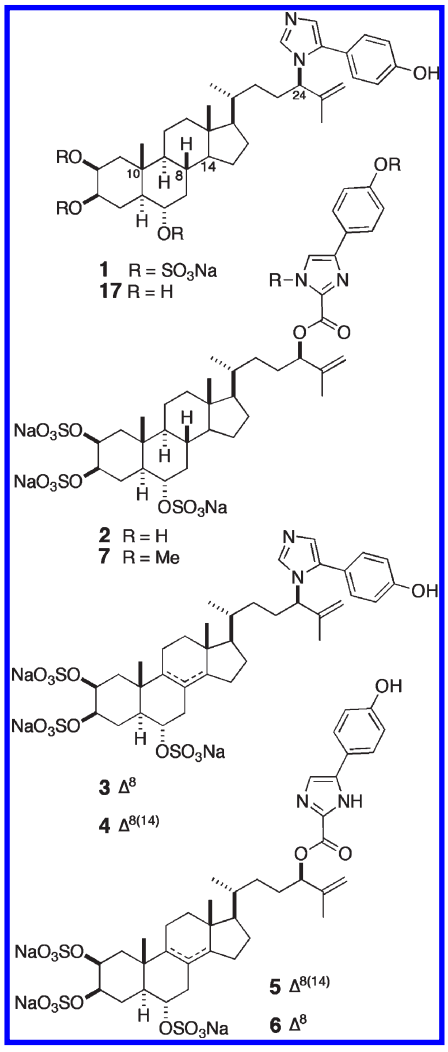


FIGURE 1. Selected gHMBC correlations and ^{13}C NMR chemical shifts for amaranzole B (**2**).

(5) Molinski, T. F. *Nat. Prod. Rep.* **2010**, *27*, 321−329.

those of **1**; however, significant differences were observed for the side chain in the vicinity of the C24 substituent compared to **1**. The H24 methine signal (δ 5.39, dd, J = 8.0, 5.6 Hz) of **2** was significantly deshielded from that of **1** (δ 4.48, m) indicating a change in the electronic environment.



Compound **2** showed only one sp^2 heteroaromatic ^1H signal (δ 7.41 ppm, Table 1) suggesting substitution or oxidation at C28 or C29 of the imidazole ring. In addition, the H24 methine signal in **2** was shifted downfield compared to that of **1** (δ 4.48, m). Coupled HSQC of **2** showed the $^1J_{CH}$ of the single heteroaromatic $^1H-^{13}C$ couplet (δ 7.41 ppm, ^1J = 187.8 Hz) was smaller than that characteristically associated with H2−C2 of five-membered ring azole heteroaromatics (^1J ~200 Hz). For example, *N*-methylimidazole shows coupling constants of ^1J = 205.7 Hz and ^1J = 187.9 Hz for the H2−C2 and H4−C4 spin pairs, respectively.[6] Thus, the heteroaromatic singlet (δ 7.41, s) was assigned to C29, and the (C=O)O group was placed at C28 (the depicted imidazole tautomer is arbitrary). Although direct observation of the C=O signal (δ 158.8, s) by ^{13}C NMR was not possible due to insufficient signal-to-noise in the mass-limited sample of **2**, the signal was observed in HMBC (Figure 1).

(6) Pachler, K. G. R.; Pachter, R.; Wessels, P. L. *Org. Magn. Reson.* **1981**, *17*, 278–284.

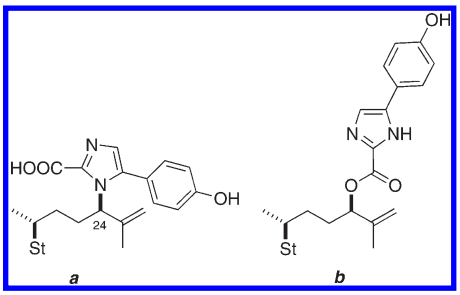


FIGURE 2. Two constitutional isomers of steroidal imidazole-2-carboxylate, **2**.

Two isomeric constitutional formulas (Figure 2) are possible for the proposed 4-(*p*-hydroxyphenyl)imidazole-2-carboxyl-substituted sterol: ***a***, a carboxylic acid with a C24−*N* linkage in which the imidazole is bonded to the side chain through a nitrogen atom and carboxylic ester ***b*** with a C24−*O* linkage. Both chemical and spectroscopic evidence pointed to ***b*** as follows.

The deshielded ^1H NMR signal for H24 in **2** (δ 5.39, dd, J = 8.0, 5.6 Hz, 1H) is more consistent with an esterified C24 carbinol than the CH−N bond of H24 in **1** (δ 4.48, m, 1H).[3] Imidazole-2-carboxylic acids are prone to spontaneous decarboxylation under neutral or acidic conditions,[7] even at room temperature, however, prolonged heating of **2** in pyridine or DMSO (>120 °C) failed to expel CO_2 and returned only starting material or small amounts of desulfated analogues of **2** (^1H NMR). This evidence suggested that the structure of amaranzole B could not contain substructure ***a*** but was an ester including substructure ***b***. Evidence for the latter was provided by a HMBC (Figure 2) which showed a weak cross peak between H24 and the carbonyl signal (δ 158.8, s, (C=O)O). The latter is consistent with the carboxyl chemical shifts of known 2-carboxyimidazoles (e.g, ethyl 1-methylimidazole-2-carboxylate, δ 159.3 ppm).[8] The structure **2** was confirmed in the present work by direct comparisons of the natural product with model compounds of defined constitution and absolute configuration prepared by asymmetric synthesis (see below).

