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Isolation and Absolute Configurations of Diversiform C$_{17}$, C$_{21}$ and C$_{25}$ Terpenoids from the Marine Sponge *Cacospongia* sp.

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Abstract: Chemical investigation of MeOH extract of a South China Sea sponge *Cacospongia* sp. yielded 15 terpenoids belonging to three different skeleton-types, including the unusual C$_{17}$ γ-lactone norditerpenoids (**1–3**), the rare C$_{21}$ pyridine meroterpenoid (**7**), and the notable C$_{25}$ manoalide-type sesterterpenoids (**4–6**, **8–10**). Compounds **1–5** were initially obtained as enantiomers, and were further separated to be optically pure compounds (**1a**, **1b**, **2a**, **2b**, **3a-r**, **3b-r**, **4a**, **4b**, **5a** and **5b**) by chiral HPLC, with a LiAlH$_4$ reduction aid for **3**. Compounds **3a**/**3b** (a pair of inseparable enantiomers), **4a**, **5a**, **6**, and **7** were identified as new compounds, while **1a**/**1b** and **2a**/**2b** were obtained from a natural source and were determined for their absolute configurations for the first time. This is also the first time to encounter enantiomers of the well-known manoalide-type sesterterpenoids from nature. The structures with absolute configurations of the new compounds were unambiguously determined by comprehensive methods including HR-ESI-MS and NMR data analysis, optical rotation comparison, experimental and calculated electronic circular dichroism (ECD), and Mo$_2$(OAc)$_4$ induced circular dichroism (ICD) methods. The cytotoxicity of the isolates against selected human tumor cell lines was evaluated, however, the tested compounds showed no activity against selected cell lines.

Keywords: marine sponge; *Cacospongia*; terpenoids

1. Introduction

Linear terpenoids, represented by linear furano- and pyrrolo-terpenoids, are a unique family of natural products with widespread bioactivities [1,2]. Among all the isolated linear terpenoids, C$_{25}$ linear sesterterpenoids are the major components with more than 200 compounds been isolated,

such as nitropyrrolins, heronapyrroles, fukanedones, and manoalide-type sesterterpenoids [3–7]. Inspiringly, the manoalide-type C_{25} sesterterpenoids, which are frequently obtained from marine sponges, have been studied as topical antipsoriatic lead drug candidates for their potent anti-inflammatory activity [8,9]. Apart from the common C_{15} sesquiterpenoids, C_{20} diterpenoids, and C_{25} sesterterpenoids, a few linear terpenoids take irregular C_{17} or C_{21} carbon skeletons [1–7]. To the best of our knowledge, there are only four C_{17} terpenoids been isolated from red algae *Laurencia viridis* [10], marine spong *Fasciospongia cavernosa* [11], and Chloranthaceae plant *Chloranthus sessilifolius* [12]. Only one of them takes the linear structure. C_{21} linear terpenoids are also a rare class of natural products which are mainly isolated from marine sponges of the genera *Cacospongia, Carteriospongia, Dysidea, Fasciospongia, Hippospongia, Leiosella, Spirastrella*, and *Spongia* [1,2].

Marine sponges of the genus *Cacospongia* (order Dictyoceratida, family Thorectidae) draw much attention for its production of diverse terpenoids [13], such as C_{21}-difuran terpenoid cacospongienone A from *C. scalaris* [14], manoalide-type sesterterpenoid cacospongionolide from *C. mollior* [15], and tetracyclic scalarane-type sesterterpenoid scalarin from *C. scalaris* [16]. However, only four out of the total 17 identified *Cacospongia* species have been studied for their chemical constituents. Besides, the unidentified species of *Cacospongia* (*Cacospongia* sp.) with potential morphological challenge are found to be significant resource for novel terpenoids. For example, two new terpenoids, namely cacofuran and (+)-isojaspic acid with novel bridged tricyclic carbon skeletons were isolated from two unidentified *Cacospongia* species collected from Okinawa and Papua New Guinea, respectively [17,18]. Furthermore, the *Cacospongia* derived terpenoids usually exhibit excellent pharmacological potentials, such as antibacterial activity, anti-inflammatory, and cytotoxicity [13–18].

