Isolation and Cytotoxic Evaluation of Marine Sponge-Derived Norterpene Peroxides

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The marine sponge *Diacarnus* cf. *spinopoculum* has provided a series of norterpenes, including five new compounds (**7**−**11**), two new *ent*-compounds [(−)-**1a** and (+)-**1b**], and three known compounds (**2a**, **2b**, and **12**). Eight of these compounds represent additional examples of the muqubilin/sigmosceptrellin classes (norsesterterpene peroxides) or the nuapapuin class (norditerpene peroxides). Also isolated were dinorditerpenones **11** and **12**, which are biosynthetically related to the muqubilin/sigmosceptrellin structure classes. In all, 11 compounds were evaluated for their cytotoxic properties using a soft agar assay system and the NCI's 60 cell-line screen. Compounds without peroxide functionality were inactive. Overall, the norsesterterpene peroxides were less selective as cytotoxins than norditerpene peroxide analogues. Two compounds, nuapapuin A methyl ester (**3**) and nuapapuin B (**7**), which were somewhat selective in their cytotoxic behavior, were selected for further *in vivo* evaluation.

Terpene peroxides are a fascinating class of compounds isolated from both plants and marine organisms. Interest has usually focused on such compounds because they frequently possess biologically active properties, but additional challenges have been addressed in defining the chirality of their multiple stereocenters. In 1979, two different groups independently reported the first examples of sponge-derived terpene peroxides. These consisted of muqubilin (**1a**)[1] from a Red Sea *Prianos* species (family Hymeniacidonidae, order Halichondrida) and sigmosceptrellin A methyl ester [(+)-**2a**] from a Papua New Guinea (PNG) *Sigmosceptrella laevis* (Lindgren) (family Latrunculiidae, order Poecilosclerida).[2] In 1982, structures were disclosed for the sigmosceptrellin epimers B (**2b**) and C (**2c**),[2b] along with an additional report describing both **1** and **2b** as antimicrobial constituents of a *Prianos* sponge.[3] Our group encountered (+)-**1a** accompanied by the norditerpene peroxide nuapapuin A (**3**) during the study of a Tongan sponge originally identified as a *Prianos* sp.,[4] but revised to *Diacarnus*[5] cf. *spinopoculum* (family Latrunculiidae, order Poecilosclerida).

In addition to compounds **1**−**3**, there are several other families of norterpene peroxides[6−8] including the monocyclic terpenes **4** and polycyclic terpenoids such as trunculin A (**5**) and mycaperoxide A (**6**). The 21 norterpene peroxides published to date (Supporting Information) reflect the considerable attention given to such compounds.

Aside from the early reports of **1a** and **2a** in connection with *Prianos* spp., all other marine-derived terpenoid peroxides were attributed to sponge families Latrunculiidae (order Poecilosclerida and/or Hadromerida)[5] and Mycalidae (order Poecilosclerida) (Supporting Information). Often, terpene peroxides have emerged as the endpoint of a bioassay-guided isolation, and the biological characteristics (Supporting Information) include antimicrobial,[3,6] ichtyotoxic,[2] sea urchin egg cell-division inhibiting,[4] cytotoxic,[7] and antiviral activities.[7] On varying occasions, we and others have assessed the cytotoxic or antitumor potential of stable sponge-derived peroxides.[7,9] The work presented below describes results for 11 sponge metabolites of the terpenoid peroxide class, including both *in vitro* and *in vivo* data.

Results and Discussion

An initial goal of this study was to use a primary screen consisting of a soft agar assay system[10] as a tool to examine the selective cytotoxic properties of norterpene peroxide compounds, or crude extracts containing such compounds. Stored in our repository were small quantities of **1a** and **3**, and these were submitted for *in vitro* cytotoxicity screening.[10] Several Latrunculiidae specimens suspected of containing additional norterpene peroxides (Supporting Information) were also selected for examination. These organisms, classified as *Latrunculia spinopoculum* at the outset of this work, are now identified as *Diacarnus* cf. *spinopoculum* in accordance with current literature.[5] The nonpolar extracts[11] of specimens from both PNG (coll. no. 95150) and the Solomon Islands (coll. no. 89042) were found to exhibit cytotoxicity during primary screens. An ensuing study of four *D.* cf. *spinopoculum* provided five new structures (**7**−**11**),[12] two *ent*-structures [(−)-**1a** and (+)-**1b**], and the known compounds (+)-**2a**, **2b**, and **12**.[2b] Upon *in vitro* evaluation of all available norterpene peroxide compounds, the nuapapuin series as exemplified by nuapapuins A (**3**) and B (**7**) demonstrated selective cytotoxicity to tumor cells and were generally

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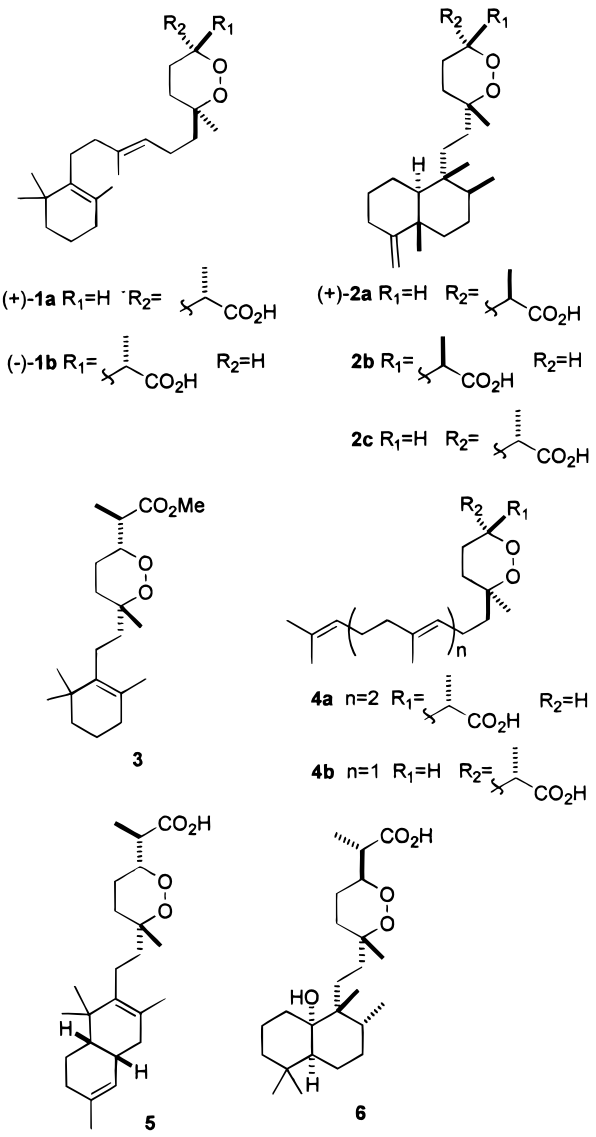