The molecular formula of amaranzole C (**3**), $C_{36}H_{47}N_2Na_3O_{13}S_3$, derived from negative-ion HRESIMS data (m/z 857.2036 $[M - Na]^-$ Δmmu +0.6 amu), is two hydrogen atoms less than the molecular formula of **1** suggesting a dehydro analogue. This was confirmed by analysis of the ^1H and 2D NMR of **3** which revealed a tetrasubstituted C8,9 C=C double bond in the sterol core. ^1H chemical shifts and coupling constants of **3** showed the substitution as well as relative stereochemistry were identical to **1** at positions H1−H6; however, the ^1H NMR signals for H7 (δ 2.10 and 2.73), H11 (δ 2.12), and H14 (δ 2.07 ppm) in amaranzole C (**3**) were shifted downfield with respect to those of **1** (H7; δ 1.01 and 2.36 ppm; H11: δ 1.31 and 1.51 ppm; H14: δ 1.06 ppm). The location of the double bond at C8, C9 in **3** was secured by a gHMBC correlation from Me-19 to C9.[9] Comparison of the ^1H NMR signals for allylic protons of the Δ^8 sterol pentasulfate ester, hamigerol B,[10] gave a good

(7) Krowicki, K.; Lown, J. W. *J. Org. Chem.* **1987**, *52*, 3493–3501.
(8) Baird, E. E.; Dervan, P. B. *J. Am. Chem. Soc.* **1996**, *118*, 6141–6146.
(9) Me-19 gave a strong gHMBC correlation to a C9 (δ 137.3, s).
(10) Chang, J.-F.; Lee, J. −S.; Sun, F.; Jares-Erijman, E. A.; Cross, S.; Rinehart, K. L. *J. Nat. Prod.* **2007**, *70*, 1195–1199.

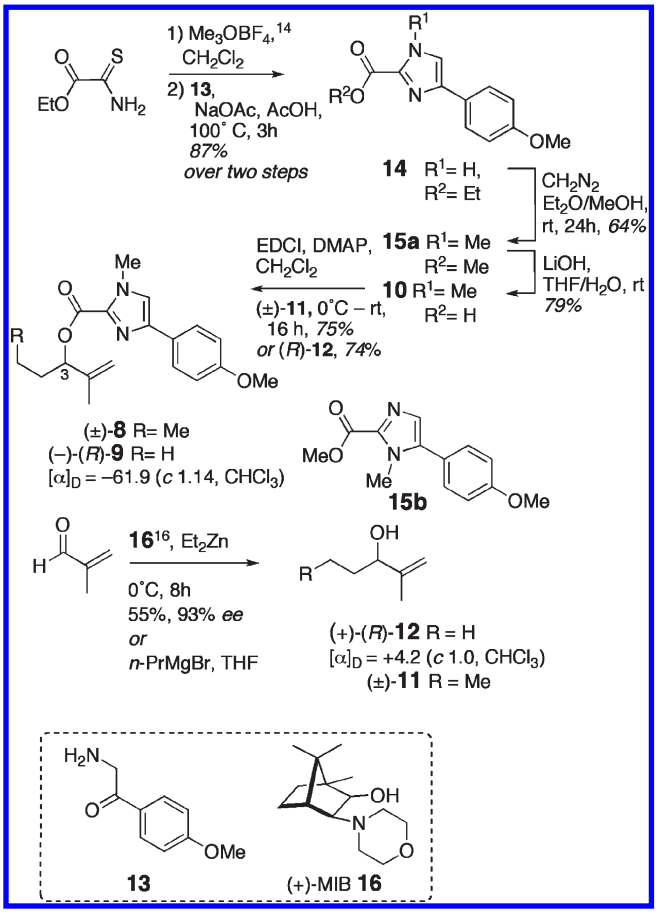
match with **3**. The side-chain linkage of **3** was shown to be of the C24-*N* type found in **1** by similarity of ^{1}H and ^{13}C NMR signals for H24 and C24.

Amaranzole D (**4**), $C_{36}H_{47}N_2N_3O_{13}S_3$, was isomeric with amaranzole C (**3**). ^{1}H and 2D NMR evidence showed that the C=C double bond in **4** was isomerized to C8,C14. Allylic proton signals H9 (δ 1.71, m, 1H) and H15 (δ 2.22, m; 2.29, m) were cross-correlated (gHMBC) to sp^2 carbons C8 (δ 125.0, d), and C14 (δ 146.3, d). Additional gHMBC cross peaks were observed from H9 to C8 and from C18 methyl group to C14. Amaranzole E (**5**) was the 8,14-dehydro derivative of amaranzole B (**2**) based on HRESITOFMS (*m*/*z* 901.1934, $[M - Na]^-$ Δmmu = 0) and nearly identical ^{1}H and ^{13}C chemical shifts for the sterol core of **4**. Similarly, amaranzole F (**6**) (*m*/*z* 901.1948 $[M - Na]^-$ Δmmu = +1.4) was assigned as the 8,9-dehydro derivative of amaranzole B (**2**) and comparison of ^{1}H, ^{13}C NMR data with amaranzole C (**3**).

Since there were no precedents for the C24−N and C24−O variants that constitute the two subfamilies of amaranzoles, we elected to determine the stereochemistry of **2** and ascertain the relationship to **1**. The *absolute* configuration of stereocenters within the sterol cores in **1**−**6** are known with certainty from firm principles of sterol biosynthesis, but the C24 stereocenter in **1** was more difficult to assign because it is located three bonds removed from the nearest stereogenic center, C21. To solve the C24 stereocenter in **1**,[3] we exploited Cotton effects observable in the CD spectrum that arise from exciton coupling of π−π* transitions of the 4-(*p*-hydroxyphenyl)imidazolyl group and the terminal vinylidene group.[11] However, the significant bond reorganizations in the side-chains of **2**, **5**, and **6** with respect to **1** required an independent solution to the configuration of C24 in the new compounds. We turned to chiroptical comparisons of **2** with a different synthetic analog of defined configuration.

In order to simplify comparative CD analyses, **2** was converted to the *N*,*O*-dimethyl compound **7** with excess CH_2N_2. Synthetic *N*,*O*-dimethyl analogues (±)-**8** and (−)-*R*-**9** were prepared by coupling (EDCI, DMAP) of the acid-sensitive imidazole-2-carboxylic acid **10** with the corresponding allylic alcohols (±)-**11** and (+)-**12** which were made available as follows. Methylation of ethyl thiooxamate[12,13] with Meerwein's salt[14] (Scheme 1) followed by condensation with 2-amino-1-(4-methoxyphenyl)ethanone (**13**)[15] gave imidazolecarboxylate ester **14**. Methylation of **14** (CH_2N_2, Et_2O−MeOH) proceeded with concomitant transesterification to afford a mixture of methyl esters. The major product **15a** (64%) was separated from the minor regioisomer **15b** (32%) by silica chromatography. Base

SCHEME 1



hydrolysis of **15a** provided imidazolecarboxylic acid **10**. The latter compound was prone to spontaneous decarboxylation at acidic pHs, even at room temperature, and required careful handling.

