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Article

Bromo-spiroisoxazoline Alkaloids, Including an Isoserine Peptide, from the Caribbean Marine Sponge *Aplysina lacunosa*

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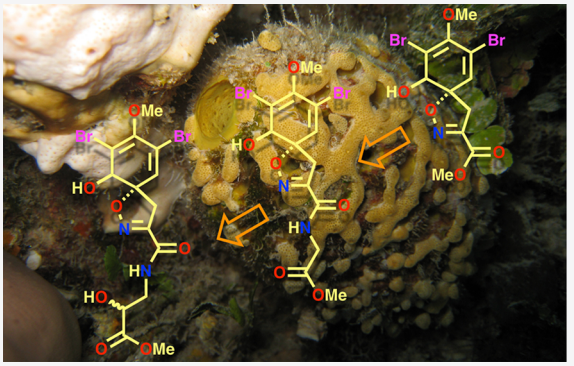
ABSTRACT: Three new bromotyrosine spiroisoxazoline alkaloids, lacunosins A and B (**1** and **2**) and desaminopurealin (**3**), were isolated from a MeOH extract of the marine sponge *Aplysina lacunosa* that showed modest α-chymotrypsin inhibitory activity. The structures of **1**−**3** share the spirocyclohexadienyl-isoxazoline ring system found in purealidin-R and several other Verongid sponge secondary metabolites. Compounds **1** and **2** are coupled to a glycine and an isoserine methyl ester, respectively. Alkaloid **3** is linked, contiguously, to an *O*-1-aminopropyl 3,5-dibromotyrosyl ether and, finally, to histamine through an amide bond. The planar structures of all three compounds were obtained from analysis of MS and 1D and 2D NMR data. The absolute configuration of the SIO unit of **1**−**3** was assigned by electronic circular dichroism (ECD). The isoserine amino acid residue in **2** was found to be a 1:1 mixture of epimers using a new Marfey's type reagent, derived from Trp-NH_2. Allylic *O*-naphthoylation of the SIO subunit enhances the ECD spectrum of SIOs and improves discrimination of enantiomorphs. A unifying hypothesis is proposed that links the biosynthesis of several of the new compounds with previously reported analogues.



Among the earliest marine natural products reported are alkaloids and peptides containing modified bromotyrosine residues from marine sponges (Porifera), mainly in the order Verongida. Approximately 300 bromotyrosine alkaloids of marine origin have been reported in the literature to date.[1,2] The formidable oxidative brominating capacity of Verongid sponges is manifested in not only secondary metabolites but also high levels of bromotyrosine amino acid residues in the structural proteins (chitin) of *Ianthella basta*[3] and *Aplysina cavernicola*.[4] Bromotyrosine secondary metabolites from marine sponges show a variety of biological properties, including antibacterial,[5] anti-inflammatory,[6] antineoplastic,[7] and antifungal activities that clearly demonstrate bio- and chemodiverse repertoires. A remarkable report by Berlinck and co-workers shows that several bromotyrosine-derived natural products, conventionally associated with Verongid sponges, are produced by fermentation of a Verongid sponge-derived bacterium, *Pseudovibrio denitrificans* Ab134.[8]

In screening for protease inhibitors, we found the MeOH extract of the marine sponge *Aplysina lacunosa* from the Bahamas exhibited significant inhibition of α-chymotrypsin activity. Here we report the structures of three new brominated spiroisoxazoline (SIO) alkaloidal peptides, lacunosins A (**1**) and B(**2**) and desaminopurealin (**3**), from an assay-guided survey of extracts with α-chymotrypsin inhibitory activity. The structure of alkaloid **2** includes a rare isoserine residue.

RESULTS AND DISCUSSION

Extracts of the sponge *A. lacunosa* were found to induce 100% inhibition of α-chymotrypsin at 50 $\mu g \cdot mL^{-1}$. The total MeOH extract of *A. lacunosa* was separated by progressive solvent partitioning into four fractions, A−D, and α-chymotrypsin inhibitory activity was found to partition into the CH_2Cl_2/MeOH-soluble "B fraction". Further purification of the latter by size exclusion chromatography further segregated activity into mid-eluting and late-eluting fractions (Figure 1). HPLC and examination by LCMS and ^1H NMR delivered three new bromotyrosine-derived metabolites (**1**−**3**) in addition to the known compounds aeroplysinin-1,[9] aerophobin-1,[10] aerophobin-2,[11] purealidin-N,[12] purealin,[13] methyl ester **4a**,[14] and aplysinin-B[7a] (for structures, see Supporting Information). MS analysis of **1** and **2** showed a $[M + Na]^+$ of 474.9113 and 504.9215, corresponding to molecular formulas of $C_{13}H_{14}Br_2N_2O_6$ and $C_{14}H_{16}Br_2N_2O_7$, respectively. The isotopic patterns of the sodium adduct ions confirmed the presence of two Br atoms in each of **1** and **2**. Additionally, FTIR absorptions at ∼3400 and 1680−1670 cm^{-1} revealed the

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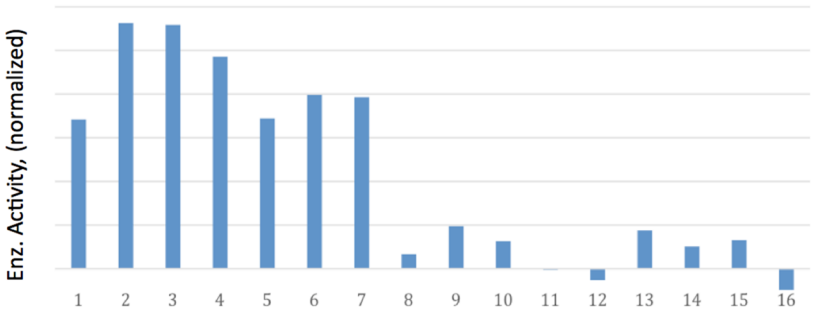

Figure 1. α-Chymotrypsin inhibition by fractions from size-exclusion chromatography (Sephadex LH-20, MeOH elution) of *Aplysina lacunosa* solvent-partitioned extract. See Experimental Section.

Table 1. ^{1}H and ^{13}C NMR Data for 1−3 in CD_3OD

	δ_C, type			δ_H (int., mult, *J*, Hz)		
no.	**1**[a,b]	**2**[a]	**3**[a]	**1**[c]	**2**[d]	**3**[d]
1	75.2, CH	74.0, CH	74.1, CH	4.08, s	4.11, s	4.08, s
2	113.9, C	112.6, C	112.9, C			
3	148.7, C	147.8, C	147.7, C			
4	122.1, C	121.3, C	121.3, C			
5	132.3, CH	130.6, CH	130.8, CH	6.41, s	6.443 [6.437[c]], s	6.42, s
6	91.9, C	91.0, C	90.9, C			
7	39.9, CH_2	38.7, CH_2	38.7, CH_2	3.76, d (18.0)	3.79, d (18.6)	3.78, d (18.6)
				3.09, d (18.0)	3.11, d (18.6)	3.10, d (18.6)
8	154.7, C	153.4, C	153.8, C			
9	160.3, C	160.2, C	160.2, C			
10	41.3, CH_2	42.5, CH_2	36.6, CH_2	4.01, s	3.600 [3.589[e]], dd (13.0, 6.0, 4.8)	3.58, t (6.9)
					3.600 [3.589[e]], dd (13.0, 6.0, 4.8)	
11	170.4, C	69.1, CH	29.2, CH_2		4.34, dd (6.0, 4.8)	2.10, pent (6.6)
12		172.8	70.8, CH_2			4.04, t (6.0)
13			151.2, C			
14			117.5, C			
14′			117.5, C			
15			133.1, CH			7.46, s
15′			133.1, CH			7.46, s
16			136.2, C			
17			27.4, CH_2			3.81, s
18			150.6, C			
19			164.2, C			
20			37.6, CH_2			3.56, t (6.7)
21			24.4, CH_2			2.93, t (6.7)
22			133.1, C			
23			116.1, CH			7.30, s
24			133.3, CH			8.77, s ($^{1}J_{CH}$ = 218 Hz[f])
3-OMe	60.2, CH_3	58.9, CH_3	59.0, CH_3	3.71, s	3.75, s	3.72, s
11-OMe	52.3, CH_3			3.72, s		
12-OMe		51.3, CH_3			3.77, s	

[a] ^{13}C chemical shifts were obtained indirectly from HSQC and HMBC data. [b] 125 MHz, $(CD_3)_2CO$. [c] 500 MHz. [d] 600 MHz. [e] Signal for the C-11 epimer. See text for discussion of multiplet complexity and Figure 3b for NMR simulation. [f] From "^{13}C satellite" measurements.

presence of OH/NH and amide carbonyl groups, respectively. Comparison of the ^{1}H and ^{13}C NMR data of **1** and **2** (Table 1) with reported literature values for (+)-purealidin-R[12] (**4b**, Figure 2) demonstrated that both **1** and **2** contained the SIO moiety with different side chains attached to the amide NH.

