Diacarperoxides, Norterpene Cyclic Peroxides from the Sponge *Diacarnus megaspinorhabdosa*

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Chemical investigation of the hexane extract of the sponge *Diacarnus megaspinorhabdosa* provided a series of new norterpene derivatives including three norditerpene cyclic peroxides, diacarperoxides A, B, and C (**1** to **3**), four norsesterterpene cyclic peroxides, diacarperoxides D to G (**4** to **7**), and the acyclic norsesterterpene diacardiol A (**8**). In addition, known norterpene peroxide congeners were also isolated, which included a known norsesterterpene cyclic peroxide (**9**), nuapapuin A methyl ester (**10**), epimuqubilin B (**11**), methyl-2-epinuapapuinoate (**12**), and methyl diacarnoate A (**13**). The structures of the new compounds were established on the basis of one- and two-dimensional NMR spectroscopic studies (^{1}H, ^{13}C, COSY, HMQC, HMBC, and ROESY) as well as by mass spectrometric analyses. The relative configuration of chiral centers at C-2, C-3, and C-6 was assigned by established empirical rules. All compounds were evaluated for their cytotoxic properties *in vitro* using several human as well as murine cancer cell lines.

Terpene peroxides are a fascinating class of compounds isolated from both plants and marine organisms.[1] There are several families of norterpene cyclic peroxides that are classified structurally according to their carbon skeleton framework as acylic, monocyclic, or bicyclic.[2] Norterpene peroxide acids and their methyl esters have been isolated frequently from marine sponges of different genera, which include *Prianos*,[3,4] *Mycale*,[5] *Sigmosceptrella*,[6] *Latrunculia*,[7] and *Diacarnus*.[8,9] Interest has been focused on terpene peroxides as they frequently possess a variety of biological activities such as ichthyotoxicity,[6,12] sea urchin egg cell division inhibitory properties,[3] cytotoxicity,[1] and antimicrobial,[10,11] antiviral,[13] antitoxoplasmodic,[14] and antimalarial activity.[8]

Here we have focused on the lipophilic fraction of the methanolic extract of the Indonesian sponge *Diacarnus megaspinorhabdosa*. The dried methanolic extract of the freeze-dried sponge tissue was subjected to solvent/solvent partitioning between *n*-hexane, EtOAc, BuOH, and H_2O. The hexane fraction was successively chromatographed over normal- and reversed-phase silica gel columns by flash and vacuum liquid chromatography to afford three new norditerpene cyclic peroxide methyl esters (**1**−**3**), four new norsesterterpene cyclic peroxides (**4**−**7**), and a new acyclic norsesterterpene (**8**). Known congeners were also isolated, which included a norsesterterpene peroxide (**9**) previously described from an unidentified Australian sponge,[10] nuapapuin A methyl ester (**10**),[3] epimuqubilin B (**11**) (formerly known as methyl nuapapuanoate),[1] methyl-2-epinuapapuanoate (**12**)[10] and methyl diacarnoate A (**13**).[9b] NMR data of the isolated known compounds were compared to previously published data (see Supporting Information). The structure determination of the new compounds was based on 1D and 2D NMR analyses and on their MS data. Capon and Macleod's empirical rules[10,15] have been extensively utilized in this study to establish the relative configuration at C-2, C-3, and C-6 of the various compounds.

Results and Discussion

Diacarperoxide A (**1**) was isolated as a colorless oil. The molecular formula $C_{20}H_{32}O_5$ for **1** was confirmed from the HREIMS molecular ion peak at *m*/*z* 352.2250 $[M]^+$, which required five degrees of unsaturation. The ^{1}H and ^{13}C NMR data (Table 1) of **1** were comparable to nuapapuin A methyl ester (**10**).[3] The presence of a peroxide ring system was evident from signals at δ 4.28/81.1 for H-3/C-3 and 79.5 for C-6. Compound **1** featured an increase in 14 mass units and an additional degree of unsaturation compared to **10**. In addition, its MS fragmentation pattern differed significantly from that of the known congener **10**. For example, the base peak at *m*/*z* 137 attesting to the cyclohexene moiety observed for **10** was absent in the EIMS of **1**. The ^{13}C NMR spectrum of **1** revealed the presence of 20 carbons as in nuapapuin A methyl ester (**10**), with the appearance of new signals at δ 164.7 (s, C-9), 130.0 (s, C-10), 199.0 (s, C-11), 34.2 (t, C-12), and 37.4 (t, C-13). These signals were virtually superimposable to those found in the cyclohexenone moiety of methyl diacarnoate A (**13**).[9b] The five degrees of unsaturation, implied by the molecular formula, were attributed in terms of the ^{13}C data to one ketone function at δ 199.0, a methyl ester at δ 174.1, a carbon−carbon double bond at δ 130.0 and 164.7 (as part of a 2,2-dimethyl-6-methylene cyclohexyl moiety), and the peroxide ring already described above.

The ^{1}H and ^{13}C NMR data of **1** were comparable but not identical to those of methyl diacarnoate A (**13**),[9b] and the COSY spectrum indicated that similar spin systems were present. The connectivity of the cyclohexenone function with that of the C_1−C_8 region was established through the HMBC correlations of CH_2-8 with C-9 and C-10 of the cyclohexenone unit. This unambiguously led to the elucidation of the planar structure of **1**, representing a new diastereoisomer of methyl diacarnoate A (**13**) and for which we propose the name diacarperoxide A.