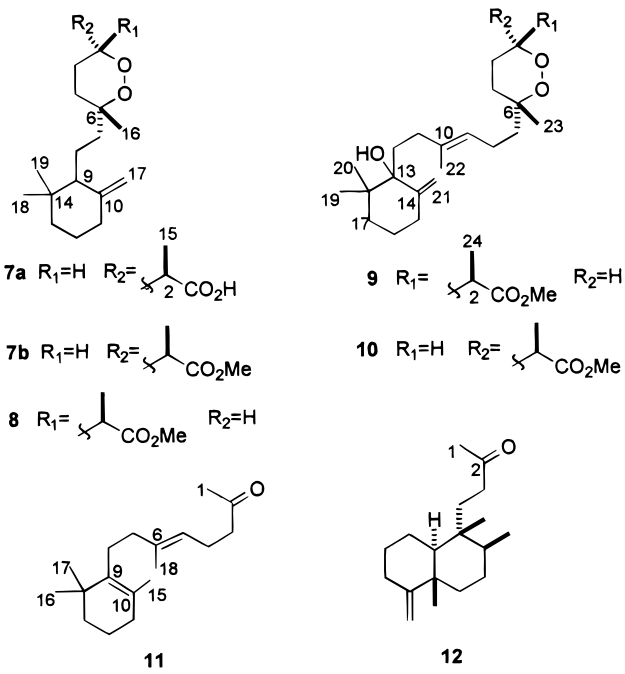

much more active than the muqubilin series [(−)-**1a**, (+)-**1b**, **9**, and **10**].

Our chemical discussion should be preceded by noting our agreement with a 1985 publication,[6a] which questions the original stereostructure we proposed[4] for **3** (revision shown in Supporting Information). Furthermore, Capon's empirical rules[6a,b] for establishing the C2, C3, and C6 relative stereochemistry were extensively used in this study (see Experimental Section). All of the crude extracts examined displayed diagnostic NMR signals for terpene peroxides. The peroxide moiety is denoted by signals at roughly δ 81/4.2 (C3/H3) and 80 (C6). Exocyclic double bonds present in such compounds are characterized by lowfield pairs of resonances in the ^{13}C NMR spectrum at approximately δ 160/102 and δ 150/110, and by peaks in the ^{1}H NMR spectrum between δ 4.4 and 4.8 (CD_3OD).

Our attention first focused on the Solomon Islands taxa (coll. no. 89042), as the NMR data intimated the presence of at least two peroxide-containing compounds. The sigmosceptrellins[2] exhibit exomethylene double-bond ^{13}C NMR resonances at δ 160 and 102 and a ^{1}H NMR at δ ca. 4.5 (br s, $CDCl_3$). While the presence of one or more sigmosceptrellins was consistently suspected,[2,6a,b] it was the compound representing the other olefinic signals (δ 150 and 110) that we sought to resolve and characterize. Sequential chromatography, first by gel permeation and second by reversed-phase HPLC, yielded the pure compound nuapapuin B (**7**). A molecular formula of $C_{19}H_{32}O_4$ was established based on APT NMR data and presumption of both the peroxide (CD_3OD, δ 82.8 d, 81.2 s) and carboxylic acid (CD_3OD, δ 178.0 s) moieties. This formula was confirmed by an HRCIMS *m*/z of 325.2379 $[M + H]^+$ (Δ −1.0 mmu). The norterpene peroxide ring system was established by comparing ^{13}C chemical shifts from C1 through C6 to known compounds such as **1b**,[6a,b] (+)-**2a**,[2] and **5**.[8] Accounting for the exomethylene, the carboxyl, and the endoperoxide left one remaining ring. Two methyl singlets (CD_3OD, δ 0.83 and 0.91), one additional methine signal (δ 55.6 d), and terpene biogenetic considerations suggested one remaining ring was present as a 2,2-dimethyl-6-methylenecyclohexyl moiety tethered to C6 by two methylenes. This substructure has appeared in several other marine metabolites,[13] which in turn served as supportive models for the NMR data of **7** (Table 1). Confirmation that **7a** was indeed the exocyclic double-bond isomer of nuapapuin A (**3**) was achieved by converting **7a** to **3** through sequential steps of carboxylic acid methylation and double-bond migration (see Experimental Section). This transformation along with characteristic NMR data—the C6 equatorial methyl (δ 23.7, C16), the axial H3 (δ 4.26 dt, $J_{3,4ax}$ = 7.5 Hz), and C2−C3 erythro configuration (δ 1.15 d, $H_3$15)—establishes the relative stereochemistry of **7a** at C2, C3, and C6 (see Experimental Section). The absolute configuration at these three centers for both **3** and **7a** is implied by a comparison of the optical rotation of **3** (lit. $[\alpha]_D$ +53.7°;[4] semisynthetic $[\alpha]_D$ +35.2°) and **7a** ($[\alpha]_D$ +45.0°) to that of (−)-**1b** ($[\alpha]_D$ −59.2°).[6a] Because these three compounds share the same relative stereochemistry, the absolute configurations opposite to (−)-**1b** (2*S*,3*S*,6*S*) must be present in **3** and **7a** (2*R*,3*R*,6*R*).