The ^{1}H and ^{13}C NMR spectra of **1** showed the characteristic features of the *O*-Me-spiroisoxazoline ring system common to purealidin-R (**4b**) and many other bromotyrosine-derived natural products from Verongid sponges. Additionally, the ^{1}H and HSQC spectra of **1** revealed an extra C-11 OMe group (δ_H 3.72, s; δ_C 52.3) and the deshielded methylene CH_2-10 (δ_H 4.01, 2H, s; δ_C 41.3). The C-9 signal (δ_C 170.4) in **1** was assigned to a carboxamide which was linked, sequentially by HMBC correlations, to the deshielded CH_2-10 (δ_H 4.02, 2H) and the terminal methyl ester group at δ_H 3.72, therefore constituting a Gly-*O*Me residue. Compound **1** is the *N*-Gly-*O*Me-extended derivative of **4b**.

The ^{1}H and HSQC spectra of **2** indicated a diastereotopic CH_2 group (δ_H 3.64, m and 3.60, m) with a complex multiplet structure (see Figure 3 and discussion, below), an *O*-CH-11

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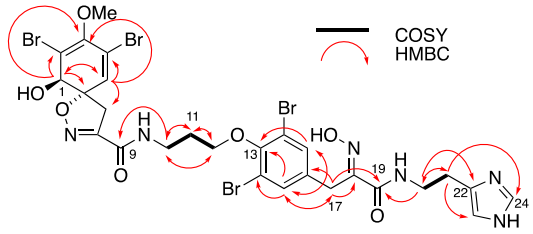
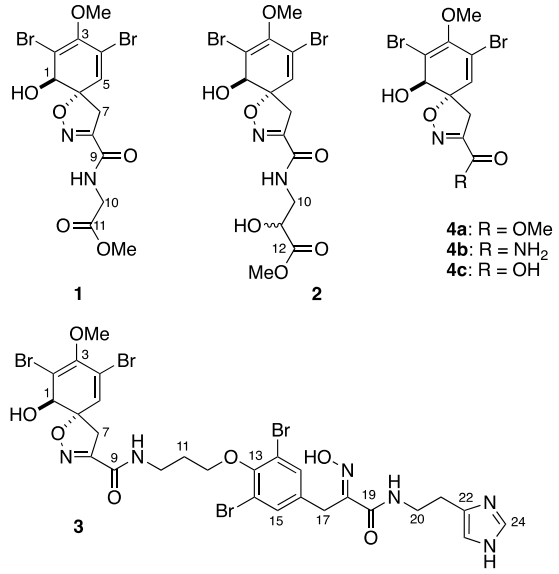


Figure 2. COSY and HMBC correlations of (−)-**3** (600 MHz, CD_3OD).



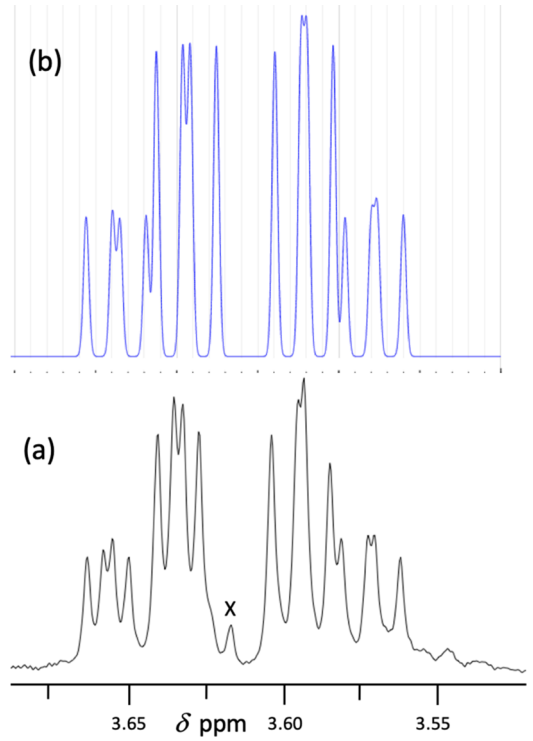


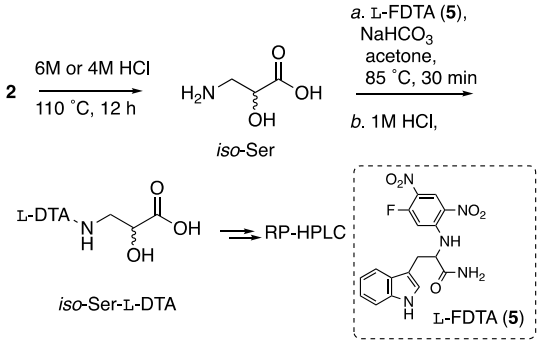
Figure 3. (a) ^{1}H NMR spectra of the diastereotopic 10-CH_2 of **2** (600 MHz, CD_3OD) and (b) simulated spectrum of ABX. Line width, 1.15 Hz, δ_A 3.589, J = 13.0, 6.0, 4.8 Hz; δ_B 3.644, J = 13.0, 6.0, 4.8 Hz. Epimer: δ_A 3.600, J = 13.0, 6.0, 4.8 Hz; δ_B 3.652, J = 13.0, 6.0, 4.8 Hz (δ_X is not shown). Ref 20.

(δ_H 4.34, dd, 6.0, 4.8), and the 13-MeO signal (δ_H 3.75, s; δ_C 58.9) in addition to the anticipated SIO ring. A COSY cross-peak between the CH_2-10 and CH-11 signals, together with HMBC correlations between CH_2-10 and C-9/C-11/C-12, between CH-11 and C-11/C-12, and between C-13-MeO and the carbonyl C-12 (δ_C 172.8) suggest **2** comprises an SIO joined to an isoserine (*iso*Ser) methyl ester residue through an amide bond.

Compound (−)-**3**, with the formula $C_{27}H_{28}Br_4N_6O_7$, is the desamino analogue of purealin, a potent inhibitor of myosin and dynein ATPase isolated from an Okinawan specimen of *Psammaplysilla purea*.[13,14b] The ^{1}H and ^{13}C NMR spectra of **3** were almost identical with those of purealin;[13] the major differences were the presence of an imidazolyl ^{1}H NMR signal H-23 (δ_H 8.77, s, $^1J_{CH}$ = 218 Hz) and other minor chemical shift changes near the histamine group. Full ^{1}H and ^{13}C NMR assignment of **3** was secured through DQF-COSY, HSQC, and HMBC (Figure 2). HMBC correlations were observed from H-21 (δ_H 2.93, 2H, t, 6.7) to C-23 (δ_C 116.1) and C-24 (δ_C 133.3). As with almost all natural products containing α-ketoxime-bromotyrosine amides, (−)-**3** has the *E*-oxime configuration.[15] The low specific rotation ($[\alpha]_D$ −11) and weak Cotton effect [λ 253 ($\Delta\varepsilon$ +0.8), 284 (+0.8)] of (−)-**3** suggest this sample is nearly racemic (see below).