To determine the relative stereochemistry, we followed a set of empirical rules developed by Capon and Macleod[10,15] for similar terpene peroxides. The stereochemistry at C-2 relative to C-3 can be attributed on the basis of the ^{1}H NMR shift of 2-CH_3. If the proton resonance for 2-CH_3 is observed at δ_H 1.13 to 1.12, the *erythro* stereochemistry is present, while a *threo* configuration is assigned when the chemical shift is between δ_H 1.24 and 1.26. On the other hand, the configuration of H-3 follows from its vicinal coupling constants. If $J_{3,4eq} + J_{3,4ax}$ amounts to approximately 8 Hz, it is equatorially oriented, while a sum of approximately 12

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Table 1. ^{1}H NMR and ^{13}C NMR Data of **1**–**3** in $CDCl_3$ at 500 and 125 MHz, Respectively

	1			**2**			**3**		
	δ_H, mult. (*J* in Hz)	δ_C	HMBC (H→C)	δ_H, mult. (*J* in Hz)	δ_C	HMBC (H→C)	δ_H, mult. (*J* in Hz)	δ_C	HMBC (H→C)
1		174.1	C		174.3	C		174.0	C
2	2.54 q (6.6)	42.7	CH 1, 4, 19	2.49 quin (7.2)	42.6	CH	2.68 m	42.7	CH
3	4.28 ddd (7.6, 6.3, 1.6)	81.1	CH 2	4.24 dt (8.8, 2.2)	81.3	CH	4.11 dt (8.8, 6.0)	81.2	CH
4	1.68 m	22.6	CH_2 5, 6, 7	1.65 m	22.5	CH_2	1.73 m	23.3	CH_2 4
5	1.73 m	31.8	CH_2 3, 4	1.73 m	33.4	CH_2	1.67 m	32.0	CH_2
6		79.5	C		79.2	C		79.4	C
7	1.46 dd (8.2, 3.8)	32.9	CH_2 18	2.24 ddd (14.5, 11.0, 5.0) 1.57 m	26.9	CH_2	1.84 m 1.68 m	33.0	CH_2
8	2.36 dt (17.0, 3.8) 2.15 ddd (17.0, 8.2, 3.8)	24.5	CH_2 5, 7, 9, 10	2.65 ddd (17.3,11.6, 6.6) 2.45 m	30.9	CH_2	2.59 ddd (15.7, 10.1,5.8) 2.50 ddd (15.8, 10.1, 5.8)	30.4	CH_2
9		164.7	C		215.7	C		215.0	C
10		130.0	C		47.6	C		47.5	C
11		199.0	C	1.48 m	39.2	CH_2 12	1.47 m	39.1	CH_2 10, 12
12	2.45 t (6.9)	34.2	CH_2 4, 9, 11	1.43 m	19.0	CH_2	1.42 m	19:0	CH_2 11, 13
13	1.82 t (6.9)	37.4	CH_2 9, 10, 11, 15, 16	2.41 dt (7.2, 1.9)	43.9	CH_2 12	2.40 t (6.9)	43.8	CH_2 12, 14
14		36.5	C		208.6	C		208.5	C
15	1.19 s	26.8	CH_3 10, 14, 16	2.12 s	29.9	CH_3	2.12 s	29.9	CH_3 14
16	1.16 s	26.8	CH_3 10, 14, 15	1.12 s	24.3	CH_3 10	1.12 s	24.5	CH_3 10, 11, 19
17	1.78 s	11.4	CH_3 9, 10, 11, 12	1.12 s	24.3	CH_3 10	1.12 s	24.5	CH_3 10, 11, 18
18	1.18 s	23.5	CH_3 5, 7	1.07 s	23.8	CH_3 6	1.26 s	20.8	CH_3 6, 7
19	1.13 d (6.9)	12.5	CH_3 1, 2, 3	1.11 d (6.9)	12.8	CH_3 1, 2	1.25 d (6.0)	13.6	CH_3 1, 2, 3
OCH_3	3.70 s	51.9	CH_3 1	3.68 s	51.9	CH_3 1	3.71 s	51.8	CH_3 1

Hz implies axial orientation. Finally, the configuration of the C-6 methyl group can be derived from its ^{13}C NMR shift, with a signal between 23.5 and 24.0 ppm indicating equatorial and a signal between 20.5 and 20.9 ppm axial orientation.[10,15] On this basis, diacarperoxide A (**1**) was assigned the 2*R**, 3*R**, 6*R** configuration, identical to that found in nuapapuin A methyl ester (**10**).[16]

Diacarperoxide B (**2**) was obtained as a colorless oil and showed a HREIMS molecular ion peak at *m*/*z* 370.2344 $[M]^+$, which confirmed the molecular formula $C_{20}H_{34}O_6$ and indicated an increase in 18 mass units compared to **1**. The ^{1}H and ^{13}C NMR data of **2** and **1** were quite similar for the C_1−C_6 cyclic peroxide moiety, but the signals associated with the cyclohexene or cyclohexenone moiety found in **1** and **10**, respectively, were not present. Instead, new signals for two aliphatic carbonyls at δ 215.7 (s, C-9) and 208.6 (s, C-14) were observed, together with a signal for a methyl ketone residue (δ 2.12 and 29.9), suggesting the presence of an open-chain dicarbonyl function,[17] which could be derived from oxidative opening of the cyclohexene ring at the C-9/C-10 olefinic bond.[14] The ^{1}H and ^{13}C NMR data of **2** were basically identical to those found in aikupikoxide C isolated from *Diacarnus erythraenus*.[8] However, the observed $[\alpha]_D$ value of **2** was −55 (*c* 0.06, $CHCl_3$), while that of aikupikoxide C was +88 (*c* 0.2, CH_2Cl_2),[8] which implied that **2** is the enantiomer of the previously reported derivative; hence **2** was assigned the 2*S**, 3*S**, 6*R** configuration.