Attention was next shifted to the PNG specimen (coll. no. 95150) from which substances (+)-**2a**, **7b**, and **9**−**12**

Table 1. ^{13}C NMR Shift Values for Nuapapuins A (**3**) and B (**7**) and Epinuapapuin B (**8**)

atom #	**3** ($CDCl_3$)	**7** (**a**, CD_3OD/**b**, $CDCl_3$)	**8** ($CD_3OD/CDCl_3$)
1	174.3	178.0/174.0	175.7/174.3
2	42.7	44.0/42.6	43.8/43.0
3	81.2	82.8/81.2	82.5/81.4
4	32.8	33.9/33.6	24.2/23.7
5	35.0	33.2/32.4	39.6/38.7
6	80.1	81.2/80.3	81.3/80.3
7	22.6	24.6/23.9	33.1/32.2
8	22.3	20.8/20.0	20.5/19.4
9	136.7	55.6/54.4	55.7/54.4
10	127.1	150.7/149.5	150.3/149.0
11	32.6	33.5/32.4	33.1/32.5
12	19.6	23.5/22.5	24.6/23.7
13	39.9	37.5/36.2	37.0/36.1
14	34.8	35.7/35.0	35.7/35.0
15	12.6	13.3/12.8	13.3/13.6
16	23.7	24.0/23.7	21.0/20.5
17	19.8	109.5/108.9	109.7/109.2
18	28.7	28.8/28.4	28.7/28.4
19	28.5	27.4/26.4	26.8/26.5

Table 2. ^{13}C NMR Shifts of (−)-Muqubilin A (**1a**), (+)-Epimuqubilin A (**1b**), and Epimuqubilin B (**10**)

atom #	**1a**[a] ($CDCl_3$)	**1b**[a] ($CDCl_3$)	**10** (C_6D_6)
1	180.0	(180.0)[b]	174.0
2	43.0	(43.0)[b]	43.0
3	81.1	81.2	81.6
4	23.5	24.0	23.0
5	39.7	32.5	33.2
6	80.3	80.2	79.4
7	32.0	34.9	35.2
8	21.7	22.1	22.6
9	123.3	123.5	124.9
10	136.6	136.4	136.3
11	39.9	39.9	34.3
12	27.9	27.9	31.7
13	137.2	137.2	79.9
14	127.0	127.0	151.1
15	32.8	32.8	34.3
16	40.3	40.2	23.3
17	19.6	19.6	38.2
18	35.0	34.9	40.0
19	28.7	28.7	24.4
20	28.7	28.7	22.4
21	19.9	19.9	108.7
22	16.1	16.0	16.4
23	20.8	23.9	24.2
24	13.3	12.7	12.9

[a] These assignments are based on published values. [b] These shifts are expected peaks, which did not appear in spectrum.

were obtained. Immediately after purification of the crude nonpolar extract, structures for (+)-**2a** and **12** were established by comparison to literature data (NMR, optical rotation).[2,6a,b] Similarly, the methyl ester **7b** was identified owing to its similar NMR spectra to that of **7a** (Table 1), which along with its measured optical rotation ($[\alpha]_D$ +39.0°) established partial absolute stereochemistry of **7b** as 2*R*,3*R*,6*R*. Full characterization of **11** was straightforward and aided by analogy of its NMR properties to those of muqubilins **1a** and **1b**. Further, the molecular formula of $C_{18}H_{30}O$ for **11** was supported by a HRCIMS *m*/z $[M - OH]^+$ of 245.2269 (Δ 0.2 mmu of calcd). Its four degrees of unsaturation could be accounted for by the presence of a ketone [δ 209; IR (C=O stretch, 1715 cm^{-1})], a diene (δ 137.5 s, 137.1 s, 127.1 s, and 122.0 d; compare to muqubilin shifts, Table 2), and one remaining ring. A LRCIMS *m*/z of 137.2 (base peak, $C_{10}H_{17}$), two equivalent methyl singlets at δ 28.7 q/1.00 s ($^{13}C/^1H$), and the vinylic methyl singlet at δ 19.9 q/1.61 s provided final confirmation for the tetrasubstituted cyclohexyl moiety.

The next task was to define the frameworks of the minor constituents, muqubilin B (**9**) and epimuqubilin B (**10**). A side-by-side comparison of the integrated ^1H NMR of each compound ($CDCl_3$) revealed their parallel identities. There were numerous diagnostic resonances suggestive of norterpene peroxides including three vinylic protons (δ 5.12 t, J = 5 Hz; 4.90 s; 4.85 s), a vinylic methyl (δ 1.62 s), a methoxy (δ 3.71 s), an oxymethine (δ 4.14 dt, J = 2.5, 5 Hz), a carbonyl α-proton (2.66 br s), and a gem dimethyl (δ 0.98 s, 0.90 s). The isolable differences in these spectra were the positions of two additional methyl shifts (**9** vs **10**: 1.16 d, J = 7 Hz vs 1.27 d, J = 7 Hz; 1.13 s vs 1.30 s). The diastereomeric nature of **9** and **10** was supported by their identical mass spectra: LRESIMS (*m*/z) of 445 $[M + Na]^+$, 461 $[M + K]^+$, and 242 $\{[M + Na + K]/2\}^{2+}$. Initial attempts to characterize **9** were thwarted because it decomposed while we were acquiring NMR data for an extended period in $CDCl_3$. Alternatively, its molecular formula ($C_{25}H_{42}O_5$) was established by obtaining an HRCIMS *m*/z of 421.2944 $[M - H]^+$ (Δ 1.0 mmu of calcd).

With **10** still in hand, efforts were made to rapidly acquire all supporting NMR data in C_6D_6. These included the ^1H (Experimental Section), ^{13}C (Table 2), DEPT, HMQC, HMBC, TOCSY, and COSY experiments. Alongside the two peroxycarbon shifts (δ 81.6 s, C3; δ 79.4 d, C6) was one additional oxygenated quaternary carbon (δ 79.9 s) and four olefinic signals, implying an exocyclic methylene (δ 151.1 s, 108.7 d) and a trisubstituted double bond (δ 124.9 d, 136.3 s). Four of the five degrees of unsaturation could be accounted for by an endoperoxide, a carboxyl, and a diene, which meant one carbocyclic ring was also present. The combination of HMBC and COSY correlation data allowed construction of two partial structures. The first consisted of C1−C11 with Me22, Me23, and Me24 attached as shown. The second contained C12−C18 with Me19, Me20, and $H_2$21 as drawn. The NMR shifts for these residues were similar to those of related partial structures in **7** (Table 1) and **1b** (Table 2). The differences between these data and those for previously disclosed (−)-**1b**[6a] was rationalized by the quaternary alcohol at C13 and the exomethylene at C14. These features were further supported by HMBC correlations, especially $H_3$19, $H_3$20, H21, H21′ → C13. That the final structure consisted of a CH_2-CH_2 connection between the two substructures was supported by the TOCSY NMR data. The gross structure of **10** could also be applied to **9**.