The configuration of the *iso*Ser in **2** was addressed by a variant of Marfey's method.[16] Total hydrolysis of **2** (6 M HCl, 110 °C, 16 h), followed by treatment of the residue with our newly described L-FDTA reagent (**5**, Scheme 1)[17] gave DTA

Scheme 1. Hydrolysis of 2 and Derivatization of *iso*Ser by L-FDTA (5)



derivatives with superior resolutions on reversed-phase HPLC.[18] Unexpectedly, two peaks corresponding to L-*iso*Ser-L-DTA (t_R = 13.63 min) and D-*iso*Ser-L-DTA (t_R = 13.94 min) were observed, suggesting the *iso*Ser residue in **2** was a mixture of epimers. When the hydrolysis was repeated under milder conditions (4 M HCl, 110 °C, 12 h), followed by derivatization by L-FDTA, HPLC revealed the same mixture. To exclude the possibility of epimerization during acid hydrolysis of **2**, authentic L-*iso*Ser was subjected to the latter conditions of hydrolysis, and upon L-FDTA derivatization and HPLC analysis, a single peak was obtained corresponding to L-*iso*Ser-L-DTA. Consequently, the above results confirm that **2** is a mixture of C-11 epimers (1:1).

Given the results above, the complexity of the ^{1}H NMR spectrum of **2**, particularly the 10-CH_2 multiplet structure, could now be interpreted in a different, consistent manner. From inspection of the structure of **2**, the expected multiplet pattern of NH−CH_2−CH(OH)− (Figure 3a) should be that

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of an ABX system (the couplings of OH and NH signals are absent due to deuterium exchange in CD_3OD: see DQF-COSY, 600 MHz, Figure S6). The CH_2-10 signal should, therefore, consist of a maximum of eight lines; yet clearly 16 lines are present. The most likely interpretation is that the ^1H NMR signals for CH_2-10 of the epimeric mixture **2** are two overlapping eight-line spin systems—the AB parts of two ABX spin systems—with virtually the same vicinal and geminal J values, but each slightly offset in chemical shift by different amounts (on average, $\Delta\delta$ ~0.01, or 6.6 Hz at 600 MHz).[19] Because the nearest stereocenter C-6 is six bonds away, the 10-CH_2 J-splitting pattern would be expected to be similar for each epimer, dictated only by local torsional parameters, solvation, and hydrogen bonding. The simulated ^1H NMR spectrum[20] (Figure 3b) supports these conclusions. In short, the deceptive spectral complexity of the 10-CH_2 ^1H NMR signal of **2** arises from overlapping spin systems of diastereomers.[21]

The absolute configurations of the brominated cyclohexadienyl-SIOs were originally assigned by Rinehart and co-workers, who correlated the ECD spectrum and X-ray crystal structure of the degenerate skewed 1,3-dienes in (+)-aerothionin,[22,23] based on earlier studies of aeroplysinin-1 by Fulmor and co-workers[24] and pioneering chiroptical studies of skewed dienes by Moscowitz[25] and others.[26] The 1,3-diene in the canonical SIO structure, verongidoic acid (**4c**), deviates from planarity by approximately +17°[27] (Figure 4) and is

(a)

#Conf.	Energy, E (kJ.mol^{-1})	Torsional Angle, θ^a (deg°)
6	6.6	8.9
5	6.4	7.3
4	5.7	8.0
3	1.4	-13.9
2	1.1	17.6
1	0.0	17.2^b

a. θ *(H-1–C-1–C-6–H6).* b. θ *'(H -1–C-1–C-6–H6) = 102.2*

(b)

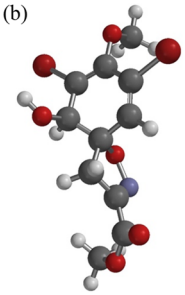

Figure 4. Energy-minimized conformers (MMFF) of (1*S*,6*R*)-verongidoic acid methyl ester (**4a**). (a) The six lowest E conformers. (b) Model of the lowest E conformer (iSpartan).

expected to manifest a positive Cotton effect (CE). In fact, two positive CEs are observed in the C_2 dimer, (+)-aerothionin (Table 2): λ 245 nm ($\Delta\varepsilon$ +23.7) and 284 (+21.4).[23] The optical rotations of the commonly configured (1*R*,6*S*)-SIOs are also consistently dextrorotatory (Table 2). Similar trends are observed in other brominated skewed 1,3-cyclohexadienes, (−)-aeroplysillin-1 (**S5**) and aplysinafulvin (**S8**), which lack an SIO ring system. The ECD spectra of **1**−**3** (Figure S14) each showed the same positive CEs, albeit of lower magnitudes than (+)-aerothionin. The strongest CEs were those of **1** [λ 253 ($\Delta\varepsilon$ +5.2), 284 (+3.6)] and the weakest, those of **3**. It can be concluded that the dominant chirality of the SIO ring in **1**−**3** is (1*R*,6*S*), as depicted, but **3** is nearly racemic. Further chiroptical determination of the %ee of **1**−**3** is impeded by lack of reliable standard SIOs of known %ee.[28]

Concern was raised about the relatively lower magnitude CEs observed in **1**−**3**. Given that $\Delta\varepsilon$ is a molar quantity, the magnitude of the CE should be relatively invariant across all similar SIOs that lack additional stereogenic centers,[29] yet this is not the case. For example, the magnitudes of the CE at λ ~250 nm observed in samples of fistularin-3 (**S2**) range from $\Delta\varepsilon$ +16 to +32.1 (Table 2). We were left to consider the possibility that **1**−**3** are not homochiral; that is, the SIO ring system is formed with imperfect stereofidelity.[30] There is precedence for this phenomenon; for example, ianthesine-A (**S14**) and -B−D, from an Australian *Ianthella* sp.,[31] purealidin B (**S15**) from an Okinawan *Psammaplysilla purea*,[32] and pseudoceratinines A and C (**S16** and **S17**) from *P. verrucosa*[33] have SIO rings of the antipodal (1*S*,6*R*) configuration. Capon and co-workers isolated (±)-purealin (**S18**) from Southern Australian specimens of *Pseudoceratina* spp. and convincingly demonstrated that coisolated (−)-pseudoceratinine A (**S16**) and (−)-aerophobin-2 were partial racemates after conversion (*R*-MTPA, DCC) to a mixture of their respective diastereomeric *R*-Mosher's esters (dr 2:1).[34] From a sample of *P. verrucosa* collected in Dampier, Western Australia, Karuso and co-workers found the (−)-enantiomer of (+)-purpuroceratic acid B (**8b**)[35,36] along with other antipodal SIO compounds.[37] Finally, the curious case of **4c** offers an even more interesting study: The EC of **4c** (Table 2) is of lower magnitude and *opposite in sign* to that of fistularin-3 (**S2**), both isolated from the *same specimen* of *Pseudoceratina* sp. from the Bahamas.[38]

The 1,3-diene twist of SIOs gives rise to a modest CE, which becomes difficult to detect in "near racemic" samples. In order to enhance the ECD signal in SIOs and provide an independent indicator of chirality, we exploited the useful cyclic allylic benzoate method developed by Nakanishi and co-workers.[39] The exciton coupling (EC) observed between the two nondegenerate chromophores—a benzoyloxy group and the adjacent C=C bond−gives rise to a split Cotton effect, of which the sign of the longer wavelength component is determined reliably from the helicity. When extended to acyclic 1,3-dienyl naphthoate esters, the magnitude of the EC is enhanced significantly. From energy-minimized models of **4a** (Figure 4), it is apparent the diene helicity (θ (H-1−C-1−C-6−H6 = 17.2°) and allylic helicity (θ (H-1−C-1−C-6−H6) = 102.2°) are of the same sign. We reasoned that the superposition of CEs arising from helicities of the 1,3-diene and 1,3-dienyl naphthoate moieties would reinforce the magnitude of the CE.

Treatment of synthetic (+)-**4a**[14c] with 6-methoxy-2-naphthoyl chloride (**6**)[40] (Et_3N, DMAP, CH_2Cl_2, Scheme 2) gave naphthoate ester (−)-**7**. The ECD spectrum of naphthoate ester (−)-**7** (Figure 5) showed additional complexity not seen in the ECD spectrum of the parent SIO including a red-shifted positive Cotton effect (λ 310 nm, $\Delta\varepsilon$ +7.6) corresponding to the forbidden *R*-band ($n \rightarrow \pi^*$) of the naphthoate chromophore. Although the magnitude of the CE in (−)-**7** is no greater than native SIOs, the "fingerprint" CE of similar 6-methoxy-2-naphthoate esters may be useful for fine-scale chiroptical analyses where longer wavelength features are preferable.