Diacarperoxide C (**3**) was obtained as a colorless oil. The HREIMS showed a molecular ion peak at *m*/*z* 370.2354 $[M]^+$, which was compatible with the molecular formula $C_{20}H_{34}O_6$ and thus identical to that of **2**. Similarly, the EIMS fragmentation of **3** was reminiscent of that of **2**, and the NMR data were similar to both **2** and aikupikoxide C.[8] These findings imply that **3** was a hitherto undescribed diastereoisomer of diacarperoxide B (**2**), with differences in the NMR spectra observed for the cyclic peroxide unit suggesting that the change occurred at this moiety. H_3-19 resonated at δ 1.25, H_3-18 appeared at δ_H 1.26 and δ_C 20.8, and the sum of the vicinal coupling constants of H-3 amounted to 6.9 Hz, thus implying a 2*R**, 3*S**, 6*S** configuration for **3** as outlined above.[10,15]

Diacarperoxide D (**4**) was isolated as a colorless oil and showed a HREIMS molecular ion peak at *m*/*z* 392.2924 $[M^+]$, which was compatible with the molecular formula $C_{24}H_{40}O_4$. This required five degrees of unsaturation, which were attributed to an olefinic double bond, one endocyclic peroxide unit, one carboxylic acid carbon, and two fused bicyclic moieties. The base peak at *m*/*z* 191 $[M - C_{14}H_{23}]^+$ could be rationalized as arising through bisallylic cleavage, indicating the presence of a bicyclic unit, tetramethyl octahydronaphthalene.[18] The ^{1}H and ^{13}C NMR data of **4** for the C_1−C_8 region were comparable to those of the previous congeners. ^{13}C NMR and DEPT spectra revealed the presence of 24 carbons consisting of six methyl carbons, nine methylenes, three methines, and six quaternary carbons. ^{1}H and ^{13}C NMR resonances of the *trans*-fused bicyclic moiety were in excellent agreement with those found in luffarins A to O,[18a] mycaperoxide G methyl ester,[2b] deoxydiacarnoate B benzyl ester,[9a] and the norsesterpene diene acid isolated from *Latrunculia brevis*.[18b] However, besides the absence of a methoxyl and benzyl signals as found in mycaperoxide G methyl ester[2b] and deoxydiacarnoate B benzyl ester,[9a] respectively, differences in the ^{13}C NMR data could be observed for the cyclic peroxide moiety. *C*H_2-7 was shifted 7 ppm upfield at δ_C 33.6, while *C*H_3-20 resonated downfield at δ_C 23.5, which suggested its equatorial orientation in **4** instead of axial, as in its known congeners.[2b,9a] Due to overlapping resonances, it was not plausible to define the relative configuration in the decalin system through its NOESY spectra. The empirical rule[10,15] described above and the molar-rotation additivity rule[19] were then utilized to determine the stereochemistry in compound **4**. The molar-rotation additivity rule has been previously applied to a group of related congeners[2b,9a,18b] to characterize their relative configuration. Compound **4** was divided

Table 2. ^{1}H NMR Data of **4**−**9** in $CDCl_3$ at 500 MHz (δ_H, mult. (*J* in Hz))

H no.	4	5	6	7	8
1a					0.95 t (8.0)
1					1.48 m
2	2.59 d (6.9)	2.54 q (7.6)	2.55 bs	2.52 q (7.6)	1.49 m
3	4.27 dt (8.8, 3.2)	4.30 dt (7.6, 6.6)	4.31 dt (7.6, 6.6)	4.26 dt (7.6, 6.6)	3.54 m
4	1.67 m	1.71 m	1.71 m	1.72 m	1.52 m
5	1.52 m	1.73 m	1.72 m	1.67 m	1.54 m
7	1.48 m	2.21 dd (4.4, 3.2)	2.21 dd (3.8, 2.9)	1.48 m	1.53 m
	1.39 m	1.45 m	1.45 m		
8	1.99 m	2.37 dd (13.2,4.1)	2.37 dd (14.5, 3.5)	2.33 ddd (17.2, 12.0, 3.8)	2.07 m
		2.16 dd (13.2, 3.5)	2.15 dd (13.9, 3.2)	2.15 ddd (17.0, 13.2, 3.8)	
9					5.15 t (6.6)
11	2.02 m				2.02 m
12	1.91 m	2.51 dd (17.3, 3.5)	2.49 dd (17.3, 3.4)	2.50 dd (17.0, 3.8)	2.00 m
		2.35 dd (17.3, 3.1)	2.33 d (17.3)	2.37 dd (17.0, 12.8)	
13	1.11 m	1.70 m	1.67 m	1.66 m	
15	1.95 m	1.49 m	1.47 m	1.46 m	1.57 m
		1.25 m	1.25 m	1.19 m	
16	1.60 m	1.66 m	1.64 m	1.58 m	1.41 m
	1.40 m	1.58 m	1.57 m		
17	1.98 m	2.07 dd (14.8, 2.5)	2.05 d (11.9)	2.05 m	1.90 t (6.3)
		1.39 dd (12.9, 3.5)	1.35 dd (13.2, 3.1)	1.35 dd (12.6, 3.0)	
19	1.17 d (6.9)	1.19 d (7.5)	1.18 d (6.9)	1.14 d (6.9)	
20	1.15 s	1.18 s	1.18 s	1.17 s	1.20 s
21	1.56 s	1.76 s	1.75 s	1.77 s	1.66 s
22	0.94 s	1.08 s	1.08 s	1.08 s	1.60 s
23	0.83 s	0.87 s	0.88 s	0.98 s	0.99 s
24	0.88 s	0.92 s	0.91 s	0.92 s	0.99 s
OCH_3				3.70 s	

into fragments I and II. Fragment I covers the chiral centers C-13 and C-18, while fragment II has C-2, C-3, and C-6. Enantiomeric structures representing a discrete chiral unit for each of the fragments were chosen and the molar-rotation angles for each of their configurations was calculated using the formula: $[M]_D = ([\alpha]_D \times MW)/100$. The $[M]_D$ value for compound **4** was calculated by arithmetically adding up the $[M]_D$ values of fragments IA and II (Tables 5 and 6). The results show that these molar-rotation calculations provide the relative configuration as 2*R**, 3*R**, 6*R**, 13*R**, 18*R**, where the *trans*-fused bicyclic unit was confirmed to be an *ent*-labdane moiety. By comparison with the literature data together with those obtained from COSY, HMQC, and HMBC spectra, the structure of **4** was unambiguously elucidated as depicted and named diacarperoxide D.