Addition of *n*-propylmagnesium bromide to methacrolein gave racemic allylic alcohol (±)-**11**, while the optically enriched homologue, (+)-*R*-**12**, was obtained by asymmetric addition of diethylzinc to methacrolein in the presence of (2*R*)-(+)-3-*exo*-*N*-morpholinoisoborneol (**16**, (+)-MIB).[16] The optical purity and assignment of configuration of (+)-*R*-**12** (93% ee) were secured by the modified Mosher's method[17] after conversion of (+)-*R*-**12** to the corresponding (*R*)- and (*S*)-MTPA esters.

Racemic (±)-**8** and (−)-*R*-**9** displayed NMR chemical shifts (Figure 3), close to those of **2** and **7**, particularly H3 (H24 steroid numbering, δ 5.45, dd, J = 8.0, 5.6 Hz, 1H and 5.36, t, J = 6.8 Hz, 1H, respectively), but significantly different from those of **1** (δ 4.48, m).[3]

As reported previously,[3,11] the dominant feature in the CD spectrum of **1** and, by extension, **2** and its dimethyl homologue **7**, is associated with a π−π* transition of imidazole chromophore; the sign and magnitude of this Cotton effect are dependent upon the configuration at C24. The CD spectra of **7** and (−)-*R*-**9** (Figure 4) showed the same weak

(11) Morinaka, B. I.; Molinski, T. F. *Chirality* **2008**, *20*, 1066–1070.
(12) Scheibye, S.; El-Barbary, A. A.; Lawesson, S.-O. *Tetrahedron* **1982**, *38*, 3753–3760.
(13) (a) Oliver, J. E.; Sonnet, P. E. *J. Org. Chem.* **1973**, *38*, 1437–1438. (b) Yamanaka, H.; Mizugaki, M.; Sakamoto, T.; Sagi, M.; Nakagawa, Y.; Takayama, H.; Ishibashi, M.; Miyazaki, H. *Chem. Pharm. Bull.* **1983**, *31*, 4549–4553.
(14) (a) Meerwein, H.; Hinz, G.; Hofmann, P.; Kroning, E.; Pfeil, E. *J. Prakt. Chem.* **1937**, *147*, 257–285. (b) Meerwein, H.; Bettenberg, E.; Gold, H.; Pfeil, E.; Willfang, G. *J. Prakt. Chem.* **1940**, *154*, 83–156.
(15) Holub, J. M.; O'Toole-Colin, K.; Getzel, A.; Argenti, A.; Evans, M. A.; Smith, D. C.; Dalglish, G. A.; Rifat, S.; Wilson, D. L.; Taylor, B. M.; Miott, U.; Glersaye, J.; Lam, K. S.; McCranor, B. J.; Berkowitz, J. D.; Miller, R. B.; Lukens, J. R.; Krumpe, K.; Gupton, J. T.; Burnham, B. S. *Molecules* **2004**, *9*, 135–157.
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(17) Kusumi, T.; Hamada, T.; Ishitsuka, M. O.; Ohtani, I.; Kakisawa, H. *J. Org. Chem.* **1992**, *57*, 1033–1035.

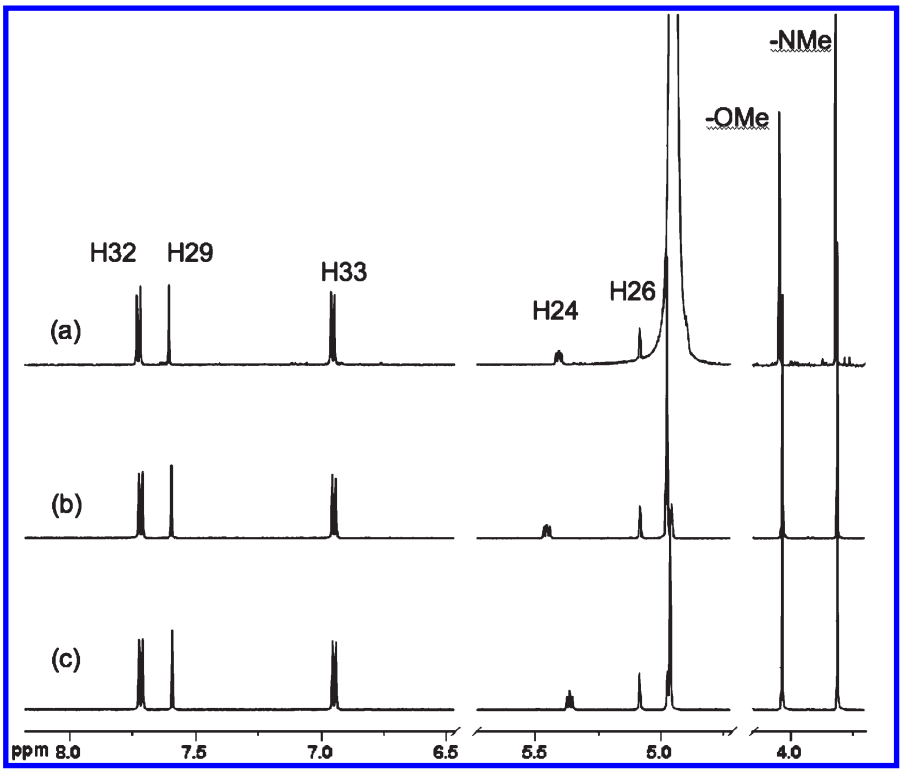

FIGURE 3. ^{1}H NMR (CD_3OD, 600 MHz) of (a) **7**, (b) (±)-**8**, and (c) (−)-**9**.

Cotton effect at λ 221 nm ($\Delta\varepsilon$ −2.8 and $\Delta\varepsilon$ −2.9, respectively); therefore, **2** and **7** have 24*R* configuration.