The analysis of the NMR and optical rotation data allowed stereochemical assignments to be made for both **9** and **10**. Thus, the ^{13}C methyl shifts in the vicinity of the peroxide ring of **10** are similar to those of **1b**[6a] but quite different from those of **1a**,[4,6a] and Me23 (δ 24.2) can be assigned as equatorial. Due to the instability problem noted previously, ^{13}C NMR data could not be obtained for **9**. A comparison of ^1H NMR resonances with those of model compounds reaffirms the Me23 of **9** as axial ($H_3$23, δ 1.30; compare to **8**) but equatorial for **10** ($H_3$23, δ 1.13; compare to **7**). The ^1H NMR data were used to establish that the oxymethine protons are

Table 3. Zone Differentials in the Disk Diffusion Soft Agar Colony Formation Assay (Solid Murine−Leukemia)[a]

class	compd	C38− L-1210	M17− L-1210
norditerpene peroxides	**3**	300	0
	7a	70	100
	7b	120	−80
	8		50
norsesterterpene peroxides	(−)-**1a**	−50	−30
	(+)-**1b**		20
	(+)-**2a**	−50	−10
	2b		0
dinorditerpenones	**11**		−60
	12		0

[a] Dose = 50 μg/disk; measured in zone units: 200 zone units = 6 mm. Murine cell lines: L-1210, lymphatic leukemia; C38, colon adenocarcinoma-38; M17, mammary-17/Adr. For comprehensive data set see Table S1.

axial ($CDCl_3$, **9**, H3, δ 4.14 dt, $J_{3,4ax} = 8$ Hz; **10**, H3 δ 4.31 dt, $J_{3,4ax} = 7$ Hz). Likewise, the ^{1}H NMR data were used to establish configurations about the C2−C3 bonds as threo in **9** ($H_3$24 δ 1.27 d, $J = 7$ Hz) and erythro in **10** ($H_3$24 δ 1.16 d, $J = 6.2$ Hz) (see Experimental Section). This provides an accordingly analogous pair to those of **1a**/**1b**, **2b**/**2a**, and **8**/**7**. These relationships, the assumption that endoperoxide formation is enzymatically mediated and thus stereocontrolled,[6a,b] and the optical rotations of **9** (−15.5°) and **10** (+14.8°) support that their respective partial absolute configurations are 2*R*,3*S*,6*R* and 2*R*,3*R*,6*R*. It is assumed that the undefined configuration at C13 is the same in **9** and **10**.

The isolation work turned next to another PNG specimen of *Diacarnus* cf. *spinopoculum* (coll. no. 91175), which yielded several pure compounds including **2b, 7a**, **7b**, **11**, the muqubilins (−)-**1a** and (+)-**1b**, and a third norditerpene epinuapapuin B (**8**). Compounds (−)-**1a** and (+)-**1b** were identified as enantiomers to the previously described structures (+)-**1a** and (−)-**1b**, respectively, because they displayed identical NMR spectra (Table 2) but opposite optical rotations. This is analogous to the isolation of both (+)-**2a**[2] and (−)-**2a**[6a,b] (Supplementary Information). It should be noted, however, that (+)-**1b** was unstable and it decomposed during analysis. The ^{13}C NMR shifts for C1 and C2 were not visible (Table 2), and an HRMS was not obtained before the sample decomposed. The identity of **8** having formula $C_{20}H_{34}O_4$, as required by the HRCIMS *m*/z of 339.2530 $[M + H]^+$ (Δ 0.5 mmu of calcd), was established by the subtle differences in the ^{13}C NMR (Table 1) and ^{1}H NMR (Experimental Section) when compared to those of nuapapuin B (**7**). For **8**, the C6 methyl is axial ($CDCl_3$, C16; δ 20.5), the oxymethine proton is axial (H3; δ 4.11 dt, $J_{3,4axial} = 8$ Hz), and there is a threo relationship about the C2−C3 bond ($H_3$15, 1.24 d, $J = 7$ Hz). A comparison of the optical rotation of **7** (−41.6°) to known compounds (+)-**1a** (+31.6°), **4b** (+52.2°), and (−)-**1a** (−35.6°) is revealing and provides support for the partial absolute stereochemistry of **8** as 2*R*,3*S*,6*R*.

All of the compounds isolated here from *Diacarnus* species, plus **3** from our repository, were evaluated *in vitro* to test for their differential cytotoxicity in the soft agar assay (Table 3).[10] Ideally, a zone differential of 250 units is expected for designation as "selective activity". The data compiled in Table 3 is normalized against the response observed for a murine leukemia cell line. The list of 11 compounds whose zones where evaluated (Supplementary Information) includes nine with the cyclic peroxide moiety and two without (**11**, **12**). Further, within the terpene peroxides, five are norsesterterpenes [(−)-**1a**, (+)-**1b**,[14] (+)-**2a**, **2b**, **9**], while four are norditerpenes (**3**, **7a**, **7b**, **8**). Interestingly, each of the norditerpene peroxides clearly demonstrated superior profiles compared to the norsesterterpene peroxides and the dinorditerpenones. It is difficult to make firm conclusions about the relationship of the norditerpene structures vs selective cytotoxicities. Apparently, variation in absolute stereochemistry at C3 from *R* (**3**, **7**) to *S* (**8**) has a small influence on the potency, as the latter compound possessed the lowest zone differential for solid murine vs leukemia cells. All past reporting of biological activities for the norterpene peroxides has been restricted to the free carboxylic acid and never observed in corresponding methyl ester compounds.

Additional evaluation of these compounds has taken place. The same battery of compounds was submitted to the NCI's 60 cell-line anticancer screen (Supporting Information). A sampling of the NCI data revealed substantial cytotoxic behavior in only the peroxides (+)-**2a**, **2b**, **7a**, and **7b** (Table 4). First, the norsesterterpene peroxides (+)-**2a** and **2b** produced 50% cell growth inhibition (GI_{50}) at submicromolar concentrations in the majority of cell types tested, including leukemic cells. Alternatively, the norditerpene peroxides **7a** and **7b** displayed submicromolar GI_{50}s, but in a selective fashion that excluded leukemic cell-lines. Both **7a** and **7b** were selected for follow-up evaluation by the NCI Biological Evaluation Committee (BEC). After first determining that **7b** was nontoxic [maximum tolerated