Diacarperoxide E (**5**) was isolated as a colorless oil and possessed a molecular formula of $C_{24}H_{38}O_5$, as evident from the HREIMS molecular ion peak at *m*/*z* 406.2717. Similar to the pair **1** and **10**, compound **5** showed an increase in 14 mass units compared to diacarperoxide D (**4**). The fragment ion at *m*/*z* 205 suggested the presence of a tetramethyl hexahydronaphthalenone moiety.[9a] The ^{13}C NMR data of **5** were comparable to those of **4** with the exception of an additional aliphatic carbonyl signal at δ 200.7, which was placed at position 11, based on its HMBC correlations with H_2-12 and H_3-21. Correspondingly, in the ^{1}H NMR spectrum downfield shifts as well as simpler multiplicities were observed for H_2-12. The ^{1}H and ^{13}C NMR resonances were almost the same as those of diacarnoate B methyl ester.[9a] The NMR data of **5** correlate very well with those of diacarnoate B methyl ester.[9a] The only difference observed was the equatorial orientation of CH_3-20 in **5**, which was axially oriented in diacarnoate B methyl ester.[9a] Following the strategy described above,[10,15,2b,9a,18b,19] **5** was assigned the 2*S**, 3*S**, 6*S**, 13*S**, 18*S** configuration, where the decalin moiety was characterized to be a labdane unit. This unambiguously led to the elucidation of the structure of **5**, which was named diacarperoxide E.

Diacarperoxide F (**6**) was isolated as a colorless oil. The molecular formula of **6** was determined as $C_{24}H_{38}O_5$, as confirmed by HREIMS through the molecular ion peak at *m*/*z* 406.2720. The fragment ion at *m*/*z* 205 suggested the presence of a tetramethyl hexahydronaphthalenone moiety as found in **5**. The ^{1}H and ^{13}C NMR spectra were very similar to those of **5**. Inspection of the ^{1}H−^{1}H COSY and HMBC spectral data (Tables 2 and 3) indicated that compound **6** has the same carbon skeleton as found for **5**. However, **6** exhibited a dextrorotatory optical rotation. Through the molar-rotation additivity rule,[10,15,2b,9a,18b,19] this indicated that diacarperoxide F was a diastereoisomer of **5** with the relative configuration 2*R**, 3*R**, 6*R**, 13*S**, 18*S**.

Diacarperoxide G (**7**) was isolated as a colorless oil. The HREIMS showed a molecular ion peak at *m*/*z* 420.2860 $[M]^+$ corresponding to $C_{25}H_{40}O_5$, which was an increase of 14 mass units compared to compounds **5** and **6**. The ^{1}H and ^{13}C NMR spectra of **7** (Tables 2 and 3) were almost identical to those of **5** and **6** except for the presence of an additional methoxy group signal at δ 3.70/51.9. The relative configuration of **7** was established as 2*R**, 3*R**, 6*R**, 13*R**, 18*R**.

Diacardiol A (**8**) was isolated as a colorless oil, and its HREIMS spectrum displayed a molecular ion peak at *m*/*z* 364.3340 $[M]^+$, indicating the molecular formula $C_{24}H_{44}O_2$. Upon inspection of the ^{13}C NMR spectrum, the resulting three degrees of unsaturation were attributed to one carbon−carbon double bond and one cyclohexene ring. The EIMS fragment ion at *m*/*z* 332 $[M^+ - 2H_2O]$ suggested the presence of two hydroxyl functions in the molecule. The base peak at *m*/*z* 137 implied the presence of a cyclohexene moiety as in a known norsesterterpene cyclic peroxide (**9**), which was also isolated in this study.[10] The ^{1}H and ^{13}C NMR data (Tables 2 and 3) were comparable to those of **9** and showed similar resonances for the C_9−C_{18} region. However, the signals associated with the cyclic peroxide and the 2-propionic acid function, which were encountered in the terpene cyclic peroxides **1**−**7**, were not observed.[1−14] Specifically, H-3 was markedly shifted upfield (δ 3.54), while the doublet observed for H_3-19 in **1**−**7** was replaced by a methyl triplet at δ 0.95 (H_3-1a). The ^{13}C NMR spectrum showed 24 carbons as in **9**, but lacking the carbonyl signal at δ 174−178 and with the appearance of shifted signals at δ 10.0 (q, C-1a), 31.0 (t, C-1), 30.3 (t, C-2), 73.7 (d, C-3), 37.8 (t, C-4), 41.7 (t, C-5), 72.7 (s, C-6), 29.0 (t, C-7), and 26.9 (q, C-20). The COSY spectrum indicated an enlarged spin system not encountered in the compounds described so far, which formally resulted from opening of the cyclic peroxide and a migration of the methyl group from C-2 to C-1. The 2-methyloctane-2,5-diol was also validated by the