The new compounds, together with **1**, derive from a polar fraction that exhibits significant feeding deterrence in assays using the blue head wrasse, *Thalasoma bifasciatum*, a common predatory reef fish. We chose to test whether amaranzoles were responsible for this activity. Two mixtures of amaranzoles, one containing **1**, **3**, and **4**, and the other containing **2**, **5**, and **6**, were tested in fish feeding assays using a protocol we reported previously.[1] The mixture containing **1**, **3**, and **4** elicited deterrent activity when tested at 16x natural concentration. We conclude that the amaranzoles are minor contributors to the chemical defense of *P. amaranthus* against *T. bifasciatum*.[18]

The structures of **1**−**6** suggest the involvement of a common intermediate in their biosynthesis. Formation of the *p*-hydroxyphenylimidazole side chain may commence by coupling of an allylic alcohol (steroid side chain) and a suitable imidazole precursor, for example, hamigeramine isolated from the marine sponge *Hamigera hamigera*.[19] Oxidative modification at C24 of the side chain is evident in the two structural subfamilies represented by amaranzoles A−F; however, the biosynthesis of **1**, **3**, and **4** clearly diverges from that of **2**, **5**, and **6**. Because all six natural products occur together in the sponge consistently, it is most likely their biosynthesis links the C24-*N* and C24-*O* subfamilies through a common pathway. One

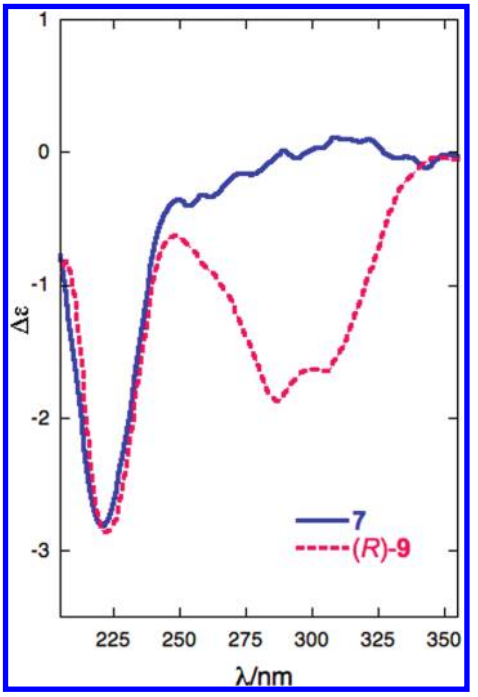

FIGURE 4. CD spectra of *N*,*O*-dimethylamaranzole B (**7**) and model compound (−)-*R*-**9** (MeOH, 23 °C).

possibility (Figure 5) invokes two allylic rearrangements of imidazole **2**: to give the primary allylic ester ***i*** that further rearranges to the transient imidazolinium-2-carboxylic acid ***ii*** with formation of a C−N bond. Facile loss of CO_2 from ***ii*** would give a relatively stable carbene ***iii*** (4-substituted imidazole-2-ylidene) which undergoes 1,2-hydride

(18) The deterrent compounds, including amaroxocanes A and B segregated into a different polar extract prepared from *P. amaranthus* and are the subjects of an earlier report. Morinaka, B. I.; Pawlik, J. R.; Molinski, T. F. *J. Nat. Prod.* **2009**, *72*, 259–264.

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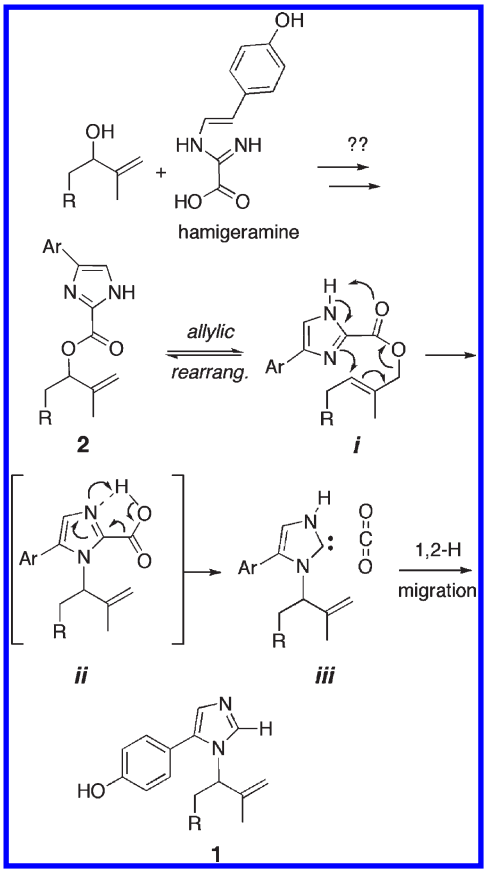

FIGURE 5. Possible biosynthetic pathway linking amaranzoles A (**1**) and B (**2**).

migration[20] to restore the aromatic imidazole ring of **1**.[21] The enthalpic cost of replacing the stronger C−O bond with a C−N bond would be paid in part by the energetically favorable loss of CO_2.

Finally, it is noteworthy that the 24*R* configuration is retained in all amaranzoles. If the allylic alcohol precursor to **5** (Figure 5) is formed with high stereochemical fidelity, the subsequent interconversion of the two subfamilies of amaranzoles must proceed through a tightly orchestrated, enantiospecific mechanism with retention of configuration.

Although insufficient amounts of amaranzoles B−F (**2**−**6**) were available for assay of biological activity, a brief evaluation cytotoxicity of amaranzole A (**1**) and the less-polar analogue **17** (obtained by acid hydrolysis of **1**; 3 M HCl−MeOH, 75 °C) toward human colon tumor cells (HCT-116) was carried out. Compound **17** exhibited significant cytotoxicity (IC_{50} = 4.4 μg/mL); however, the natural product **1** was essentially inactive (IC_{50} > 32 μg/mL). Presumably, cell permeability of **1** is restricted by the highly charged sulfate groups, which is relaxed in the more lipophilic parent molecule **17**.

(20) The carbene, 2,3-dihydroimidazole-2-ylidene, is calculated to be 26.3 kcal·mol^{-1} higher in energy than its isomer, imidazole. (a) Maier, G.; Endres, J. *Eur. J. Chem.* **1998**, 1571–1520. (b) Freeman, F.; Lau, D. J.; Patel, A. R.; Pavia, P. R.; Willey, J. D. *J. Phys. Chem. A* **2008**, *112*, 8775–8784.

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Conclusions

We have isolated and structurally characterized five new amaranzoles B−F (**2**−**6**) that belong to two related families with different side-chain bond constitutions incorporating *p*-hydroxyphenylimidazole. Structure elucidation of **2**, **5**, and **6** revealed an unexpected C−O bond at C24 instead of the C−N imidazole found in the other amaranzoles A (**1**), C (**3**), and D (**4**). The two amaranzole families may be linked through allylic rearrangements that interchange C24−N and C24−O bonds with concomitant loss of CO_2. The polar sulfated **1** showed no activity against HCT-116 tumor cells; however, the more liphophilic derivative **17** was cytotoxic (IC_{50} = 4.4 μg/mL).