Table 4. *In Vitro* Growth Inhibition (GI_{50}, μM) from NCI's 60 Cell-Line Screen[a]

class[b]	compd	HL−60 (TB)	MOLT−4	A549/ATCC	KM12	LOX IMVI	IGROV1	786−0	BT−549
ND P	**3**	>5.0	>5.0	>5.0	>5.0	>5.0	>5.0	>5.0	>5.0
	7a	1.63	2.16	3.05	4.82	0.25	0.63	0.94	1.05
	7b	1.60	>5.0	0.64	0.40	0.47	0.50	0.27	4.95
	8	2.06	>5.0	>5.0	>5.0		1.73	>5.0	>5.0
NS P	(−)-**1a**	1.77	>5.0	>5.0	>5.0	2.17	1.11	>5.0	>5.0
	(+)-**1b**	>5.0	>5.0		>5.0		>5.0		
	(+)-**2a**	0.14	0.98	1.45	0.94		0.12	0.61	1.81
	2b	0.14	0.84	0.94	0.95	0.16	0.10	0.50	0.96
	9	>5.0	>5.0	>5.0	>5.0	>5.0	2.42	>5.0	
DNDT	**11**	2.88	2.08	>5.0	>5.0		>5.0	>5.0	>5.0
	12	>5.0	>5.0	>5.0	>5.0		>5.0	>5.0	>5.0

[a] Cell-lines: HL-60 (TB)/MOLT-4, leukemia; A549/ATCC, nonsmall cell lung cancer; KM12, colon cancer; LOX IMVI, melanoma; IGROV1, ovarian cancer; 786−0, renal cancer; BT-549, breast cancer. For comprehensive data set including all cell lines from NCI screen see Table S2. [b] Compound classes: ND P, norditerpene peroxide; NS P, norsesterterpene peroxide; DNDT, dinorditerpenone.

dose (MTD) of >400 mg/kg], it was later also determined inactive in an *in vivo* hollow fiber assay.[15] The free acid **7a** is still under BEC evaluation and is scheduled for an acute toxicity test for determining its MTD. Perhaps representing further reinforcement of the theme observed above, the norsesterterpene mycaperoxide B (Supporting Information), isolated by Tanaka et al.[7,9i] in 1993, was also selected for *in vivo* cytotoxicity evaluation by the NCI−BEC.[16] With **3** emerging as the leading candidate from the soft agar assay (zone differential = 300, Table 3), *in vivo* tests were performed. No antitumor activity or toxicity, however, was observed after 424 mg/kg was administered on an iv split-dose schedule to four mice.

Conclusions

This study on *Diacarnus* Poecilosclerids has added an interesting theme about how the norterpene peroxide constituents can vary among the same sponge species while also providing new structures to the norterpene peroxide class. The occurrence of norterpene peroxides with enantiomeric configurations at C2/C3/C6 is striking and is represented by our isolation of (−)-muqubilin A (**1a**) and (+)-epimuqubilin A (**1b**). Muqubilin B (**9**) and epimuqubilin B (**10**) are equally interesting in that they are the first marine natural products found to contain the 2,2-dimethyl-1-hydroxy-6-methylenecyclohexyl unit. To the best of our knowledge, the only natural product previously found to contain this substructure was a constituent of the Greek tobacco *Nicotiana tabacum*.[17] While **9** and **10** were isolated as minor components, their most obvious precursors would be structures (−)-**1a** and (+)-**1b**, respectively. A cytochrome P_{450}-mediated hydroxylation, which involves a similar double-bond migration, was recently described as a critical step in taxol biosynthesis.[18] It is plausible that a similar pathway employing a monooxygenase is also at work in converting **1a** to **9** and **1b** to **10**. A cytochrome P_{450}-like monooxygenase may also be involved in endoperoxide cleavage of **1** to form ketone **11** and cleavage of **2** to form **12**. Finally, we believe that further study of the antitumor potential of additional norterpene peroxides, especially those with C3 *R* stereochemistry is warranted.

Experimental Section

General Experimental Procedures. The NMR were recorded at 250, 300, or 500 MHz for ^{1}H NMR and 62.9 and 125.7 MHz for ^{13}C NMR. Multiplicities of ^{13}C NMR were determined from APT data, DEPT data, or HMQC (500 MHz). MS data were obtained on a VG 70-VSE (LRCI, HRCI), a VG ZAB-SE (LRFAB), a VG 70-SE-4F (HRFAB), and a VG Quattro (LRESI, LRAPCI). Chromatography was performed using Sephadex LH-20 (gel permeation), Si gel (normal phase: flash, HPLC), and ODS (reversed-phase: flash, HPLC). Optical rotations were measured on a JASCO DIP-370 digital polarimeter in $CHCl_3$. IR spectra were measured on a Varian 1600 series FTIR spectrometer.

Collection and Identification. All sponge specimens were collected using scuba from locations in the Solomon Islands (coll. no. 89042) and PNG (coll. no. 95150, 91175, 91172). The sponges *Diacarnus* cf. *spinopoculum* were identified by Dr. M. C. Diaz (UCSC). They were massive−amorphous, subspherical to tubular in shape, with one large atrium, and oscules on the walls of the atrium. The surface was tuberculate, rubbery, and sometimes with regularly distributed holes, 1−3 mm in diameter. The sponges were of a tough consistency, reddish pink and yellow-tan internally. The skeleton consists of styloids (length: 300−600 μm, width: 8−10 μm), radially arranged toward the surface and arranged in loose tracts in the choanosome, and sanidasters (= spinorhabds) with four whorls of spines (60−80 × <10 μm), highly concentrated at the surface or dispersed in the choanosome. All specimens belong to the genus *Diacarnus*.[5] Two species names could be associated with the specimens studied: *D. spinopoculum* (Carter, 1879) and *D. tubifera* (Kelly-Borges and Vacelet, 1995), mostly distinguished by the predominance of a barrel-to-spherical shape on the former and single tubes or vases on the latter. We found a high plasticity in the growth of the specimens studied, making it very hard to assign some of them to one or the other growth-form category. Considering that otherwise the specimens were very similar, we assigned them as tentatively representing specimens of *D.* cf. *spinopoculum*. Underwater photographs and vouchers of these specimens are available from UCSC. Similar rationale was used in reassigning a prior Tongan specimen (coll. no. 83015)[4] from *Prianos* sp. to *D.* cf. *spinopoculum*.