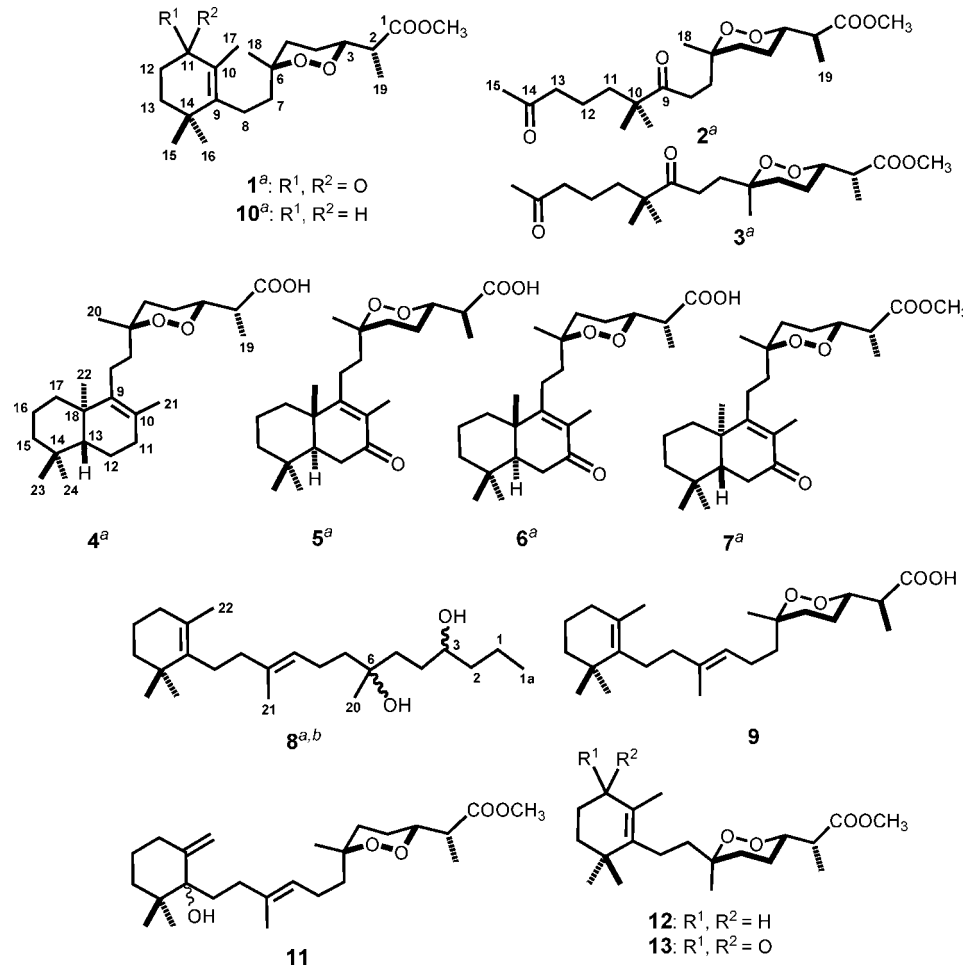
Table 3. ^{13}C NMR and HMBC Data of Compounds **4**−**9** in $CDCl_3$ at 125 and 500 MHz, Respectively

	4			**5**			**6**			**7**			**8**		
	δ_C		HMBC (H→C)	δ_C		HMBC (H→C)	δ_C		HMBC (H→C)	δ_C		HMBC (H→C)	δ_C		HMBC (H→C)
1a													10.0	CH_3	3, 4
1	178.0	C		178.1	C		178.1	C		174.1	C		31.0	CH_2	4, 5
2	42.3	CH	1, 3, 4, 19	42.9	CH	3	42.7	CH		42.7	CH	3,1	30.3	CH_2	4, 5
3	80.8	CH	1, 2, 19	81.4	CH		80.9	CH		81.0	CH		73.7	CH	
4	22.4	CH_2	2, 3	23.1	CH_2		22.5	CH_2		22.6	CH_2		37.8	CH_2	
5	32.5	CH_2	6	33.4	CH_2		32.8	CH_2		33.0	CH_2		41.7	CH_2	
6	80.2	C		80.4	C		79.6	C		79.6	C		72.7	C	
7	33.6	CH_2		33.5	CH_2		33.1	CH_2		32.8	CH_2		29.0	CH_2	9, 24
8	19.0	CH_2	7	23.6	CH_2		23.2	CH_2	6,7,9,10	23.2	CH_2		22.6	CH_2	10, 12
9	140.1	C		168.8	C		168.4	C		168.3	C		123.5	C	9, 12, 23
10	125.7	C		130.6	C		130.0	C		129.9	C		136.5	C	
11	33.6	CH_2	9, 10, 13	200.7	C		200.3	C		200.2	C		40.2	CH_2	10, 13
12	19.1	CH_2	10, 14, 18	35.3	CH_2	11,18	35.2	CH_2	11,13,18	35.2	CH_2	11,13	27.8	CH_2	11, 12
13	51.9	CH	9, 12, 18, 22	50.7	CH		50.3	CH	12,22,24	50.3	CH	14	137.0	CH	
14	33.8	C		33.7	C		33.5	C		33.1	C		35.0	C	
15	41.8	CH_2	13	41.9	CH_2		41.4	CH_2		41.1	CH_2		39.8	CH_2	13, 14, 16, 17, 23, 24
16	21.6	CH_2	18	19.1	CH_2		18.5	CH_2	18	18.5	CH_2		19.5	CH_2	14, 18
17	36.8	CH_2		36.1	CH_2		35.7	CH_2		35.7	CH_2		32.7	CH_2	13, 15, 16, 18
18	39.1	C		41.5	C		41.1	C		41.1	C		126.9	C	
19	12.3	CH_3	1, 2, 3	12.8	CH_3	1, 2, 3	12.5	CH_3		12.5	CH_3	1, 2, 3			
20	23.5	CH_3	5, 6, 7	23.8	CH_3	5, 6	23.5	CH_3	6,7	23.5	CH_3	6, 7	26.9	CH_3	5, 6, 7, 8
21	19.4	CH_3	9, 10, 11	11.6	CH_3	9, 10, 11	11.2	CH_3	9,10,11	11.2	CH_3	9, 10, 11	16.0	CH_3	10, 11
22	20.0	CH_3	9, 13, 17, 18	18.5	CH_3	9, 13, 17,18	17.9	CH_3	9,13,18,17	17.9	CH_3	9, 13, 17, 18	19.8	CH_3	13, 17, 18
23	32.5	CH_3	13,14,15, 24	32.9	CH_3	13,14,15, 24	32.5	CH_3	13, 14, 15, 24	32.5	CH_3	13, 14, 15, 24	28.6	CH_3	14, 18, 19
24	21.7	CH_3	13, 14, 15	21.8	CH_3	13,14,15	21.3	CH_3	13, 14, 15, 23	21.3	CH_3	13, 15, 23	28.6	CH_3	13, 14, 15, 23
OCH_3										51.9	CH_3	1			