Experimental Section

General Experimental Procedures. ^{1}H and 2D NMR spectra were acquired using a 600 MHz NMR spectrometer equipped with a $\{^{13}C\}^1H$ 1.7 mm microcryoprobe or 500 MHz spectrometers $\{^{13}C\}^1H$ and $\{^1H\}^{13}C$ probes. All ^{1}H spectra were acquired in MeOH-d_4 and referenced to δ 3.31 ppm. UV spectra were recorded on a double-beam spectrophotometer. Optical rotations were measured using a digital polarimeter. Circular dichroism (CD) measurements were acquired on a grating spectropolarimeter using dilute solutions in spectroscopic-grade solvent and quartz cells (1 mm path length). IR spectra were obtained using an FTIR spectrometer equipped with a ZnSe ATR plate. HPLC was carried out using a dual-pump preparative instrument equipped with a high-dynamic range UV−vis detector set to λ 260 nm. For semipreparative HPLC, an RP column (5 μm C_{18}-bonded silica, 10 × 250 mm) was used. Preparative HPLC was carried on radial compression cartridges (6 μm C_{18}-bonded silica 25 × 100 mm) and commercial HPLC grade solvents were used for liquid chromatography. THF, CH_2Cl_2, and DMF were dried by passage through dual-alumina cartridges under an atmosphere of Ar, and reagent-grade chemicals were used as purchased.

Animal Material. Extraction and Isolation. *P. amaranthus* Duchassaing & Michelotti 1864 was collected in November 2006 using scuba from Dry Rocks Reef, Key Largo, Florida (25°7.850′ N, 80°17.521′ W), at a depth of 20−25′. Specimens were identified by J.R.P and stored at −20 °C until required. Vouchers samples are archived at UCSD. Two specimens (06-04-004a and 06-04-004b) of *P. amaranthus* collected at the same site were analyzed separately. The first sample (06-04-004a, 88.3 g) was lyophilized, extracted with water (1 L), followed by MeOH (1 L), and finally CH_2Cl_2 (1 L). The organic extracts were combined, dried, and partitioned between hexane and H_2O/MeOH 1:9. The hexane layer ("A") was separated, and the MeOH layer was adjusted to 1:1 by addition of water and then partitioned against CH_2Cl_2. The CH_2Cl_2 layer ("B") was separated from the aqueous MeOH layer ("C"), and the solvents were removed under reduced pressure. The crude MeOH extract C (33.5 g) was subjected to filtration through reversed-phase silica (C_{18} cartridge, conditioned with H_2O/MeOH 19:1) eluting with water and then MeOH/CH_3CN 1:1 to give two fractions. The second fraction (7.03 g, 1.46 g further purified) was then subject to reversed-phase chromatography (C_{18} cartridge) using a stepwise gradient (H_2O/MeOH 4:1, 7:3, 3:2, 1:1, 2:3, 3:7, 1:4, 1:9, then MeOH) to attain nine fractions. A portion (42.5 mg) of the fraction (127.3 mg) eluting with H_2O/MeOH (3:2) was subject to gradient semipreparative reversed-phase HPLC (flow rate: 2.5 mL/min; mobile phase: H_2O/CH_3CN containing 0.5 M $NaClO_4$; gradient: 7:3 isocratic 15 min to 2:3 over 30 min; λ = 254 nm) to give five fractions. The third fraction contained amaranzole A (4.2 mg, **1**). The fourth fraction was subjected to

gradient semipreparative reversed-phase HPLC (same conditions as above, except mobile phase: H_2O/CH_3CN containing 0.75 M $NaClO_4$) to give amaranzole B (1.2 mg, **2**).

A second lyophilized sample of *P. amaranthus* (06-04-004b, 131.2 g) was extracted with 1 L of H_2O/MeOH 1:1 (23 °C, overnight). The aqueous−MeOH phase was removed, and extraction was repeated twice, first with 1 L H_2O/MeOH 1:1 and then 1 L MeOH. The sponge tissue was then blended at high speed and extracted once more with 1 L of MeOH (23 °C, overnight), and the combined extracts were concentrated under reduced pressure. The remaining sponge tissue was extracted with CH_2Cl_2 (2 × 1 L). The CH_2Cl_2 extract was dried and partitioned between hexane and H_2O/MeOH 1:9. The aqueous MeOH layer was dried and combined with the previous MeOH extracts to give a crude aqueous MeOH extract (48.6 g). A portion (33.9 g) of the aqueous MeOH crude extract was filtered through a reversed-phase cartridge (C_{18}, conditioned with H_2O/MeOH 19:1) eluting with H_2O/MeOH 9:1, H_2O/MeOH 1:9, and *i*-PrOH to give three fractions. A portion (1.16 g) of the second eluting fraction (5.82 g) was subjected to gradient preparative reversed-phase HPLC (flow rate: 25 mL/min; mobile phase: H_2O/CH_3CN containing 1.5 M $NaClO_4$; gradient: 73:27 isocratic 10 min to 23:77 over 30 min; $\lambda = 240$ nm) to give seven fractions. A portion (4.6 mg) of the third fraction (61.6 mg) was subjected to a repeated gradient reversed-phase HPLC (flow rate: 2.5 mL/min; mobile phase: H_2O/CH_3CN containing 0.5 M $NaClO_4$; gradient: 7:3 isocratic 15 min to 2:3 over 30 min; λ = 254 nm) to give amaranzole C (0.19 mg, **3**), amaranzole D (0.32 mg, **4**), and amaranzole A (0.35 mg, **1**). A portion (1.4 mg) of the fourth fraction (8.4 mg) was subjected to repeated gradient reversed phase C_{18} HPLC (H_2O/CH_3CN containing 1.5 M $NaClO_4$, flow rate: 2.5 mL/min; gradient: 61:39 isocratic 15 min to 31:69 over 30 min; λ = 260 nm) to give amaranzole F (0.10 mg, **6**), amaranzole E (0.19 mg, **5**), and amaranzole B (0.15 mg, **2**).