Extraction and Isolation. Overall the following compounds were isolated as a function of the sponge examined: (a) coll. no. 89042, (+)-**2a**, **7a**; (b) coll. no. 91172, **7a**; (c) coll. no. 91175, (−)-**1a**, (+)-**1b**, **2b**, **7a**, **7b**, **8**, **11**; (d) coll. no. 95150, **7b**, **9**, **10**, **11**, **12**. All sponges were preserved and extracted according to our standard procedures described elsewhere.[11] Starting with the CH_2Cl_2 extract (0.20 g) of coll. no. 89042 (Solomon Island), sequential chromatography, first by gel permeation (2:3 CH_2Cl_2−MeOH) and second by reversed-phase HPLC (80:20 MeOH−H_2O; RI detection), yielded pure **7a** and nearly pure (+)-**2a**. Subsequent work was conducted on the hexane extract of coll. no. 95150 (PNG). Subjecting the extract to flash column chromatography in (95:5 hexane−EtOAc) yielded several fractions, including pure (+)-**2a**. The second-eluting fraction (0.30 g) was subjected to normal-phase HPLC (95:5 hexane−EtOAc) affording **7b**, (+)-**2a**, and a mixture of **11** and **12**. After two more rounds of HPLC purification (reversed-phase: step 1: 95:5 MeOH−H_2O; step 2: 90:10 MeOH−H_2O), the two pure methyl ketones **11** and **12** were obtained. During the second round of HPLC purification of **11** and **12** (i.e., reversed-phase, step 1), diastereomers **9** and **10** were also isolated. Next work was conducted on a second PNG organism, coll. no. 91175. The hexane extract (0.7 g) was subjected to gel permeation chromatography (2:3 CH_2Cl_2−MeOH) followed by reversed-phase HPLC of the fourth-eluting fraction (0.30 g), affording several pure compounds including (−)-**1a**, (+)-**1b**, **2b**, **7a**, **7b**, **8**, and **11**.

Stereochemical Analysis. The relative stereochemistry proposed in all of the norterpene peroxides reported is based on the empirical rules for C2, C3, and C6 relative stereochemistry introduced by Capon and MacLeod.[6a,b] Additional insights came by extrapolation from diastereomeric, enantiomeric, and analogous structures of known absolute stereochemistry. The empirical

rules we adopted from those originally proposed are as follows. First, the ^{13}C NMR shift of the C6 tertiary methyl reveals axial (20.5−20.9 ppm) or equatorial (δ 23.5−24.0) orientation. Second, the ^1H NMR shift of C2 secondary methyl reveals whether C2 and C3 are in an erythro configuration (*R*,*R* or *S*,*S*; δ 1.13−1.14) or a threo configuration (*R*,*S* or *S*,*R*; δ 1.22−1.24). Last, the $^3J_{HH}$ values to H3 establishes its axial or equatorial nature.

(−)-Muqubilin A (1a): yellow oil, 93 mg (15.8% of 91175 hexane extract); $[\alpha]_D$ −35.6° (*c* 9.8, $CHCl_3$); IR (neat) ν 2936, 1712, 1455, 1377, 1239, 1202, 1170, 1013 cm^{-1}; HRCIMS *m*/z $[M - H]^+$ 391.2831 ($C_{24}H_{39}O_4$, Δ 1.8 mmu of calcd); ^1H NMR ($CDCl_3$) spectrum is identical to that previously published;[4] ^{13}C NMR data shown in Table 2.

(+)-Epimuqubilin A (1b): yellow oil, 8.8 mg (1.5% of 91175 hexane extract); $[\alpha]_D$ +61.7° (*c* 0.7, $CHCl_3$); IR (neat) ν: 2938, 1713, 1454, 1378, 1266, 1197, 1087, 1000, 737 cm^{-1}; LRAPCIMS *m*/z $[M - H]^+$ 391 (23); ^1H NMR ($CDCl_3$) looks identical to that for (−)-**1a**, with the exception of δ 1.15 s (Me23), 1.20 br s (Me24); ^{13}C NMR is shown in Table 2.

Nuapapuin B (7a,b): 7a: clear colorless oil; 8.9 mg (4.4% of 89042 CH_2Cl_2 extract); $[\alpha]_D$ +45.0° (*c* 0.46, $CHCl_3$); IR (neat) ν 2934, 1714, 1455, 1374, 1251, 1213,-1010, 886 cm^{-1}; HRCIMS *m*/z $[M + H]^+$ 325.2379 ($C_{19}H_{33}O_4$, Δ −1.0 mmu of calcd); ^1H NMR (CD_3OD) δ 2.39 m (H2), 4.13 dt, *J* = 3.5, 8.5 Hz (H3), 1.19−1.80 (H4−H13), 2.02 m ($H_2$11), 1.09 d (Me15), 1.04 s (Me16), 4.76 d/4.57 d, *J* = 2.5 Hz (H17), 0.91 s (Me18), 0.83 s (Me19); ^{13}C NMR are shown in Table 1.

7b: clear colorless oil; 61.0 mg (6.1% of 95150 hexane extract); $[\alpha]_D$ +39.0° (*c* 1.74, $CHCl_3$); IR (neat) ν 2933, 1742, 1454, 1375, 1261, 1199, 1162, 1008, 888 cm^{-1}; HRCIMS *m*/z $[M - H]^+$ 337.2379 ($C_{20}H_{33}O_4$, Δ 0.0 mmu of calcd); ^1H NMR ($CDCl_3$) δ 2.58 p, *J* = 7.5 Hz, 4.22 m (H3), 1.20−2.01 (H4−H13), 1.15 d, *J* = 7.5 Hz (Me15), 1.10 s (Me16), 4.75 d/4.57 d, *J* = 2.5 Hz (H17), 0.92 s (Me18), 0.85 s (Me19), 3.69 s (OMe); ^{13}C NMR data are shown in Table 1.

Epinuapapuin B (8): clear colorless oil; 18 mg (3.0% of 91175 hexane extract); $[\alpha]_D$ −41.6° (*c* 1.5, $CHCl_3$); IR (neat) ν 2940, 1734, 1454, 1375, 1252, 1194, 1161 cm^{-1}; HRCIMS *m*/z $[M + H]^+$ 339.2530 ($C_{20}H_{35}O_4$, Δ 0.5 mmu of calcd); ^1H NMR (CD_3OD) δ 2.65 t, *J* = 6.5 Hz (H2), 4.06 dt, *J* = 3.0, 8.5 Hz (H3), 1.22−1.66 ($H_2$4-$H_2$8, H_2-12, $H_2$13), 1.65 m (H9), 2.02 m ($H_2$11), 1.18 d, *J* = 7.0 Hz (Me15), 1.24 s (Me16), 4.76 d/4.53 d, *J* = 2.5 Hz (H_2-17), 0.92 s (Me18), 0.85s (Me19), ($CDCl_3$) δ 2.62 p, *J* = 7.5 Hz (H2), 4.11 dt, *J* = 3.5, 8.0 Hz (H3), 1.99 m (H_2-11), 1.20−1.65 ($H_2$4−$H_2$8, $H_2$12, $H_2$13), 1.24 d, *J* = 7 Hz (Me15), 1.28 s (Me16), 4.74 d/4.51 d, *J* = 2.4 Hz (H_2-17), 0.90 s (Me18), 0.83 s (Me19), 3.69 s (OMe); ^{13}C NMR data shown in Table 1.