In the course of our continuing search for new bioactive metabolites from Xisha Island sponges [19,20], an unidentified *Cacospongia* species was collected and investigated for its chemical components, yielding three pairs of rare C_{17} γ-lactone norditerpenoid enantiomers (**1a**, **1b**, **2a**, **2b** and the inseparable **3a**/**3b**), an infrequent C_{21} pyridine terpenoid (**7**), and six notable C_{25} manoalide-type sesterterpenoids (**4–6**, **8–10**) including two pairs of enantiomers (**4a**, **4b**, **5a** and **5b**) (Figure 1). Among them, (±)-8,13-secoepicavernosine (**3a**/**3b**), (+)-hippolide E (**4a**), (+)-(6*E*)-neomanoalide (**5a**), (3*R*,4*R*)-14,18-secoluffariolide C (**6**), and cacospongine A (**7**) were identified as new compounds, while the enantiomers **1a**/**1b** and **2a**/**2b** were separated by chiral HPLC. This is the first time to obtain manoalide-type enantiomers from nature. The structures with absolute configurations of the new compounds were unambiguously elucidated by a combinatorial methods, including HR-ESI-MS, 1D and 2D NMR spectra analysis, optical rotation comparison, experimental and calculated ECD comparison, and $Mo_2(OAc)_4$ induced circular dichroism (ICD) method. Furthermore, cytotoxicity of these compounds was tested against selected tumor cell lines. Herein, we report the isolation, chiral resolution, structure elucidation, and bioactivity of the isolated linear terpenoids.
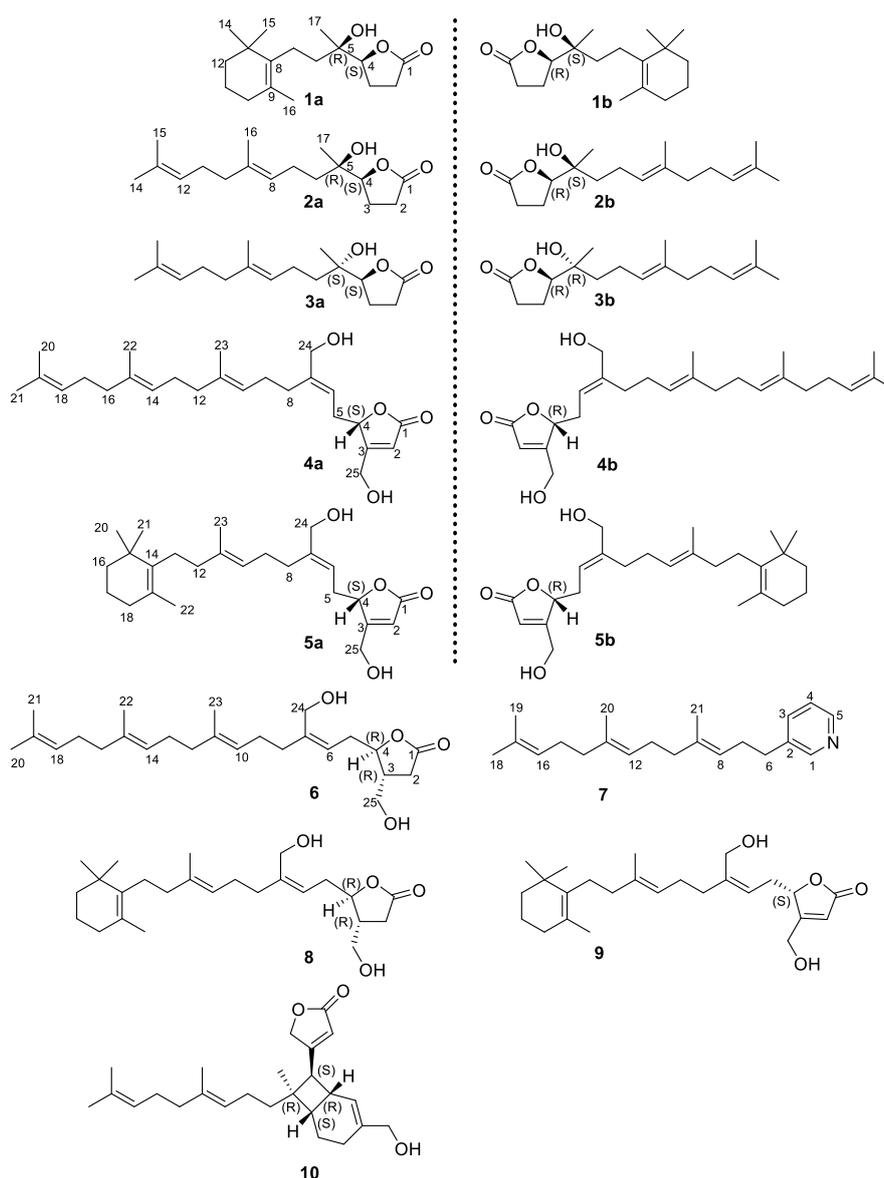
Figure 1. Structures of compounds **1–10**.