Amaranzole B (2): colorless glass; $[\alpha]^{21}_D$ +21.8 (*c* 0.52, MeOH); FTIR (ATR, ZnSe) ν_{max} 3487, 2945, 1652, 1449, 1232, 1065, 1001, 915, 839 cm^{-1}; UV (H_2O-CH_3CN 7:3) λ_{max} 256 nm (7800), 289 (9300), 310 (8000); CD (H_2O-CH_3CN 7:3) 218 nm ($\Delta\varepsilon$ −1.0); ^1H NMR, see Table 1; ^{13}C NMR (from 600 MHz, HSQC/gHMBC data, CD_3OD) δ 160.2 (C34), 158.8 (C35), 144.3 (C25), 142.2 (C30), 138.4 (C28), 127.5 (C32), 122.5 (C31), 120.6 (C29), 116.9 (C33), 113.3 (C26), 79.6 (C24), 78.5 (C3), 77.9 (C6), 76.2 (C2), 56.9 (C17), 55.2 (C9), 51.6 (C5), 43.4 (C13), 42.2 (C1), 40.6 (C12), 39.6 (C7), 37.8 (C10), 36.1 (C20), 34.8 (C8), 32.3 (C22), 29.9 (C14), 29.9 (C23), 28.5 (C16), 25.4 (C4), 24.3 (C15), 21.8 (C11), 18.6 (C21), 17.9 (C27), 15.6 (C19), 11.8 (C18); HRESITOFMS *m/z* 903.2108 $[M - Na]^-$ calcd for $C_{37}H_{49}N_2Na_2O_{15}S_3$, 903.2090.

Amaranzole C (3): colorless glass; FTIR (ATR, ZnSe) ν_{max} 3454, 2947, 1631, 1230, 1110, 1002, 949 cm^{-1}; UV (H_2O/CH_3CN 7:3) λ_{max} 250 nm (ε 8300); CD (H_2O-CH_3CN 7:3) 201 nm ($\Delta\varepsilon$ +3.5), 217 ($\Delta\varepsilon$ −8.0); ^1H NMR, see Table 1; HRESITOFMS *m/z* 857.2036 $[M - Na]^-$ calcd for $C_{36}H_{47}N_2Na_2O_{13}S_3$, 857.2030.

Amaranzole D (4): colorless glass; FTIR (ATR, ZnSe) ν_{max} 3487, 2951, 1651, 1450, 1327, 1230, 1072, 1001, 964, 916, 840 cm^{-1}; UV (H_2O-CH_3CN 7:3) λ_{max} 250 nm (ε 8300); CD (H_2O-CH_3CN 7:3) λ 201 nm ($\Delta\varepsilon$ +4.7), 215 ($\Delta\varepsilon$ −1.9); ^1H NMR, see Table 1; HRESITOFMS *m/z* 857.2051 $[M - Na]^-$ calcd for $C_{36}H_{47}N_2Na_2O_{13}S_3$, 857.2030.

Amaranzole E (5): colorless glass; FTIR (ATR, ZnSe) ν_{max} 3559, 2952, 1651, 1453, 1386, 1232, 1113, 1001, 918, 843 cm^{-1}; ^1H NMR, see Table 1; HRESITOFMS *m/z* 901.1941 $[M - Na]^-$ calcd for $C_{37}H_{47}N_2Na_2O_{15}S_3$, 901.1934.

Amaranzole F (6): colorless glass; FTIR (ATR, ZnSe) ν_{max} 3523, 2948, 1651, 1451, 1233, 1069, 1002, 915, 841 cm^{-1}; HRESITOFMS *m/z* 901.1948 $[M - Na]^-$ calcd for $C_{37}H_{47}N_2Na_2O_{15}S_3$, 901.1934).

***N,O*-Dimethylamaranzole B (7).** Excess ethereal diazomethane was added dropwise to a mixture of amaranzoles B, E, and F (∼1:1:1, 1.5 mg) in 0.5 mL of MeOH. The mixture was stirred at room temperature for 1.5 h. The solvent was evaporated under a stream of N_2. The mixture was subject to three rounds of reversed-phase HPLC; first with a gradient of 10−100% (CH_3CN/H_2O + 1 M $NaClO_4$, over 40 min) followed by two passages with 50−100% (CH_3CN/H_20 + 1 M $NaClO_4$ over 40 min) to give **7** (ca. 30 μg, based on comparison of UV to (−)-**9**): UV (MeOH) λ_{max} 261 nm (ε 16200), 286 nm (ε 15400), 309 nm (ε 8600); CD (MeOH) 221 nm ($\Delta\varepsilon$ −2.8); ^1H and ^{13}C NMR chemical shifts for the steroidal ABCD ring system are nearly identical to those of amaranzole B (**1**); ^1H NMR (600 MHz, CD_3OD) δ 7.73 (d, 2H, 8.8 Hz), 7.61 (s, 1H), 6.95 (d, *J* = 8.8 Hz, 2H), 5.41 (dd, *J* = 8.0, 5.4 Hz, 1H), 5.08 (m, 1H), 4.90 (m, 1H), 4.05 (s, 3H), 3.82 (s, 3H), 1.84 (s, 3H); ^{13}C NMR (150 MHz, CD_3OD, partial data) δ 160.2 (C34), 144.2 (C25), 142.6 (C30), 137.2 (C28), 127.4 (C32), 126.6 (C31), 123.4 (C29), 114.7 (C33), 113.6 (C26), 79.9 (C24), 55.4 (OMe), 36.5 (NMe).

Ethyl 4-(4-Methoxyphenyl)-1*H*-imidazole-2-carboxylate (14). Trimethyloxonium tetrafluoroborate (3.31 g, 22.4 mmol) was added to a stirred solution of ethyl thiooxamate[12] (2.59 g, 19.4 mmol) in CH_2Cl_2 (60 mL) in three portions over 1 h at room temperature. After the mixture was stirred for an additional 30 min, the solvent was evaporated and the crude product used immediately in the next step. Sodium acetate (3.18 g, 38.8 mmol), 2-amino-1-(4-methoxyphenyl)ethanone (**13**, 6.5 g, 19.3 mmol), and acetic acid (60 mL) were added, and the mixture was stirred for 3 h at 100 °C. The mixture was allowed to cool to room temperature, and the solvent was removed under reduced pressure. Water (400 mL) was added, and the mixture was extracted with ethyl acetate (3 × 100 mL). The combined organic extracts were dried ($MgSO_4$), and the solvent was evaporated. The crude product was recrystallized from MeOH/H_2O to give pure **14** (4.18 g, 87% yield): mp 132−134 °C; FTIR (ATR, ZnSe) ν_{max} 2981, 2938, 2837, 1715, 1615, 1479, 1458, 1441, 1379, 1288, 1248, 1178, 1150, 1131, 1027, 795 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$ + 0.1% TFA-*d*) δ 7.67 (d, *J* = 8.4 Hz, 2H), 7.42 (s, 1H), 6.92 (d, *J* = 8.4 Hz, 2H), 4.42 (q, *J* = 7.2 Hz, 2H), 1.38 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$+0.1% TFA-*d*) δ 159.6 (C), 158.6 (C), 139.5 (C), 136.8 (C), 126.9 (CH), 122.9 (C), 120.4 (CH), 114.2 (CH), 62.2 (CH_2), 55.2 (CH_3), 14.0 (CH_3); HRESITOFMS *m/z* 247.1079 $[M + H]^+$ (calcd for $C_{13}H_{15}N_2O_3$, 247.1077)