Brominated SIOs arise from bromination−oxidation of tyrosine, as shown by Rinehart and co-workers using ^{14}C-labeling.[47] Much speculation has appeared on the biosynthesis of the SIO and subsequent transformation, the consensus being that the SIO ring system arises from oxidation of a 3,5-Br_2Tyr-α-ketoxime to an 1,2-arene epoxide followed by intramolecular S_N2 attack−ring opening by the C=NOH group. The dominance of (+)-(1*R*,6*S*)-SIO stereoisomers in Nature attests to *re*-facial selectivity of the putative arene epoxidase, with the less common (−)-(1*S*,6*R*) enantiomorphs arising from variant enzymes exhibiting preferential *si*-facial

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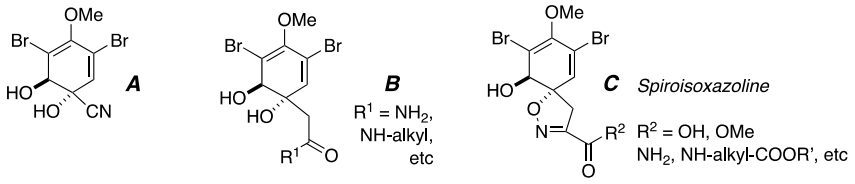
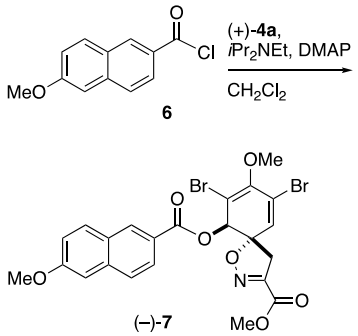
Table 2. Chiroptical Data ($[\alpha]_D$, ECD) for Three Chemotypes (*A*−*C*) of Dibromocyclohexa-1,3-dienes



compound	type	origin	species	$[\alpha]_D$	λ/nm ($\Delta\varepsilon$)[a]	λ/nm ($\Delta\varepsilon$)[a]	ref
1	*C*	Bahamas	*Ap. lacunosa*[b]	+71.8	253 (+5.2)	284 (+3.6)	[c]
2	*C*	Bahamas	*Ap. lacunosa*[b]	+32	253 (+3.1)	284 (+2.8)	[c]
3	*C*	Bahamas	*Ap. lacunosa*[b]	−11	253 (+0.8)	289 (+0.8)	[c]
(+)-aerothionin (**S1**)	*C*	Mexico	*Ap. fistularis*[d]	+210[e]	245 (+23.7)	284 (+21.4)	22
fistularin-3 (**S2**)	*C*	Belize	*Ai. crassa*[f]		273 (+32.1)	287 (+27.6)	5c
araplysillin III (**S3b**)	*C*	Belize	*Ai. crassa*[f]	+96.3[g]			5c
hexadellin C (**S4**)	*C*	Belize	*Ai. crassa*[f]	+102[h]			5c
(−)-aeroplysinin-1 (**S5**)	*A*	Caribbean	*Ia. ardis*[ij]	−198[k]	282 (−15)		24
(+)-aeroplysinin-1 (**S6**)	*A*	Bahamas	*Ia.* sp.	+182[k]			24
"verongidoic acid" (**4c**)	*C*	Bahamas	*Ps. crassa*[li]		245 (−9.1)	284 (−6.4)	48
S2	*C*	Virgin Islands	*Ap.* f. *fulva*[m]	+104.2[p]			41
S2	*C*	Bahamas	*Ps. crassa*[i,l]		245 (+16)	284 (+15)	48
S2	*C*	Brazil	*Ap. cauliformis*[o]		252 (+19.7)	286 (+19.7)	52
S2	*C*	Bahamas	'*Ap. fulva*'[p]		251 (+19.0)	285 (+17.8)	52
11-*epi*-fisturalin-3[q] (**S7**)	*C*	Australia	*Ag. oroides*[r]	+65.2[s]	248 (+23.7)	284 (+20.6)	42
aplysinafulvin (**S8**)	*B*	Brazil	*Ap. fulva*[t]	+130[u]	251 (+1.63)	285 (+0.79)	43
(12*S*)-hydroxy-11-oxo-aerothionin (**S9**)	*C*	Bahamas	*Ap. fistularis*[d]	+152.5	250 (+18.2)	288 (+15.2)	44
(12*R*)-hydroxy-11-oxo-aerothionin (**S10**)	*C*	Bahamas	*Ap. fistularis*[d]	+162.7	251 (+18.3)	288 (+15.0)	44
11-hydroxyaerothionin (**S11**)	*C*	Brazil	*Ap. caissara*[v]	+178[w]	248 (+24)	284 (+24)	45
Caissarine C (**S12**)	*C*	Brazil	*Ap. caissara*[v]	+175[x]	248 (+26)	290 (+27)	45
N-sulfatoaraplysillin I (**S13**)	*C*	Bahamas	'*Ap. fulva*'[t]	+100[y]	244 (+8.3)	285 (+8.0)	50
(+)-purpuroceratic acid B (**8b**)	*C*	Bahamas	'*Ap. fulva*'[t]	+140[z]	243 (+7.0)	289 (+5.2)	50
(−)-purpuroceratic acid B (**8b**)		Australia	*Ps. verr*[aa]	−9[ab]			37
ianthesine A (**S14**)[ac]	*C*	Australia	*Ianthella* sp.	−118[ad]	248 (−10.2)	285 (−9.94)	31
purealdin B (**S15**)[ac]	*C*	Japan	*Psa. pur.*[ae]	−4.5[af]	252 (−2.5)	290 (−2.4)	32
pseudoceratinine A (**S16**)[z]	*C*	New Caledonia	*Ps. verr.*[aa]	−158[ag]	248 (−9.66)	290 (−8.48)	33
(−)-purealin (**S18**)[z]	*C*	Japan	*Psa. pur.*[ae]	−85[ah]	245 (−9.51)	284 (−9.15)	13
(±)-purealin (**S18**)	*C*	Australia	*Ps.* sp	[ai]			34
subereamolline A (**S19a**)	*C*	Egypt	*Suberea mollis*	+156.5[aj]			46
subereamolline A (**S19a**)	*C*	Egypt	*Suberea mollis*	+22.9[ak]			46

[a]MeOH. Literature values of molar ellipticities, $[\theta]$, are normalized: $\Delta\varepsilon = 3300[\theta]$. See Supporting Information for the structures **S1**−**S19b**. [b]*Aplysina lacunosa*. [c]This work. See Experimental Section. [d]*Aplysina fistularis*. [e](*c* 1.0, MeOH). [f]*Aiolochroia crassa*. [g](*c* 0.19, MeOH). [h](*c* 0.067, MeOH). [i]Renamed *Aiolochroia crassa*. [j]*Ianthella ardis*. [k](*c* 0.5, acetone). [l]*Pseudoceratina crassa*. [m]*Pseudovibrio denitrificans* Ab134 isolated from the sponge *Arenosclera braziliensis*. [n]*Aplysina* forma fulva. [o]*Aplysina cauliformis*. [p]Originally identified as *Aplysina fulva*, but upon review, it is more likely *Aplysina fistularis*. [q]The configuration at C-11 is variable.[52] [r]*Agelas oroides*. [s](*c* 1.04, acetone). [t]*Aplysina fulva*. [u](*c* 0.002, MeOH). [v]*Aplysina caissara*. [w](*c* 0.0014, MeOH). [x](*c* 0.0021, MeOH). [y](*c* 0.06, MeOH). [z](*c* 0.04, MeOH). [aa]*Pseudoceratina verrucosa*. [ab](*c* 0.4, MeOH). [ac]Less common (1*S*,6*R*) configuration. [ad](*c* 1.02, MeOH). [ae]*Psammaplysilla purea* (likely *P. purpurea*). [af](*c* 1.3, MeOH). [ag](*c* 1, MeOH). [ah](*c* 2.1, MeOH). [ai]No chiroptical data; presumably "0". [aj](*c* 0.55, MeOH). [ak](*c* 6.25, CH_2Cl_2).

Scheme 2. Naphthoate Ester (−)-7 from Acylation of (+)-4a



selectivity or even nonselectivity in the cases of near-racemic SIOs. Another way to view this outcome is imperfect desymmetrization by O atom addition to the aryl ring of the 3,5-Br_2Tyr-α-ketoxime precursor.