2. Results and Discussion

Compound **1** was obtained as colorless oil, and its molecular formula was determined as $C_{17}H_{28}O_3$ by HR-ESI-MS (*m/z* 303.1936, [M+Na]⁺, calcd 303.1931, Supplementary Figure S8). A comparison of the ¹H and ¹³C NMR data (Table 1) with literature suggested **1** to be the reported cavernosine definitely [11]. However, compound **1** showed neither optical activity ($[\alpha]_D^{20} \sim 0$) nor Cotton effect on its CD spectra, just as cavernosine did in the original report ($[\alpha]_D^{20} - 1.8$) [11], which suggested **1** to be a racemic mixture. Then, chiral HPLC resolution of **1** afforded a pair of stereoisomers **1a** ($[\alpha]_D^{20} + 7.5$) and **1b** ($[\alpha]_D^{20} - 10.1$) with a ratio of approximately 1:1 (Supplementary Figure S3). The relative configuration of the C-4–C-5 fragment in **1a** and **1b** were both assigned as *erythro* type (4*S**,5*R**) by comparing the ¹H and ¹³C NMR data (Table 1, Supplementary Figures S9 and S10) with those of the synthesized *erythro* (±)-cavernosine and *threo* (±)-epicavernosine [21]. The absolute configurations of **1a** and **1b** were established by ECD methods. According to literature, the γ-chiral center of a saturated γ lactone would induce a Cotton effect at 213 nm, which is mainly attributed to the $\pi_x \rightarrow \pi_x^*$ electronic transition of the carbonyl group in the γ-lactone ring [22]. Accordingly, **1a** and **1b** were found to take a negative and positive Cotton effects at 213 nm, respectively. Thus, **1a** and **1b** were proposed to have

the 4*S* and 4*R* absolute configurations [22]. The result was further confirmed by a comparison of the experimental ECD spectra with the calculated curves of the four possible candidate stereoisomers of **1**. The calculated ECD data suggested that the valuence of the Cotton effect at 213 nm was solely related to the absolute configuration of C-4 (negative-4*S*, positive-4*R*) (Figure 2), while the Cotton effect intensity could be affected by the auxochromic hydroxy group at C-5. Finally, the absolute configurations of **1a** [(+)-cavernosine] and **1b** [(−)-cavernosine] were assigned as 4*S*,5*R* and 4*R*,5*S*, respectively. This is the first time to separate enantiomers of cavernosine and to determine their absolute configurations.

Table 1. ^{1}H and ^{13}C NMR Data for Compounds **1**–**3** in CDCl$_3$ (500 MHz for ^{1}H and 125 MHz for ^{13}C).

| No.	1a/1b		2a/2b		3a/3b	
	δ_C, Type	δ_H (*J* in Hz)	δ_C, Type	δ_H (*J* in Hz)	δ_C, Type	δ_H (*J* in Hz)
1	177.3, C		177.5, C		177.2, C	
2	28.9, CH$_2$	2.56, m	28.8, CH$_2$	2.51, m	29.0, CH$_2$	2.56, m
3	21.8, CH$_2$	2.15, m; 2.27, m	21.7, CH$_2$	2.11, m; 2.22, m	22.2, CH$_2$	2.26, m; 2.16, m
4	85.6, CH	4.35, t (7.6)	85.6, CH	4.31, t (7.5)	85.0, CH	4.37, t (7.7)
5	73.0, C		72.6, C		73.0, C	
6	36.9, CH$_2$	1.41, m	36.9, CH$_2$	1.38, m; 1.48, m	39.2, CH$_2$	1.60, m; 1.68, m
7	21.9, CH$_2$	1.89, t (6.2)	21.6, CH$_2$	2.02, m; 2.11, m	22.2, CH$_2$	2.10, m; 2.05, m
8	136.2, C		123.5, CH	5.08, t (7.1)	123.6, CH	5.12, t (6.6)
9	127.5, C		135.6, C		135.9, C	
10	32.7, CH$_2$	2.00, td (13.0, 5.6) 2.15, m	39.5, CH$_2$	1.94, m	39.6, CH$_2$	1.98, m
11	19.4, CH$_2$	1.55, m; 1.68, m	26.4, CH$_2$	2.02, m	26.6, CH$_2$	2.05, m
12	39.7, CH$_2$	1.48, m; 1.55, m	124.0, CH	5.04, t (6.9)	124.2, CH	5.08, t (6.7)
13	35.0, C		131.2, C		131.5, C	
14	28.6, CH$_3$	0.99, s	25.5, CH$_3$	1.63, s	25.7, CH$_3$	1.68, s
15	28.5, CH$_3$	0.97, s	17.5, CH$_3$	1.56, s	17.7, CH$_3$	1.60, s
16	19.7, CH$_3$	1.58, s	15.8, CH$_3$	1.58, s	16.0, CH$_3$	1.62, s
17	23.24, CH$_3$	1.34, s	23.0, CH$_3$	1.26, s	20.9, CH$_3$	1.14, s
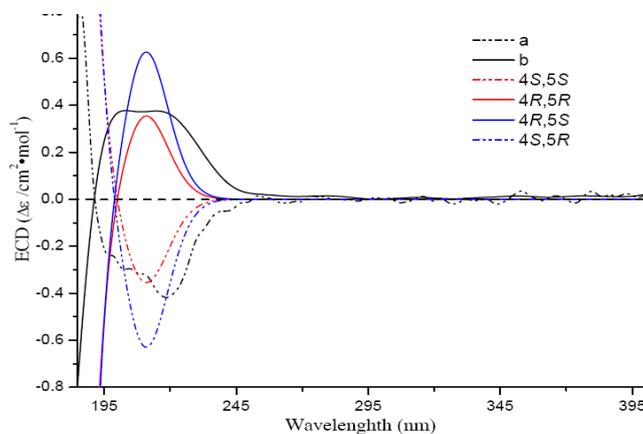
Figure 2. Experimental CD spectra of **1a** and **1b** overlaid with calculated spectra for the candidate stereostructures.