Table 4. Assignment of the Relative Stereochemistry at C-2, C-3, and C-6 According to the Empirical Method

		empirical method according to Capon and McLeod						
		stereochemistry of 2-CH_3 relative to H-3 *threo* or *erythro*? as based on the ^{1}H NMR shift of 2-CH_3			relative stereochemistry of H-3 axial or equatorial? as based on the ^{1}H NMR coupling constant of H-3		relative stereochemistry at C-6 axial or equatorial? as based on the ^{13}C NMR shift of 6-CH_3	
compound	$[\alpha]_D$							
diacarperoxide A (**1**)	+53.4	2*R*, 3*R*, 6*R*	1.13	*erythro*	ddd, J = 7.6, 6.3, 1.6 Hz	[axial]	23.5	equatorial
diacarperoxide B (**2**)	−55.0	2*S*, 3*S*, 6*R*	1.11	*erythro*	dt, J = 8.8, 2.2 Hz	[axial]	23.8	equatorial
diacarperoxide C (**3**)	−35.0	2*R*, 3*S*, 6*S*	1.25	*threo*	dt, J = 8.0, 6.0 Hz	[axial]	20.8	axial
diacarperoxide D (**4**)	−11.3	2*R*, 3*R*, 6*R*,	1.17	*erythro*	dt, J = 8.8, 3.2 Hz	[axial]	23.5	equatorial
diacarperoxide E (**5**)	−12.6	2*S*, 3*S*, 6*S*	1.19	*erythro*	dt, J = 7.6, 6.6 Hz	[axial]	23.8	equatorial
diacarperoxide F (**6**)	+40.5	2*R*, 3*R*, 6*R*	1.18	*erythro*	dt, J = 7.6, 6.6 Hz	[axial]	23.5	equatorial
diacarperoxide G (**7**)	+12.6	2*R*, 3*R*, 6*R*	1.14	*erythro*	dt, J = 7.6, 6.6 Hz	[axial]	23.5	equatorial
unnamed cyclic peroxide (**9**)	−20.5	2*S*, 3*S*, 6*S*	1.17	*erythro*	bm	[axial]	23.6	equatorial
nuapapuin A methyl ester (**10**)	+52.7	2*R*, 3*R*, 6*R*	1.12	*erythro*	dt, J = 6.9, 2.8 Hz	[axial]	23.6	equatorial
epimuqubilin B (**11**)	+3.1	2*R*, 3*R*, 6*R*	1.14	*erythro*	q, J = 8.2 Hz	[axial]	24.1	equatorial
methyl-2-epinuapapuinoate (**12**)	−30.3	2*R*, 3*S*, 6*R*	1.25	*threo*	dt, J = 8.2, 4.4	[axial]	20.5	axial
methyl diacarnoate A (**13**)	−52.0	2*R*, 3*S*, 6*R*	1.26	*threo*	dt, J = 8.0, 6.0 Hz	[axial]	20.6	axial

Chart 1



[a]Only the relative stereochemistry is shown for the new compounds **1**−**8**. [b]For convenience, the numbering scheme used is in accordance to known compounds described in the literature.

correlations observed in the HMBC spectrum. The triplet methyl signal showed correlations with C-2 and a four-bond coupling to C-3. H_2-2 correlated with the oxygen-bound C-3 and the methylene C-4. The methyl singlet at δ 1.20 (H_3-20) correlated with C-5, C-6, and C-7. These resonances were comparable to those found in the triol derivatives of muqubilin.[4] The connectivity of 2-methyloctane-2,5-diol with that of C-8 to C-18 was established through the HMBC correlations of H_2-7 with C-20 and C-8. By comparison of the literature data with the data obtained from the COSY, HMQC, and HMBC, the structure of **8** was unambiguously elucidated. Thus, **8** was identified as a novel, rearranged norditerpene for which we propose the name diacardiol A.

The terpene cyclic peroxides previously isolated from the genus *Diacarnus* were described to exhibit significant cytotoxicity.[1] In our search for new biologically active natural products, all of the isolated norterpene peroxides were evaluated for their cytotoxic

Table 5. Calculated Molar Rotations for Fragments in their Specific Enantiomeric Configurations for Compounds **4**, **5**, **6**, and **7**

fragment	configuration, Sign	enantiomeric structure	$[M]_D$
IA-1	(13*R*, 18*R*)−ve	12-oxolabda-8,13(16)-dien-15-oic acid[a]	±222
IA-2	(13*S*, 18*S*)+ve		
IB-1	(13*R*, 18*R*)−ve	15-acetoxy-8,13E-labdadiene-7-one[b]	±155
IB-2	(13*S*, 18*S*)+ve		
II-1	(2*S*, 3*S*, 6*S*)−ve	cyclic peroxide (**9**)	±80.3
II-2	(2*R*, 3*R*, 6*R*)+ve		

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activity. In the brine shrimp assay, nuapapuin A methyl ester (**10**) proved to be the most active congener encountered, while diacardiol A (**8**) was the least active derivative, which was probably due to the lack of the cyclic peroxide moiety in **8** (Table 5). It was also interesting to note that, in further experiments, most of the compounds showed strong or moderate activity against different cancer cell lines such as HeLa human cervix carcinoma, L5178Y mouse lymphoma, and PC12 rat brain tumor cells. The tests were performed using the microculture tetrazolium (MTT) assay,[20] and the results are listed in Table 5. As described in the literature,[9b,21] it was also observed that insertion of a prenyl unit in the central portion of the molecule made norsesterterpenes cyclic peroxides (compounds **4**−**9** and **11**) more active than their norditerpene congeners (compounds **1**−**3**, **12**, and **13**). It has been reported previously that a longer side chain allowed the norsesterterpenes to cross the cell membranes (lipophilicity effect) and afforded a better fit to the receptor (size effect).[9b] Although in our present study we cannot make any concrete conclusion on the SAR of the different norterpene peroxide congeners we have tested, the results of the bioassay clearly showed that the derivatives were quite selective rather than generally cytotoxic.