In this report, our interest was on the origin of *iso*Ser, a rare nonproteinogenic amino acid observed in **2** and the first time in an SIO. Although it is possible that *iso*Ser derives from an independent, discrete biosynthetic pathway like most other AAs, the *rac*-modification seen in **2** suggests an alternative explanation: a postassembly oxidation of the SIO-containing compound (Scheme 3). Condensation of **4c** (reported from *Pseudoceratina* sp.[48] and as a fermentation product of *Pseudovibrio denitrificans* Ab1348[49] and given the name "verongidoic acid") with β-alanine would give purpuroceratic acid A (**8a**),[36] a likely intermediate also found with

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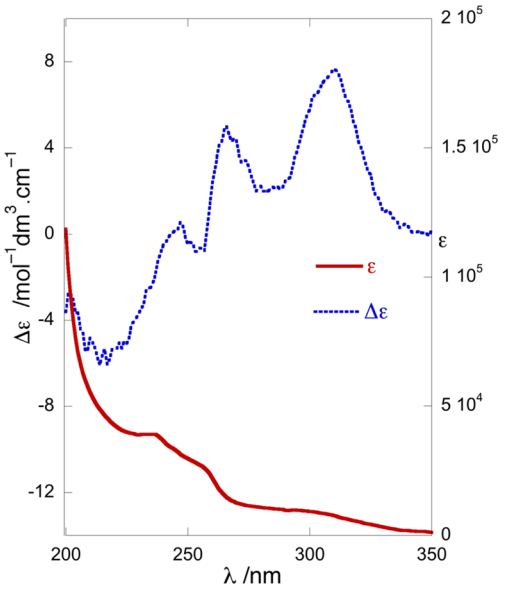
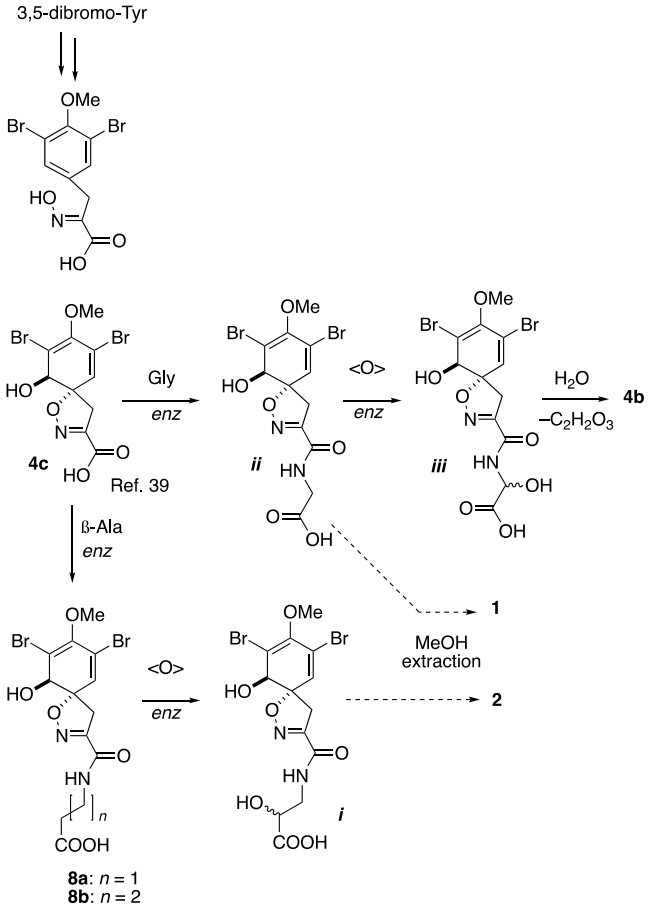


Figure 5. UV−vis and ECD spectra (c 2.49 × 10^{-5} M, MeOH, 23 °C) of 6′-methoxy-2′-naphthoate ester (−)-**7** derived from (+)-**4a**.

Scheme 3. Biosynthetic Hypothesis for 1, 2, and 4b



homologues (e.g., purpuroceratic acid B, **8b**), in at least two other Verongid sponges.[14a,50] Compound **8a** would then suffer stereorandom α-hydroxylation by a monooxygenase enzyme (Scheme 3), giving carboxylic acid ***i***; the corresponding methyl ester **2** arises as an artifact of adventitious Fischer−Speier esterification during extraction with MeOH.[51] Earlier observations of nonspecific hydroxylation of spiroisoxazolines from *Aplysina* sp. support the notion that such "biotransformations" may be commonplace in the biosyntheses of brominated SIOs.[52] Finally, a unifying hypothesis comes to mind. The structure of purealidin-R (**4b**), itself, may arise from a well-known pathway to *C*-terminal primary amides:[53] α-hydroxylation of the terminal Gly residue of ***ii*** (again, **1** arises from ***ii*** by adventitious methylation of ***ii***) to give the carbinolamine ***iii*** followed by hydrolytic loss of glyoxylic acid to give **4b**.

Several peptides with *iso*Ser residues have been shown to be protease inhibitors. For example, a series of synthetic *iso*Ser-containing peptides showed significant inhibition of amino-peptidase-N.[54] Upon assay, none of the new compounds **1**−**3** or the other seven known secondary metabolites from the active extract of *A. lacunosa* showed inhibition of α-chymotrypsin at concentrations up to 25 $\mu g \cdot mL^{-1}$, suggesting the chemical entity responsible for inhibitory activity is a very minor, potent congener that escaped detection or decomposed during purification.

In conclusion, three new brominated spiroisoxazoline alkaloids (**1**−**3**) were isolated from extracts of *Aplysina lacunosa*, and their structures determined by integrated analysis of MS, NMR, and ECD data. The rare amino acid (±)-*iso*Ser was found as a residue in **2**, a 1:1 mixture of epimers. Configurational heterogeneity is noted in spiroisoxazolines, both in ECD and the range of magnitude of the Cotton effects ($\Delta\varepsilon$) and preparation of diastereomeric amides from **4c**. None of the new or known compounds were responsible for activity in the α-chymotrypsin inhibition assay.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured on a JASCO P-2000 polarimeter at the D-double emission line of Na. UV−vis spectra were measured on a JASCO V-630 spectrometer. ECD spectra were measured on a JASCO J-810 spectropolarimeter at 23 °C in quartz cells of 1, 2, or 5 mm path length. FTIR spectra were collected from thin film samples using a JASCO FTIR-4100 fitted with an ATR ZnSe plate. 1D and 2D NMR spectra were measured on a JEOL ECA (500 MHz) spectrometer, equipped with a 5 mm $^1H\{^{13}C\}$ room-temperature probe, or a Bruker Avance III (600 MHz) NMR spectrometer with a 1.7 mm ^1H-$\{^{13}C/^{15}N\}$ microcryoprobe (23 °C). Chemical shifts were referenced to internal solvent or residual ^1H signals ($CDCl_3$, δ_H 7.26 ppm; δ_C 77.16. CD_3OD, δ_H 3.31; δ_C 49.00). LC-MS measurements were performed with a Thermoelectron Surveyor UHPLC coupled to an MSD single-quadrupole detector. HR-ESI-TOF mass spectroscopy analyses were conducted on an Agilent 1200 HPLC connected to an Agilent 6350 TOF-MS at the Small Molecule Mass Spectrometry Facility at the Department of Chemistry and Biochemistry at UCSD. Preparative, semipreparative, and analytical HPLC purifications were carried out on a JASCO system consisting of a UV−vis detector (UV-2075), dual pumps (PU-2086 Plus), and a dynamic mixer (MX-2080-32).

Biological Material. The sponge *Aplysina lacunosa* (11-14-018) was collected in 2011 from Little San Salvador Island in the Bahamas (lat. 24° 35.242′ N, long. 75° 58.495′ W) at a depth of −23 m using scuba and kept frozen (−20 °C) until needed. A type sample (MeOH) is archived in the Department of Chemistry and Biochemistry, UCSD.