Muqubilin B (9): clear colorless oil, 0.9 mg (0.09% of 95150 hexane extract); $[\alpha]_D$ −15.5° (*c* 0.54, $CHCl_3$); IR (neat) ν 3446, 2940, 1731, 1453, 1378, 1254, 1200, 1160, 1046 cm^{-1}; HRCIMS *m*/z $[M - H]^+$ 421.2944 ($C_{25}H_{41}O_5$, Δ 1.0 mmu of calcd); ^1H NMR ($CDCl_3$) δ 2.66 br s (H2), 4.14 dt, *J* = 4.0, 8.0 Hz (H3), 5.12 t, *J* = 5.0 Hz (H9), 0.98 s (Me19), 0.90 s (Me20), 4.90 s/4.85 s (H_2-21), 1.62 s (Me22), 1.30 s (Me23), 1.27 d, *J* = 7.0 Hz (Me24), 3.71 s (OMe).

Epimuqubilin B (10): clear colorless oil, ca. 1.5 mg (0.15% of 95150 hexane extract); $[\alpha]_D$ +14.8° (*c* 0.27, $CHCl_3$); IR (neat) ν 3452, 2931, 1737, 1737, 1456, 1376, 1254, 1195, 1158, 1052 cm^{-1}; ESMS *m*/z $[M + Na]^+$ 445.3 (85), $[M + K]^+$ 461.3 (100), $\{[M + Na + K]/2\}^{2+}$ 242.3 (30); ^1H NMR (C_6D_6) δ 2.47 p, *J* = 7.0 Hz (H2), 4.31 dt, *J* = 2.8, 9.4 Hz (H3), 1.20−1.50 ($H_2$4, $H_2$5, H_2-16, $H_2$17), 2.03 m/1.45 m ($H_2$7), 2.28 m/2.03 m ($H_2$8), 5.35 br t *J* = 5.0 Hz (H9), 2.19 m/1.89 m ($H_2$11), 1.89 m/1.73 m ($H_2$12), 2.19 m/1.89 m ($H_2$15), 0.98 s (Me19), 0.90s (Me20), 4.98 br t/4.91 br t *J* = 1.5 Hz ($H_2$21), 1.66 s (Me22), 1.00 s (Me23), 0.94 d, *J* = 7.5 Hz (Me24), 3.32 s (OMe), ($CDCl_3$) δ 2.59 p, *J* = 6.2 Hz (H2), 4.25 q, *J* = 6.7 Hz (H3), 5.17 t, *J* = 6.2 Hz (H9), 0.99 s (Me19), 0.91 s (Me20), 4.96 s/4.86 s ($H_2$21), 1.64 s (Me22), 1.13 (Me23), 1.16 d, *J* = 6.2 Hz (Me24), 3.71 s (OMe); ^{13}C NMR are shown in Table 2.

Muquketone (11): clear colorless oil; 7.0 mg (0.7% of 95150 hexane extract); IR (neat) ν 3401, 2930, 1715, 1667, 1454, 1360, 1160 cm^{-1}; HRCIMS *m*/z $[M - OH]^+$ 245.2267 ($C_{18}H_{29}$, Δ 0.2 mmu of calcd); ^1H NMR ($CDCl_3$) δ 2.15 s (Me1), 2.48 t, *J* = 7.5 Hz ($H_2$3), 2.28 q, *J* = 7.5 Hz ($H_2$4), 5.11 t, *J* = 7.5 Hz (H5), 2.05 t, *J* = 6.0 Hz ($H_2$7), 2.04 t, *J* = 6.0 Hz ($H_2$8), 1.91 t, *J* = 6.0 Hz (H_2-11), 1.58 m ($H_2$12), 1.42 m ($H_2$13), 1.61 s (Me15), 1.00 s (Me16), 1.00 s (Me17), 1.67 s (Me18); ^{13}C NMR ($CDCl_3$) δ 30.1 q (C1), 209.0 s (C2), 43.9 t (C3), 22.5 t (C4), 122.0 d (C5), 137.5 s (C6), 40.3 t (C7), 27.9 t (C8), 137.1 s (C9), 127.1 s (C10), 32.9 t (C11), 19.6 t (C12), 40.0 t (C13), 35.1 s (C14), 19.9 q (C15), 28.7 q (C16), 28.7 q (C17), 16.1 q (C18).

Conversion of 7a to 3. NMR spectra of the hexane extract (10 g) of a fourth specimen of *D.* cf. *spinopoculum* (PNG, coll. no. 91172) suggested **7a** was present as a minor component. The entire extract was treated with CH_2N_2, and subsequent reversed-phase HPLC yielded **7b** (800 mg; $[\alpha]_D$ +43.7°: *c* 3.2, $CHCl_3$). An aliquot (360 mg) of this oil was covered with CH_2Cl_2 containing a stoichiometric amount of *p*-TsOH and refluxed (2 h) until the solution turned black. The organic layer was washed with 5% $NaHCO_3$ and filtered through Si gel. HPLC purification (92:8 hexane−EtOAc) afforded pure **3** (98 mg; $[\alpha]_D$ +35.2° *c* 0.2, $CHCl_3$).

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Supporting Information Available: Table of all zones of inhibition measured for 11 structures against eight tumor cell-lines in the soft agar assay, table of GI_{50}s measured in the NCI assay for more than 60 cell lines, table of all published norterpene peroxides from marine sponges, and taxonomy of sponges producing related compounds (5 pages). Ordering information is given on any current masthead page.

References and Notes

(1) Kashman, Y.; Rotem, M. *Tetrahedron Lett.* **1979**, 1707−1708.