Methyl 4-(4-Methoxyphenyl)-1-methyl-1*H*-imidazole-2-carboxylate (15a). A solution of ethereal diazomethane (∼0.2 M) was added dropwise to a stirred solution of **5** (100 mg, 0.406 mmol) in MeOH/Et_2O (8:3, 5.5 mL) at room temperature. When a yellow color persisted, excess diazomethane (∼10 equiv) was added, and the reaction was stirred for 24 h. The solvent was evaporated under a stream of nitrogen, and the mixture was subjected to silica flash chromatography (1:4 EtOAc/hexanes) to give **15a** (64 mg, 64% yield) and **15b** (34 mg, 34% yield).

Methyl 4-(4-Methoxyphenyl)-1-methyl-1*H*-imidazole-2-carboxylate (15a): FTIR (ATR, ZnSe) ν_{max} 2952, 2838, 1709, 1613, 1443, 1419, 1248, 1179, 1151, 1116, 1047, 1024, 930, 832 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.72 (d, 2H, 8.8 Hz), 7.23 (s, 1H), 6.91 (d, 2H, 8.8 Hz), 4.04 (s, 3H), 3.96 (s, 3H), 3.82 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.6 (C), 161.2 (C), 143.9 (C), 138.0 (C), 128.5 (CH), 127.7 (C), 123.4 (CH), 115.9 (CH), 57.2 (CH_3), 54.3 (CH_3), 38.0 (CH_3); HRESITOFMS *m/z* 247.1080 $[M + H]^+$ (calcd for $C_{13}H_{15}N_2O_3$, 247.1077).

Methyl 5-(4-Methoxyphenyl)-1-methyl-1*H*-imidazole-2-carboxylate 15b: FTIR (ATR, ZnSe) ν_{max} 2952, 1711, 1453, 1290, 1252, 1201, 1129, 1029, 949, 839, 789 cm^{-1}; ^1H NMR (400 MHz,

$CDCl_3$) δ 7.30 (d, J = 8.0 Hz, 2H), 7.14 (s, 1H), 6.98 (d, J = 8.0 Hz, 2H), 3.94 (s, 3H), 3.91 (s, 3H), 3.85 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 160.1 (C), 159.9 (C), 138.5 (C), 136.7 (C), 130.6 (CH), 128.5 (CH), 120.8 (C), 114.3 (CH), 55.3 (CH_3), 52.1 (CH_3), 33.7 (CH_3); HRESITOFMS m/z 247.1076 $[M + H]^+$ (calcd for $C_{13}H_{15}N_2O_3$, 247.1077).

4-(4-Methoxyphenyl)-1-methyl-1*H*-imidazole-2-carboxylic Acid (10). A solution of LiOH (10.4 mg, 0.248 mmol) in THF/H_2O (8:3, 3.2 mL) was added to ester **15a** (43 mg, 0.165 mmol), and the mixture was stirred overnight at room temperature. The THF was evaporated under reduced pressure, and the solution was cooled to 0 °C. Aqueous HCl (0.1 M) was added dropwise until pH ~4, at which point the free acid precipitated as a white solid. The mixture was centrifuged, the supernatant removed, and the solid washed with H_2O (2 mL) and dried under high vacuum to give the free acid **10** (30.3 mg, 79%) as a colorless solid. **10**: FTIR (ATR, ZnSe) ν_{max} 2909, 2834, 1659, 1327, 1270, 1254, 1023, 910, 811, 796 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.81 (s), 7.72 (d, J = 8.8 Hz, 2H), 6.97 (d, J = 8.8 Hz, 2H), 3.95 (s, 3H), 3.77 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 159.3 (C), 158.7 (C), 138.3 (C), 137.5 (C), 126.0 (CH), 125.1 (C), 121.8 (CH), 114.1 (CH), 55.1 (CH_3), 35.7 (CH_3); HRESITOFMS m/z 233.0923 $[M + H]^+$ (calcd for $C_{12}H_{13}N_2O_3$, 233.0921).

2-Methylhex-1-en-3-yl 4-(4-Methoxyphenyl)-1-methyl-1*H*-imidazole-2-carboxylate (8). A solution of EDCI (30.0 mg, 0.156 mmol) in CH_2Cl_2 (3 mL) was added dropwise to a stirred solution of the **10** (21.1 mg, 0.091 mmol), (±)-2-methylhex-1-en-3-ol[22] (26 mg, 0.228 mmol), and DMAP (2.4 mg, 0.020 mmol) in CH_2Cl_2 (2 mL) at 0 °C. The mixture was stirred for an additional 2 h and then at room temperature for 18 h before removal of solvent under reduced pressure and purification of the residue by silica flash chromatography (15:85 EtOAc/hexanes) to give the ester (±)-**8** (22.1 mg, 74% yield): FTIR (ATR, ZnSe) ν_{max} 2958, 1707, 1450, 1281, 1262, 1123, 1105, 836, 786 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.73 (d, J = 8.8 Hz, 2H), 7.20 (s, 1H), 6.91 (d, J = 8.8 Hz, 2H), 5.43 (t, 1H, 6.8 Hz), 5.09 (m, 1H), 4.95 (m, 1H), 4.00 (s, 3H), 3.82 (s, 3H), 1.93−1.85 (m, 1H), 1.83 (s, 3H), 1.79−1.70 (m, 1H), 1.50−1.34 (m, 2H), 0.96 (t, J = 7.2 Hz, 3H); ^{1}H NMR (400 MHz, CD_3OD) δ 7.70 (d, J = 8.8 Hz, 2H), 7.55 (s, 1H), 6.93 (d, J = 8.8 Hz, 2H), 5.45 (dd, J = 8.0, 5.6 Hz, 1H), 5.08 (m, 1H), 4.95 (m, 1H), 4.00 (s, 3H), 3.80 (s, 3H), 1.93−1.85 (m, 1H), 1.83 (s, 3H), 1.80−1.71 (m, 1H), 1.50−1.32 (m, 2H), 0.98 (t, J = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 160.8 (C), 159.4 (C), 144.7 (C), 143.0 (C), 137.7 (C), 127.7 (CH), 127.0 (C), 123.7 (CH), 115.0 (CH), 113.8 (CH_2), 79.8 (CH), 55.7 (CH_3), 36.7 (CH_3), 35.9 (CH_2), 19.9 (CH_2), 18.4 (CH_3), 14.1 (CH_3); HRESITOFMS m/z 329.1866 $[M+H]^+$ (calcd for $C_{19}H_{25}N_2O_3$, 329.1860).