Extraction and Isolation. A sample of *A. lacunosa* (11-14-018) was lyophilized (dry wt 24.0 g) and extracted twice with MeOH (2 × 400 mL). The combined, filtered extracts were concentrated under reduced pressure to yield an extract (5.17 g), which was dissolved in $MeOH/H_2O$ (9:1, 400 mL) before repeated partitioning against hexanes (3 × 400 mL) to provide the hexane-soluble "A" fraction (0.2517 g). The $MeOH/H_2O$ solution (6:4, 600 mL) was adjusted to

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40% v/v H_2O, and the mixture repeatedly partitioned against CH_2Cl_2 (2 × 400 mL). The combined CH_2Cl_2 layers were concentrated to deliver the CH_2Cl_2-soluble "B-fraction" (0.8412 g) after removal of volatiles. The MeOH/H_2O layer was concentrated under reduced pressure, and the residual aqueous layer extracted with *n*-BuOH (2 × 300 mL) to yield the *n*-BuOH-soluble "C" layer (1.2460 g) and H_2O-soluble "D" layer (2.8300 g) after removal of volatiles.

The "B-fraction" (0.7342 g) was separated by size-exclusion chromatography (Sephadex LH-20, MeOH), and the eluate grouped into 16 fractions by TLC profiling (9:1 of CH_2Cl_2/MeOH, UV-activity, *p*-anisaldehyde stain). The tenth fraction (18.5 mg) was further purified by reversed-phase preparative HPLC (Phenomenex C_{18} column, 150 × 21.2 mm, 5 μm, under gradient elution: initial conditions 10% CH_3CN/H_2O−0.1% TFA for 3 min to 60% CH_3CN/H_2O−0.1% TFA over 20 min, 13 mL·min^{-1} flow rate) to yield lacunosin A (**1**, 3.2 mg) eluting at t_R = 17.19 min. The 11th fraction (10.0 mg) was also purified by reversed-phase semipreparative HPLC (Phenomenex C_{18} column, 250 × 10 mm, 5 μm, step gradients, initial conditions: 30% CH_3CN/H_2O−0.1% TFA for 5 min to 75% CH_3CN/H_2O−0.1% TFA for an additional 25 min, 2.5 mL·min^{-1} flow rate) to yield 13 fractions. Fraction 6 (0.3 mg) contained lacunosin B (**2**) eluting at t_R = 18.95 min. Fraction 9 (0.2 mg), which eluted at t_R = 21.38 min, was further purified by reverse-phase analytical HPLC (Synergi Hydro-RP column, 150 × 4.6 mm, 4 μm, step gradients, initial conditions 20% CH_3CN/H_2O−0.1% TFA for 3 min to 70% CH_3CN/H_2O−0.1% TFA for 32 min, 0.7 mL·min^{-1} flow rate) to yield desaminopurealin **3** (0.1 mg), which eluted at t_R = 24.50 min. An additional 0.7 mg of compound **3** was purified from the 12th fraction (11.7 mg) of the LH-20 column under similar semi-preparative HPLC conditions as the 11th fraction. Additional repeated HPLC separations delivered aeroplysinin-1,[9] aerophobin-1,[10] aerophobin-2,[11] purealidin-N,[12] methyl ester **4a**,[14a,b] and aplysinin-B[7a] and whose identities were confirmed by MS and ^{1}H NMR.

*Lacunosin A, **1**:* pale yellow solid; $[\alpha]^{23.8}_D$ +71.8 (*c* 1.00, MeOH); ECD (*c* 3.93 × 10^{-4} M, MeOH) λ_{max} (Δε) 214 (−1.8), 227 (0), 253 (+5.08), 282 (+3.7) nm; UV (MeOH) λ_{max} (log ε) 194 (3.48), 230 (3.37), and 282 (3.09); FTIR (ATR, film, ZnSe) ν 3353, 1744, 1671, 1599, 1541, 1438, 1205, 1133, 1047, 1025, 988, 916, 837, 801, 766, and 722 cm^{-1}; ^{1}H and ^{13}C NMR data, Table 1; HRMS (ESI-TOF) *m*/*z* 474.9113 [M + Na$^+$] (calcd for $C_{13}H_{14}Br_2N_2O_6Na^+$, 474.9111).

*Lacunosin A, **2**:* white solid; $[\alpha]^{23.9}_D$ +31.8 (*c* 0.15, MeOH); UV (MeOH) λ_{max} (log ε) 194 (3.68), 225 (3.48), and 281 (3.15) nm; ECD (*c* 3.56 × 10^{-4} M, MeOH) λ_{max} 214 (Δε −1.4), 227 (0), 251 (+3.1), and 282 (+2.7) nm; FTIR (ATR, film, ZnSe) ν 3427, 1685, 1207, 1141, 805, and 724 cm^{-1}; ^{1}H and ^{13}C NMR data, Table 1; HRMS (ESI-TOF) *m*/*z* 504.9215 [M + Na]$^+$ (calcd for $C_{14}H_{16}Br_2N_2O_7Na^+$, 504.9216).

*Desaminopurealin, **3**:* white solid; $[\alpha]^{23.5}_D$ −10.5 (*c* 0.13, MeOH); UV (MeOH) λ_{max} (log ε) 205 (3.88), 221 (4.56), and 275 (3.04) nm; ECD (*c* 4.63 × 10^{-4} M, MeOH) λ_{max} (Δε) 214 (−0.34), 227 (0), 251 (+0.8), and 282 (+0.7); FTIR (ATR, film, ZnSe) ν 3405, 1684, 1207, 1142, 804, and 722 cm^{-1}; ^{1}H and ^{13}C NMR data, Table 1; HRMS (ESI-TOF) *m*/*z* 864.8828 [M + H]$^+$ (calcd for $C_{27}H_{29}Br_4N_6O_7^+$, 864.8826).

*Compound **4a**:* white solid; $[\alpha]^{21.4}_D$ +145 (*c* 0.44, MeOH), lit.[14c] $[\alpha]^{27.9}_D$ +165.5 (*c* 0.325, C_6H_6); ECD (1.07 × 10^{-3} M, MeOH) λ (Δε) 222 (4.48), 252 (12.58), and 289 (−4.83) nm; ^{1}H NMR and HRMS of **4a** were consistent with literature values.[14b,c]

*Acid Hydrolysis of **2**.* A sample of **2** (0.1 mg, 0.2 μmol) was dissolved in 4 M HCl (100 μL, 400 μmol) and stirred in a sealed vial at 110 °C for 12 h. The solution was cooled to room temperature, dried under a stream of N_2, and derivatized with L-FDTA (**5**)[17] to yield the *iso*Ser-DLT derivatives of **2** for LC-MS analysis (see below).

*Absolute Configuration of isoSer in Compound **2**.* To the acid hydrolysate of **2** in H_2O (50 μL) were added 1 M $NaHCO_3$ (50 μL, 50 μmol) and L-FDTA[17] (**5**, 0.25 mg, 0.65 μmol) in acetone (75 μL). The reaction was stirred at 85 °C for 30 min, cooled, and neutralized with 1 M HCl (50 μL, 50 μmol). The derivatized hydrolysate (20 μL) was diluted with MeOH (80 μL), centrifuged, and analyzed by LC-MS (Hypersil Gold, C_{18} column, 50 × 2.1 mm, 1.9 μm, linear gradient, initial conditions 15−45% CH_3CN/H_2O−0.1% HCOOH over 25 min, 0.5 mL·min^{-1} flow rate). L-DTA derivatives of authentic standards of L- and DL-*iso*Ser eluted gave peaks at t_R = 13.63 and 13.94 min, respectively. The LC-MS analysis of the L-DTA derivative of the acid hydrolysate of **2** gave both L-*iso*Ser-L-DTA and D-*iso*Ser-L-DTA (ratio ∼1:1).