Compounds **2** and **3**, having the same molecular formula of C$_{17}$H$_{28}$O$_3$ with **1**, were isolated as colorless oil. A comparison of the 1D NMR data of **2** with those of **1** (Table 1, Supplementary Figures S13–S15, S21 and S22) suggested that they both take the same γ-lactone ring and C-4-C-7 fragments. However, the rest fragment of **2** is obviously different with that of **1** according to their 1D data, especially the two methine signals (δ_H 5.08, t, *J* = 7.1 Hz and δ_H 5.04, t, *J* = 6.9 Hz) observed in **2**. HMBC correlations from H$_3$-14/H$_3$-15 to two alkenyl carbon signals at δ_C 124.0 (C-12) and δ_C 131.2 (C-13) strongly suggested the formation of a double bound of C-12 = C-13 in **2** (Supplementary Figure S18). Thus compound **2** was determined as a 8,13-seco product of **1**. Compound **2** has been synthesized as a side product [23]. The mostly identical 1D and 2D NMR data of **3** and **2** indicated that they both take the same planar structure. The slight difference of Me-17 in **2** and **3** (δ_H 1.14, δ_C 20.9 in **3** vs. δ_H 1.26, δ_C 23.0 in **2**) suggested they may be a pair of epimers. Geometrical configurations

of double bonds Δ^8 in **2** and **3** were both deduced as *E* geometry, which was deduced from NOE correlations of H_3-16/H_2-7 and H_2-10/H-8, and from the chemical shifts of C-16 (<20 ppm, a methyl resonance appearing at a chemical shift less than 20 ppm is indicative of an 'E' configuration, whereas a value larger than 20 ppm indicates a 'Z' configuration) [24]. Comparisons of chemical shifts of H_3-17 in **2** (δ_H 1.02; C_6D_6) and **3** (δ_H 0.74; C_6D_6) with that of the synthesized *erythro* 8,13-secocavernosine (δ_H 1.08; C_6D_6) revealed **2** possessing a *erythro*-type relative configuration for the C-4–C-5 fragment [23]. While chemical shifts of Me-17 in **3** (δ_H 1.14 vs. δ_H 1.26 in **2**; $CDCl_3$) was in agreement with that of the synthesized *threo*-type analog [(S)-5-((S)-2-hydroxy-6-methylhept-5-en-2-yl)-dihydrofuran-2(3H)-one] (δ_H 1.16; $CDCl_3$) [25]. Thus, the relative configurations of **2** and **3** were assigned as *erythro* (4S*,5R*) and *threo* (4S*,5S*), respectively. Optical rotation value and ECD experiment disclosed that **2** and **3** were both optical inactive. Further chiral resolution of **2** afforded a pair of stereoisomers **2a** ($[\alpha]_D^{20}$ + 6.5) and **2b** ($[\alpha]_D^{20}$ − 10.4) (Supplementary Figure S4). The absolute configurations of **2a** and **2b** were established as 4S,5R and 4R,5S, respectively, by experimental and calculated ECD spectra comparison (Figure 3) Thus, **2a** and **2b** were finally established as (+)-8,13-secocavernosine and (−)-8,13-secocavernosine, respectively.
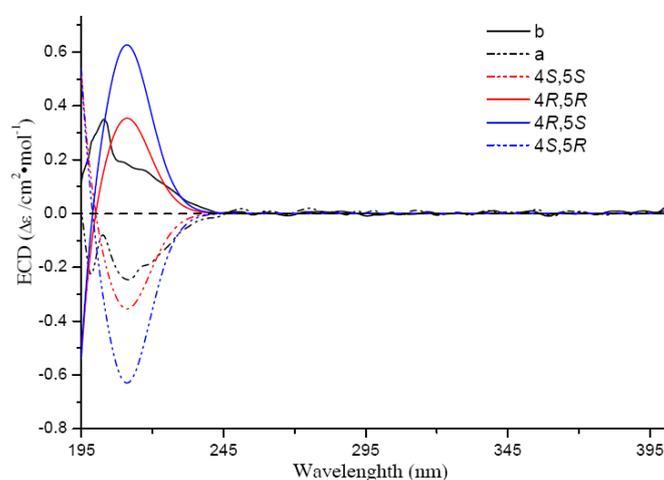