Experimental Section

General Experimental Procedures. Optical rotation was recorded on a Perkin-Elmer model 341 LC polarimeter. HREIMS were measured on a Finnigan MAT TSQ-7000 mass spectrometer. 1D and 2D NMR spectra (chemical shifts in ppm, coupling constants in Hz) were recorded on Bruker DRX 400, 500, and DMX 600 NMR spectrometers using standard Bruker software and $CDCl_3$ as solvent. NMR spectra were referenced to the solvent signal. Solvents were distilled prior to use, and spectral grade solvents were used for spectroscopic measurements. TLC was performed on precoated TLC plates with silica gel 60 F_{254} (layer thickness 0.2 mm, E. Merck, Darmstadt, Germany).

Materials. The sponge was 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 sponge was of a tough consistency, reddish-pink and yellow color internally. The sponge was collected by diving at a depth of 8 m from Pulau Baranglompo Island close to Makassar in Indonesia in July 1997. The sample was frozen directly after collection and stored at −20 °C. A voucher specimen has been deposited in the Zoological Museum, University of Amsterdam, under the registration no. ZMA POR. 17057.

Isolation. The freeze-dried sponge (200 g dry weight) was extracted several times with MeOH. The total MeOH extract was evaporated to dryness and partitioned between aqueous MeOH, *n*-hexane, EtOAc, and BuOH. The *n*-hexane fraction (4 g) was subjected to normal-phase (Si 60) vacuum liquid chromatography (VLC) by gradient elution using hexane/EtOAc as solvents, and 10 fractions were obtained. The third fraction, obtained with a 80:20 mixture, was subjected to normal-phase flash chromatography again by gradient elution utilizing hexane/CH_2Cl_2/acetone as solvents, and five subfractions were collected. The fractions were further purified using a silica gel column using hexane/EtOAc (90:10) as eluents to afford **1** (3 mg) and **2** (2.5 mg).

The fifth VLC fraction, obtained with a 60:40 mixture, was chromatographed over Sephadex LH-20 using MeOH/CH_2Cl_2 (9:1) as eluent to afford eight subfractions. Subfractions 3 to 6 were subjected to reversed-phase silica gel column chromatography and eluted with MeOH/H_2O (9:1) to obtain **3** (1.8 mg) and **4** (2 mg). Subfractions 7 and 8 were further chromatographed over a silica gel column using hexane/CH_2Cl_2/acetone (9:3:3) as eluting solvents to yield congener **10** (20 mg). The sixth VLC fraction, obtained with a 50:50 mixture, was subjected to silica gel flash column chromatography to yield **12** (12 mg) and **5** (3 mg). The seventh and eighth VLC fractions, obtained

Table 6. Molar Rotations of Different Stereoisomers for Diacarperoxides **4** to **7** Calculated from Fragment Increments According to the Molar-Rotation Additivity Rule

fragments	configuration	calcd $[M]_D$	obsd $[M]_D$	
II-1 + IA-1	2S, 3*S*, 6*S*, 13*R*, 18*R*	−408		
II-1 + IA-2	2S, 3*S*, 6*S*, 13*S*, 18*S*	+36		
II-2 + IA-1	2R, 3*R*, 6*R*, 13*R*, 18*R*	−36	− 44.2	diacarperoxide D (**4**)
II-2 + IA-2	2R, 3*R*, 6*R*, 13*S*, 18*S*	+408		
II-1 + IB-1	2S, 3*S*, 6*S*, 13*R*, 18*R*	−235.3		
II-1 + IB-2	2S, 3*S*, 6*S*, 13*S*, 18*S*	−74.7	−51.9	diacarperoxide E (**5**)
II-2 + IB-1	2R, 3*R*, 6*R*, 13*R*, 18*R*	+74.7	+52.9	diacarperoxide G (**7**)
II-2 + IB-2	2R, 3*R*, 6*R*, 13*S*, 18*S*	+235.3	+164.4	diacarperoxide F (**6**)

Table 7. Cytotoxicity and Brine Shrimp Bioassay Results for Norterpene Peroxides

	EC_{50} (μg/mL)			brine shrimp bioassay results (10 μg/mL)	
compound	L5178Y	HeLa cell	PC12	% mortality (24 h)	% mortality (48 h)
diacarperoxide A (**1**)	>10	not active	not active	35	55
diacarperoxide B (**2**)	10.0	not active	not active	45	55
diacarperoxide C (**3**)	>10	not active	not active	35	55
diacarperoxide D (**4**)	<0.10	0.17	8.10	not tested	not tested
diacarperoxide E (**5**)	<3.0	not active	not active	not tested	not tested
diacarperoxide F (**6**)	0.06	0.60	0.80	not tested	not tested
diacarperoxide G (**7**)	2	>10	>10	50	60
diacardiol A (**8**)	4.80	>10	>10	20	40
unnamed cyclic peroxide (**9**)	0.28	7.20	>10	25	50
nuapapuin A methyl ester (**10**)	0.76	5.50	6.20	100	100
epimuqubilin B (**11**)	<0.10	1	6	45	60
methyl-2-epinuapunoate (**12**)	not active	not active	not active	25	65
methyl diacarnoate A (**13**)	<3.0	not active	not active	not tested	not tested

with 40:60 and 30:70 mixtures, respectively, were pooled and subjected to normal-phase flash column chromatography to afford derivatives **6** (2.8 mg) and **11** (4 mg). The ninth VLC fraction, obtained with a 20:80 mixture, was chromatographed over a silica gel column by gradient elution with hexane/CH_2Cl_2/acetone, and the resulting fractions were purified by HPLC using a reversed-phase column (C18) to yield **7** (4 mg), **8** (4.5 mg), and **9** (3 mg). The tenth VLC fraction, obtained with a 10:90 mixture of hexane/EtOAc, was further purified over a silica gel column to yield **13** (7 mg).

Hydrogenation of the Peroxide Ring. Compounds **10**, **12**, and **13** were hydrogenated to yield their diol derivatives. To a solution of the respective norterpene peroxide derivative [4 mg each in 1 mL of dry diethyl ether] was added $LiAlH_4$ (4 mg) in excess, and the resulting mixture was stirred under reflux for 2 h. The reaction was quenched by the addition of 0.4 mL of 10% HCl and extracted with EtOAc. The reaction products were concentrated under vacuum, then purified by a silica gel column using hexane/EtOAc (9:1) to yield the corresponding diol derivatives.