*6′-Methoxy-2′-naphthoate Ester (±)-**7**.* A solution of (±)-**4a**[14c] (1.0 mg, 2.5 μmol) in CH_2Cl_2 (0.2 mL) was treated sequentially with 6-methoxy-2-naphthoyl chloride (0.55 mg, 10 μmol, prepared from the corresponding carboxylic acid and oxalyl chloride), Et_3N (1.0 mg, 10 μmol), and DMAP (∼0.5 mg) at 0 °C, then stirred for 16 h at 23 °C. The mixture was concentrated, applied to a preparative TLC plate (silica, 20 × 20 × 0.1 cm), and the plate eluted (40:60 EtOAc/hexanes) to give a nonpolar UV-active fraction (1.4 mg) containing (±)-**7** (R_f = 0.26, 35:65 EtOAc/hexanes). The latter was further purified by HPLC (normal phase, Rainin Dynamax 10 × 250 mm, 5 μ, 35% EtOAc/hexanes, 4.0 mL·min^{-1}) to give (±)-**7** as a straw-yellow solid (0.73 mg, 49%): UV−vis (MeOH) λ_{max} 209 nm ($\log_{10}$ ε 4.16), 241 (4.17), 255 (4.18), 310 (3.48); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.49 (1H, s), 7.97 (1H, dd, *J* = 8.4, 1.2 Hz), 7.85 (1H, d, *J* = 9.0 Hz), 7.77 (1H d, *J* = 8.4 Hz), 7.21 (1H, dd, *J* = 9.0, 2.4 Hz), 7.16 (1H, d, *J* = 2.4 Hz), 6.40 (1H, s), 6.18 (1H, s), 3.96 (3H, s, 6′-OMe), 3.85* (3H, s, 3-OMe), 3.82* (3H, s, COOMe), 3.61 (1H, d, *J* = 18.6 Hz, H-7b), 3.10 (1H, d, *J* = 18.6 Hz, H-7a); HRMS (ESI-TOF) *m*/*z* 601.9418 [M + Na]$^+$ (calcd for $C_{14}H_{16}Br_2N_2O_7Na^+$, 601.9420).

*(1R,6S)-6′-Methoxy-2′-naphthoate Ester (1R,6S)-**7**).* A sample of (±)-**4a** (4.2 mg) was separated by semipreparative chiral-phase HPLC (Phenomenex, Amylose-2, 10 × 250 mm, 30:70 CH_3CN/H_2O, 4.0 mL·min^{-1}) to give pure (+)-**4a** (t_R = 18.2 min, 1.5 mg) and (−)-**4a** (t_R = 30.6 min, 1.5 mg). A sample of (+)-**4a** (0.78 mg, 2.0 μmol) was acylated with freshly prepared 6-methoxy-2-naphthoyl chloride (7.9 μmoL) and Et_3N (7.9 μmol) in CH_2Cl_2 (80 μL) to give (1*R*,6*S*)-**7a** (0.29 mg) after purification by HPLC (normal phase, Rainin Dynamax 10 × 250 mm, 5 μ, 35% EtOAc/hexanes, 4.0 mL·min^{-1} t_R = 7.4 min). (1*R*,6*S*)-**7**: colorless solid; $[\alpha]^{23}_D$ −21 (*c* 0.058, MeOH); UV−vis (MeOH) λ_{max} ($\log_{10}$ ε) 210 (4.11), 237 (4.37), 253 (4.20), and 310 (3.82) nm; ECD (2.49 × 10^{-5} M, MeOH) λ (Δε) 217 (−5.7), 267 (+4.7), and 310 (+7.6) nm; ^{1}H NMR (600 MHz), identical with that of (±)-**7**; HRMS (ESI-TOF) *m*/*z* 596.9860 [M + NH_4]$^+$ (calcd for $C_{27}H_{19}Br_2N_2O_7^+$, 596.9867).

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jnatprod.9b01286.

Structures of several known cyclohexa-1,3-dienyl SIOs (see Table 2), UV-ECD, 1D and 2D NMR, and HRMS data for compounds **1**−**3**, ^{1}H HNMR of (−)-**7**, UV−vis, ECD for **4a**, *S*-**6**, and *R*-**18**, HRMS data, and description of the α-chymotrypsin assay (PDF)

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Peng, J.; Li, J.; Hamann, M. T. *Alkaloids Chem. Biol.* **2005**, *61*, 59−262.
(2) Lira, N. S.; Montes, R. C.; Tavares, J. F.; da Silva, M. S.; da Cunha, E. V.; de Athayde-Filho, P. F.; Rodrigues, L. C.; da Silva Dias, C.; Barbosa-Filho, J. M. *Mar. Drugs* **2011**, *9*, 2316−2368.
(3) Ueberlein, S.; Machill, S.; Schupp, P. J.; Brunner, E. *Mar. Drugs* **2017**, *15*, 34−49.
(4) Ueberlein, S.; Machill, S.; Niemann, H.; Proksch, P.; Brunner, E. *Mar. Drugs* **2014**, *12*, 4417−4438.
(5) (a) Kobayashi, J.; Tsuda, M.; Agemi, K.; Shigemori, H.; Ishibashi, M.; Sasaki, T.; Mikami, Y. *Tetrahedron* **1991**, *47*, 6617−6622. (b) Andersen, R. J.; Faulkner, D. J. *Tetrahedron Lett.* **1973**, *14*, 1175−1178. (c) Gao, H.; Kelly, M.; Hamann, M. T. *Tetrahedron* **1999**, *55*, 9717−9726.
(6) De Medeiros, A.; Gandolfi, R. C.; Secatto, A.; Falcucci, R. M.; Faccioli, L. H.; Hajdu, E.; Peixinho, S.; Berlinck, R. G. S. *Immunopharmacol. Immunotoxicol.* **2012**, *34*, 919−924.
(7) (a) Shaala, L. A.; Youssef, D. T. A.; Badr, J. M.; Sulaiman, M.; Khedr, A. *Mar. Drugs* **2015**, *13*, 1621−1631. (b) Göthel, Q.; Sirirak, T.; Köck, M. *Beilstein J. Org. Chem.* **2015**, *11*, 2334−2342.
(8) Nicacio, K. J.; Ióca, L. P.; Fróes, A. M.; Leomil, L.; Appolinario, L. R.; Thompson, C. C.; Thompson, F. L.; Ferreira, A. G.; Williams, D. E.; Andersen, R. J.; Eustaquio, A. S.; Berlinck, R. G. S. *J. Nat. Prod.* **2017**, *80*, 235−240.
(9) Fattorusso, E.; Minale, L.; Sodano, G. *J. Chem. Soc. D* **1970**, 751−752.
(10) Gunasekera, M.; Gunasekera, S. P. *J. Nat. Prod.* **1989**, *52*, 753−756.
(11) Cimino, G.; De Rosa, S.; De Stefano, S.; Self, R.; Sodano, G. *Tetrahedron Lett.* **1983**, *24*, 3029−3032.
(12) Kobayashi, J.; Honma, K.; Sasaki, T.; Tsuda, M. *Chem. Pharm. Bull.* **1995**, *43*, 403−407.
(13) (a) Nakamura, H.; Wu, H.; Kobayashi, J. i.; Nakamura, Y.; Ohizumi, Y.; Hirata, Y. *Tetrahedron Lett.* **1985**, *26*, 4517−4520. (b) Nakamura, Y.; Kobayashi, M.; Nakamura, H.; Wu, H.; Kobayashi, J.; Ohizumi, Y. *Eur. J. Biochem.* **1987**, *167*, 1−6.
(14) (a) Ciminiello, P.; Fattorusso, E.; Magno, S.; Pansini, M. *J. Nat. Prod.* **1994**, *57*, 1564−1569. (b) Zhu, G.; Yang, F.; Balachandran, R.; Höök, P.; Vallee, R. B.; Curran, D. P.; Day, B. W. *J. Med. Chem.* **2006**, *49*, 2063−2076. (c) Shearman, J. W.; Myers, R. M.; Brenton, J. D.; Ley, S. V. *Org. Biomol. Chem.* **2011**, *9*, 62−65.
(15) Two lines of evidence to support the presence of thermodynamically more stable *E*-geometry include the C-17 chemical shift (CCCCCCCC$_{13C}$ 27.4; cf. *E,E*-psammaplyin A, δ 27.1). (a) Arabshahi, L.; Schmitz, F. J. *J. Org. Chem.* **1987**, *52*, 3584−3586 and the consistent presence of a hydrogen bond between the oxime N and the amide NH observed in X-ray structures. (b) Kazlauskas, R.; Lidgard, R. O.; Murphy, P. T.; Wells, R. J.; Blount, J. F. *Aust. J. Chem.* **1981**, *34*, 765−786. Natural products with the rare less-stable *Z*-geometry (viz. bastadin isomers) have been documented. (c) Calcul, L.; Inman, W. D.; Morris, A. A.; Tenney, K.; Ratnam, J.; McKerrow, J. H.; Valeriote, F. A.; Crews, P. *J. Nat. Prod.* **2010**, *73*, 365−372.
(16) Marfey, P. *Carlsberg Res. Commun.* **1984**, *49*, 591−196.
(17) Salib, M. N.; Molinski, T. F. *J. Org. Chem.* **2017**, *82*, 10181−10187.
(18) The Marfey's derivatives L- and D-*iso*Ser-L-DAA,[16] prepared from standard *iso*Ser samples and L-FDAA, failed to separate under the same reversed-phase HPLC conditions.
(19) Duplication of the H-5 vinyl ^{1}H NMR signal ($\Delta\delta$ = 0.003; Table 1) is also observed.
(20) Castillo, A. M.; Patiny, L.; Wist, J. *J. Magn. Reson.* **2011**, *209*, 123−130.
(21) This is not contradictory to our independent findings that the epimer ratio of *iso*Ser in **2** is 1:1 but 67:33 in the SIO heterocycle (see Note 27 below and discussion in the text). For simplicity of argument, if the latter ratio is approximated to 2:1 and the components permuted, the four outcomes are two enantiomeric pairs of diastereomers: (1*S*,6*R*,11*S*), (1*S*,6*R*,11*R*) and (1*R*,6*S*,11*R*), (1*R*,6*S*,11*S*) in the proportions 3:3:2:2. The NMR-distinguishable diastereomers, therefore, are present in the ratio of 5:5 or 1:1.
(22) McMillan, J. A.; Paul, I. C.; Goo, Y. M.; Rinehart, K. L.; Krueger, W. C.; Pschigoda, L. M. *Tetrahedron Lett.* **1981**, *22*, 39−42.
(23) Fattorusso, E.; Minale, L.; Sodano, G. *Chem. Commun.* **1970**, 752−753.
(24) Fulmor, W.; Van Lear, G. E.; Morton, G. O.; Mills, R. D. *Tetrahedron Lett.* **1970**, *11*, 4551−4552.
(25) Moscowitz, A.; Charney, E.; Weiss, U.; Ziffer, H. *J. Am. Chem. Soc.* **1961**, *83*, 4661−4663.
(26) Burgstahler, A. W.; Ziffer, H.; Weiss, U. *J. Am. Chem. Soc.* **1961**, *83*, 4660−4661.
(27) MMFF. Molecular modeling of aeroplysillin-1 shows the diene has similar helicity (this work, MMFF, dihedral $\theta = \pm 17.6°$), in good correspondence with the X-ray crystal structure assignments of both (+)- and (−)-aeroplysillin-1 ($\theta = 17.3 \pm 1$). Cosulich, D. B.; Lovell, F. M. *J. Chem. Soc. D* **1971**, 397−398.
(28) If one assumes, for example, highly dichroic (+)-aerothionin (**S2**, Table 2) is optically pure ("100% ee", also see ref 38), the ratio of CE magnitudes of **S2** and **2** implies the latter is only 19.3% ee (er = 63:37). Lack of precision of ECD measurements on a sub-milligram sample of **2** limits the accuracy of this conclusion.
(29) We have found the effects of more distal stereocenters in SIOs upon the net ECD spectrum, e.g., fistularin-3 and 11-hydroxyfistularin-3, to be negligible.
(30) Another possibility we considered is that **4c** is formed as a racemic or near-racemic modification and kinetically resolved in downstream enzymatic coupling reactions. This possibility would explain enantiomorphic **4c** and fistularin-3 in the same sample.[48]
(31) Okamoto, Y.; Ojika, M.; Kato, S.; Sakagami, Y. *Tetrahedron* **2000**, *56*, 5813−5818.
(32) Kobayashi, J. i.; Tsuda, M.; Agemi, K.; Shigemori, H.; Ishibashi, M.; Sasaki, T.; Mikami, Y. *Tetrahedron* **1991**, *47*, 6617−6622.
(33) Benharref, A.; Païs, M.; Debitus, C. *J. Nat. Prod.* **1996**, *59*, 177−180.
(34) Salim, A. A.; Khalil, Z. G.; Capon, R. J. *Tetrahedron* **2012**, *68*, 9802−9807.