(*R*)-2-Methylpent-1-en-3-yl 4-(4-Methoxyphenyl)-1-methyl-1*H*-imidazole-2-carboxylate (−)-9. A solution of EDCI (32.0 mg, 0.166 mmol) in CH_2Cl_2 (4 mL) was added dropwise to a mixture of **7** (32.0 mg, 0.138 mmol), (*R*)-2-methylpent-1-en-3-ol (27.6 mg, 0.276 mmol), and DMAP (1.7 mg, 0.014 mmol) in CH_2Cl_2 (1 mL) at 0 °C with stirring. The mixture was stirred at 0 °C for 2 h and then at room temperature for 18 h. The solvent was removed under reduced pressure and the residue subjected to silica flash chromatography (15:85 EtOAc/hexanes) then reversed-phase HPLC (C_{18}, 3:1 CH_3CN/H_2O) to give the ester (−)-**9** (32.5 mg, 75% yield): $[\alpha]^{24}$ −61.9 (*c* 1.14, $CHCl_3$); FTIR (ATR, ZnSe) ν_{max} 2966, 1705, 1505, 1448, 1400, 1246, 1120, 1089, 1030, 949, 903, 835, 794 cm^{-1}; UV (MeOH) λ_{max} 261 nm (ε 16200), 286 nm (ε 15200), 309 nm (ε 8600); CD (MeOH) λ 222 nm ($\Delta\varepsilon$ −2.9), 287 ($\Delta\varepsilon$ −1.9), 306 ($\Delta\varepsilon$ −1.6); ^{1}H NMR (400 MHz, CD_3OD) δ 7.71 (d, J = 8.8 Hz, 2H), 7.57 (s, 1H), 6.94 (d, J = 8.8 Hz, 2H), 5.36 (t, J = 6.8 Hz, 1H), 5.09 (m, 1H), 4.97 (m, 1H), 4.03 (s, 3H), 3.81 (s, 3H), 1.95−1.80 (m, 2H), 1.83 (s, 3H), 0.98 (t, J = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 160.8 (C), 159.4 (C), 144.3 (C), 143.0 (C), 137.7 (C), 127.7 (CH), 127.0 (C), 123.7 (CH), 115.0 (CH), 114.0 (CH_2), 81.5 (CH), 55.7 (CH_3), 36.7 (CH_3), 26.7 (CH_2), 18.4 (CH_3), 10.4 (CH_3); HRESITOFMS m/z 315.1703 $[M+H]^+$ (calcd for $C_{18}H_{23}N_2O_3$, 315.1703)

(*R*)-2-Methylpent-1-en-3-ol (+)-12. Diethylzinc (8 mmol, 1 M in hexanes) was added to a stirred solution of (+)-MIB (48.5 mg, 0.2 mmol) in hexanes (20 mL) at 0 °C. Freshly distilled methacrolein (280 mg, 4 mmol) was added dropwise to the reaction and stirred for 8 h at 0 °C. The reaction was quenched with saturated ammonium chloride (40 mL) and extracted with pentane (3 × 100 mL). The combined organic layers were dried by filtration through $MgSO_4$, and filtered, and the solvent evaporated under reduced pressure at 0 °C. The mixture was subjected to silica flash chromatography (1:9 diethyl ether/pentane) to give (+)-**12** (220 mg, 55% yield, 93% ee): $[\alpha]^{24}_D$ = +4.2 (*c* 1.0, $CHCl_3$) [lit.[22b] +4.1 (CH_2Cl_2, 90% ee)]; (−)-**12**, $[\alpha]^{24}_D$ = −5.6 (*c* 1.0, $CHCl_3$, >98% ee).[22a] The % ee was determined by ^{1}H NMR analysis of the both (+) and (−)-MTPA esters. ^{1}H and ^{13}C NMR data were identical to literature values.[22]

Desulfation of Amaranzole A (1). A solution of amaranzole A (**1**, 1 mg) was heated in 3 M HCl (MeOH−H_2O, 2 mL) at 75 °C for 45 min. The cooled solution was concentrated and passed through a SiO_2 cartridge, which was which was eluted with MeOH−CH_2Cl_2 to give the desulfated compound **17** (quant): ^{1}H NMR (500 MHz, CD_3OD) (selected) δ 3.93 (brq, J = 4.0 Hz, H2), 3.50 (dt, J = 10.9, 4.0 Hz, H3), 3.39 (td, J = 10.9, 4.6 Hz, H6), 1.72 (s, H27), 1.00 (s, H19), 0.92 (d, J = 6.9 Hz, H21), 0.63 (s, H18). The remainder of the ^{1}H NMR signals were essentially identical to those of **1**. HRESITOFMS: m/z 577.3996 $[M + H]^+$, calcd for $C_{36}H_{53}N_2O_4$ 577.4005.

Cytotoxicity Assay. Amaranzole A (**1**) and the des-sulfato derivative **17** were evaluated for in vivo cytotoxicity against cultured human colon tumor cells (HTC-116) using a cell viability assayed based on a colorimetric end point of the soluble formazan dye from coincubated MTS [(3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium, inner salt] as described elsewhere.[23] Amaranzole A (**1**) was inactive (IC_{50} >32 μg/mL), and **17** gave an IC_{50} of 4.4 μg/mL.

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Supporting Information Available: ^{1}H, ^{13}C, and 2D NMR spectra of **2**−**6** and synthetic compounds **8**−**10**, **14**, **15a**,**b**, and **17**. This material is available free of charge via the Internet at http://pubs.acs.org.

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