Mosher Derivatization. Aliquots of the diol derivatives **10**, **12**, and **13** (2 mg each) were dissolved in 0.7 mL of pyridine-d_5 and transferred to NMR tubes. ^{1}H and COSY NMR spectra were measured prior to adding 5 μL of (*R*)-MTPA-Cl and (*S*)-MTPA-Cl reagent (Fluka, Germany), respectively. The tubes were shaken thoroughly and were allowed to stand at room temperature for 72 h. The reaction was monitored by ^{1}H NMR and COSY spectroscopy after every 24 h.[22]

Diacarperoxide A (1): clear, colorless oil; $[\alpha]_D$ +53.4 (*c* 0.25, $CHCl_3$); ^{1}H NMR, ^{13}C NMR, and HMBC data, see Table 1; EIMS *m/z* 352 $[M]^+$ (1.7), 334 (6.2), 265 (8.8), 207 (20), 189 (18), 165 (79), 149 (65), 135 (45), 115 (32), 109 (40), 93 (32), 81 (40), 69 (47), 57 (28), 43 (100); HREIMS *m/z* 352.2250 $[M]^+$ (calcd for $C_{20}H_{32}O_5$ 352.2250).

Diacarperoxide B (2): colorless oil; $[\alpha]_D$ −55 (*c* 0.06, $CHCl_3$); ^{1}H NMR, ^{13}C NMR, and HMBC data, see Table 1; EIMS *m/z* 370 $[M]^+$ (7.7), 339 (6.2), 286 (6.8), 254 (10), 209 (20), 193 (18), 165 (10), 149 (25), 135 (28), 127 (42), 109 (70), 98 (22), 69 (40), 57 (22), 43 (100); HREIMS *m/z* 370.2344 (calcd for $C_{20}H_{34}O_6$ 370.2355).

Diacarperoxide C (3): colorless oil; $[\alpha]_D$ −35 (*c* 0.11, $CHCl_3$); ^{1}H NMR, ^{13}C NMR, and HMBC data, see Table 1; EIMS *m/z* 370 $[M]^+$ (7.5), 339 (6.2), 286 (18), 254 (32), 209 (20), 193 (18), 165 (10), 149 (25), 135 (28), 127 (42), 109 (70), 98 (22), 69 (40), 57 (22), 43 (100); HREIMS *m/z* 370.2354 (calcd for $C_{20}H_{34}O_6$ 370.2355).

Diacarperoxide D (4): colorless oil; $[\alpha]_D$ −11.3 (*c* 0.10, $CHCl_3$);); ^{1}H and ^{13}C NMR data, see Tables 2 and 3; EIMS *m/z* 392 $[M]^+$ (20), 319 (10), 302 (10), 269 (15), 229 (8.2), 205 (24), 191 (100), 163 (18), 149 (25), 135 (28), 121 (38), 109 (70), 95 (52), 69 (48), 57 (50), 43 (52); HREIMS *m/z* 392.2924 (calcd for $C_{24}H_{40}O_4$ 392.2927).

Diacarperoxide E (5): colorless oil; $[\alpha]_D$ −12.6 (*c* 0.3, $CHCl_3$); ^{1}H and ^{13}C NMR data, see Tables 2 and 3; EIMS *m/z* 406 $[M]^+$ (1.7), 344 (6.2), 233 (8.8), 205 (73.6), 135 (49), 57 (47), 43 (100); HREIMS *m/z* 406.2720 (calcd for $C_{24}H_{38}O_5$ 406.2719).

Diacarperoxide F (6): clear, colorless oil; $[\alpha]_D$; +40.5 (*c* 0.1, $CHCl_3$): ^{1}H and ^{13}C NMR data, see Tables 2 and 3; EIMS *m/z* 406 $[M]^+$ (1.7), 344 (6.2), 233 (8.8), 205 (73.6), 135 (49), 57 (47), 43 (100); HREIMS *m/z* 406.2717 (calcd for $C_{24}H_{38}O_5$ 406.2719).

Diacarperoxide G (7): colorless oil; $[\alpha]_D$ +12.6 (*c* 0.1, $CHCl_3$); ^{1}H and ^{13}C NMR data, see Tables 2 and 3; EIMS *m/z* 420 $[M^+]$, 233 (17), 205 (100), 135 (55), 57 (30), 43 (79); HREIMS *m/z* 420.2860 (calcd for $C_{25}H_{40}O_5$ 420.2876).

Diacardiol A (8): colorless oil; $[\alpha]_D$ −9.8 (*c* 0.25, $CHCl_3$); ^{1}H and ^{13}C NMR data, see Tables 2 and 3; EIMS *m/z* 332 $[M - 2H_2O]^+$ (2), 177 (5), 137 (100), 123 (22), 110 (20), 95 (78), 55 (67); HREIMS *m/z* 364.3340 (calcd for $C_{24}H_{44}O_2$ 364.3341).

Cytotoxicity Assay. Antiproliferative activity was examined against several cell lines and was determined through an MTT assay as described earlier.[23] Activity against brine shrimp, *Artemia salina*, was determined as previously outlined.[24]

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Supporting Information Available: This material is available free of charge via the Internet at http://pubs.acs.org.

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(16) As in previously reported norterpene peroxide congeners, the advanced Mosher procedure has been used to determine the absolute stereochemistry for the known compounds **10**, **12**, and **13**, which confirmed the relative configurations obtained by Capon and Macleod's empirical method[10,15] as well as their being identical to those described in the literature. This was achieved by hydrogenation of the peroxide rings to yield the corresponding diol derivatives, which were subsequently esterified to their R and *S* MTPA-Cl esters, respectively. Due to the low yields of the new derivatives **1**−**8**, only their relative configurations were assigned using the empirical method.
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