(35) Kijjoa, A.; Bessa, J.; Wattanadilok, R.; Sawangwong, P.; Nascimento, M. S. J.; Pedro, M.; Silva, A. M. S.; Eato, G.; van Soest, R.; Herz, W. *Z. Naturforsch., B: J. Chem. Sci.* **2005**, *60*, 904−908.
(36) From values of $[\alpha]_D$, we estimate the Karuso sample of (−)-**8b** from NW Australia to be only 7% ee—rather than a different ionization state, as speculated by the authors—to our sample of (+)-**8b** from the Bahamas.[48]
(37) Ragini, K.; Fromont, J.; Piggott, A. M.; Karuso, P. *J. Nat. Prod.* **2017**, *80*, 215−219.
(38) It is perhaps no coincidence that earlier-reported SIOs, purified by crystallization (e.g., (+)-aerothionin[20]), are optically enriched through the process and have the highest values.
(39) (a) Gonnella, N. C.; Nakanishi, K.; Martin, V. S.; Sharpless, K. B. *J. Am. Chem. Soc.* **1982**, *104*, 3775−3776. (b) Harada, N.; Iwahuchi, J.; Yokota, Y.; Uda, H. A. *J. Am. Chem. Soc.* **1981**, *103*, 5590−5591.
(40) The 6-methoxy-substituted naphthoic acid was chosen for its stronger electronic transition dipole moment (donor−acceptor effect).
(41) Gopichand, Y.; Schmitz, F. J. *Tetrahedron Lett.* **1979**, *20*, 3921−3924.
(42) König, G. M.; Wright, A. D. *Heterocycles* **1993**, *36*, 1351−1358.
(43) Nuñez, C. V.; de Almeida, E. V. R.; Granato, A. C.; Marques, S. O.; Santos, K. O.; Pereira, F. R.; Macedo, M. L.; Ferreira, A. G.; Hajdu, E.; Pinheiro, U. S.; Muricy, G.; Peixinho, S.; Freeman, C. J.; Gleason, D. F.; Berlinck, R. G. S. *Biochem. Syst. Ecol.* **2008**, *36*, 283−296.
(44) Ciminiello, P.; Costantino, V.; Fattorusso, E.; Magno, S.; Mangoni, A.; Pansini, M. *J. Nat. Prod.* **1994**, *57*, 705−712.
(45) Lira, T. O. d.; Berlinck, R. G. S.; Nascimento, G. G. F.; Hajdu, E. *J. Braz. Chem. Soc.* **2006**, *17*, 1233−1240.
(46) Abou-Shoer, M. I.; Shaala, L. A.; Youssef, D. T. A.; Badr, J. M.; Habib, A.-A. M. *J. Nat. Prod.* **2008**, *71*, 1464−1467.
(47) Carney, J. R.; Rinehart, K. L. *J. Nat. Prod.* **1995**, *58*, 971−985.
(48) Aiello, A.; Fattorusso, E.; Menna, M.; Pansini, M. *Biochem. Syst. Ecol.* **1995**, *23*, 377−281.
(49) Compound **4c** is likely the precursor to all SIOs.
(50) Rogers, E. W.; Molinski, T. F. *J. Nat. Prod.* **2007**, *70*, 1191−1194.
(51) Counter examples of biosynthesized carboxylic acid methyl esters have been described. (a) Molinski, T. F.; Ireland, C. J. *J. Org. Chem.* **1988**, *53*, 2103−2105. (b) Ferreira, E. L. F.; Williams, D. E.; Ióca, L. P.; Morais-Urano, R. P.; Santos, M. F. C.; Patrick, B. O.; Elias, L. M.; Lira, S. P.; Ferreira, A. G.; Passarini, M. R. Z.; Sette, L. D.; Andersen, R. J.; Berlinck, R. G. S. *Org. Lett.* **2015**, *17*, 5152−5155.
(52) Rogers, E. W.; Fernanda de Oliveira, M.; Berlinck, R. G. S.; König, G. M.; Molinski, T. F. *J. Nat. Prod.* **2005**, *68*, 891−896.
(53) Owen, T. C.; Merkler, D. J. *Med. Hypotheses* **2004**, *62*, 392−400.
(54) Yang, K.; Fen, J.; Fang, H.; Zhang, L.; Gong, J.; Xu, W. *J. Enzyme Inhib. Med. Chem.* **2012**, *27*, 302−310.

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