(2) (a) Albericci, M.; Collart−Lempereur, M.; Braekman, J. C.; Daloze, D.; Tursch, B. *Tetrahedron Lett.* **1979**, 2687−2690. (b) Albericci, M.; Braekman, J. C.; Daloze, D.; Tursch, B. *Tetrahedron* **1982**, *38*, 1881−1890.
(3) Sokoloff, S.; Halevy, S.; Usieli, V.; Colorni, A.; Sarel, S. *Experientia* **1982**, *38*, 337−338.
(4) Nuapapuin A was formerly referred to as methyl nuapapuanoate; Manes, L. V.; Bakus, G. J.; Crews, P. *Tetrahedron Lett.* **1984**, *25*, 931−934.
(5) Reexamination of our voucher by Dr. M. C. Diaz (UCSC, Institute of Marine Sciences) indicates it to be *Diacarnus* cf. *spinopoculum* (family Latrunculiidae, order Poecilosclerida). The family Latrunculiidae can arguably be placed in orders Hadromerida or Poecilosclerida: Kelly-Borges, M.; Vacelet, J. *Mem. Queensl. Mus.* **1995**, *38*, 477−503.
(6) (a) Capon, R. J.; MacLeod, J. K. *Tetrahedron* **1985**, *41*, 3391−3404. (b) Capon, R. J. In *Studies in Natural Products Chemistry*; Atta-ur-Rahman, Ed.; Elsevier Science: Amsterdam, 1991, Vol. 9; pp 15−33. (c) Faulkner, D. J.; He, H.; Lu, H. S. M.; Clardy, J. *J. Org. Chem.* **1991**, *56*, 2112−2115. (d) Capon, R. J. *J. Nat. Prod.* **1991**, *54*, 190−195.
(7) Tanaka, J.; Higa, T.; Suwanborirux, K.; Kokpol, U.; Bernardinelli, G.; Jefford, C. W. *J. Org. Chem.* **1993**, *58*, 2999−3002.
(8) Capon, R. J.; MacLeod, J. K.; Willis, A. C. *J. Org. Chem.* **1987**, *52*, 339−342.
(9) Polyketide peroxides: (a) Quiñoà, E.; Kho, E.; Manes, L. V.; Crews, P.; Bakus, G. J. *J. Org. Chem.* **1986**, *51*, 4260−4264. (b) Varoglu, M.; Peters, B. M.; Crews, P. *J. Nat. Prod.* **1995**, *58*, 27−36. (c) Ichiba, T.; Scheuer, P. J.; Kelly-Borges, M. *Tetrahedron* **1995**, *51*, 12195−12202; U.S. patent 5514705 A. (d) Horton, P. A.; Longley, R. E.; Kelly-Borges, M.; McConnell, O. J.; Ballas, L. M. *J. Nat. Prod.* **1994**, *57*, 1374−1381. (e) Rudi, A.; Kashman, Y. *J. Nat. Prod.* **1993**, *56*, 1827−1830. (f) Davidson, B. S. *Tetrahedron Lett.* **1991**, *32*, 7167−7170. (g) Gunasekera, S. P.; Gunasekera, M.; Gunawardana, G. P.; McCarthy, P.; Burres, N. *J. Nat. Prod.* **1990**, *53*, 669−674. (h) Toth, S. I.; Schmitz, F. J. *J. Nat. Prod.* **1994**, *57*, 123−127. Norterpene Peroxides: (i) See Tanaka *et al.*[7]; patent appl., EP 610076 A1.
(10) (a) Valeriote, F.; Corbett, T.; LoRusso, P.; Moore, R. E.; Scheuer, P.; Patterson, G.; Paul, V.; Grindey, G.; Bonjouklian, R.; Pearce, H.; Suffness, M. *Intl. J. Pharmacog.* **1995**, *33 (Suppl)*, 59−66. (b) Valeriote, F.; Corbett, T.; Edelstein, M.; Baker, L. *Cancer Invest.* **1996**, *14*, 124−141. (c) Corbett, T. H.; Valeriote, F. A.; Polin, L.; Panchapor, C.; Pugh, S.; White, K.; Lowichik, N.; Knight, J.; Bissery, M.-C.; Wozniak, A.; LoRusso, P.; Biernat, L.; Polin, D.; Knight, L.; Biggar, S.; Looney, D.; Demchik, L.; Jones, J.; Jones, L.; Scott, B.; Palmer, K.; Essenmacher, S.; Lisow, L.; Mattes, K. C.; Cavanaugh, P. R.; Rake, J. B.; Baker, L. In *Cytotoxic Anticancer Drugs: Models and Concepts for Drug Discovery and Development*; Valeriote, F. A., Corbett, T. H., Baker, L. H., Eds.; Kluwer Academic: Norwell, MA, 1992; pp 35−87.
(11) For our standard extraction procedure see: Rodríguez, J.; Nieto, R. M.; Crews, P. *J. Nat. Prod.* **1993**, *56*, 2034−2040.
(12) Structure **11** was previously described as a synthetic product: Semenovski, A. V.; Smit, V. A.; Kucherov, V. F. *Dokl. Akad. SSSR.* **1965**, *160*, 1097−1101.
(13) A partial listing of these models includes the following: (a) zaatirin: Rudi, A.; Kashman, Y. *J. Nat. Prod.* **1992**, *55*, 1408−1414. (b) oceanapamine: Boyd, K. G.; Harper, M. K.; Faulkner, D. J. *J. Nat. Prod.* **1995**, *58*, 302−305. (c) agellesine E: Wu, H.; Nakamura, H.; Kobayashi, J.; Ohizumi, Y.; Hirata, Y. *Tetrahedron Lett.* **1984**, *25*, 3719−3722. (d) pallescensone: Cambie, R. C.; Craw, P. A.; Bergquist, P. R.; Karuso, P. *J. Nat. Prod.* **1987**, *50*, 948−949.
(14) Cytotoxicity measurements for (+)-**1b** may be unreliable because of its instability.
(15) Hollingshead, M. G.; Alley, M. C.; Camalier, R. F.; Abbot, B. J.; Mayo, J. G.; Malspeis, L.; Grever, M. R. *Life Sci.* **1995**, *57*, 131−141.
(16) Munro, M. H. G.; Blunt, J. W.; Lake, R. J.; Litaudon, M.; Battershill, C. N.; Page, M. J. In *Sponges in Time and Space*; van Soest, R. W. M., van Kempen, Th.M. G., Braekman, J. C., Eds.; A. A. Balkema: Rotterdam, 1994; pp 473−484.
(17) Behr, D.; Wahlberg, I.; Nishida, T.; Enzell, C. R. *Acta Chem. Scand. B* **1977**, *31*, 609−613.
(18) Hefner, J.; Rubenstein, S. M.; Ketchum, R. E. B.; Gibson, D. M.; Williams, R. M.; Croteau, R. *Chem. Biol.* **1996**, *3*, 479−489.

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