Figure 3. Experimental CD spectra of **2a** and **2b** overlaid with calculated spectra for the candidate stereostructures.

However, the optical inactive compound **3** could not be further separated by chiral HPLC (Supplementary Figure S5). To address the absolute configuration of **3**, several chemical tailoring reactions were tried. During the NaOH hydrolysis of **3**, the hydrolytic product **3-h** was unstable and spontaneously formed the original γ-lactone **3**, even using CH_2N_2 as a protective agent for the carboxyl of **3-h** (Supplementary Scheme S1). Finally, compound **3** was successfully reduced to acyclic 1,4,5-triol derivative using $LiAlH_4$ (Scheme 1) [11]. The reduced product was further separated to be a pair of isomers **3a-r** ($[\alpha]_D^{20}$ − 7.8) and **3b-r** ($[\alpha]_D^{20}$ + 11.1), with a ratio of 1:1 on chiral HPLC (Scheme 1). On the basis of Snatzke's theory [26,27], $Mo_2(OAc)_4$ induced circular dichroism (ICD) spectra of acyclic adjacent diol substrate (relative configuration determined) could be used to established the absolute configurations of the diol fragment according to the key diagnostic Cotton effect at 310 nm (band IV). Thus, $Mo_2(OAc)_4$ ICD experiments was carried out, and a positive and a negative Cotton effect at 310 nm on the ICD spectra of **3a-r** and **3b-r** were observed, respectively. The absolute configurations of **3a-r** and **3b-r** were determined to be 4S,5S and 4R,5R (Figure 4), respectively [26,27]. This result confirmed **3** to be a pair of inseparable new enantiomers bearing the 4S,5S and 4R,5R absolute configurations.
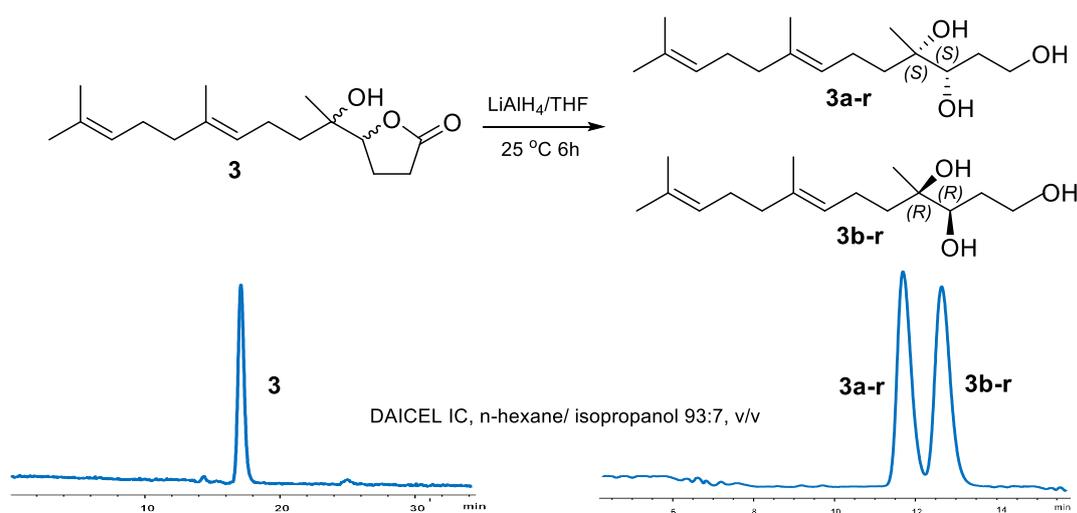
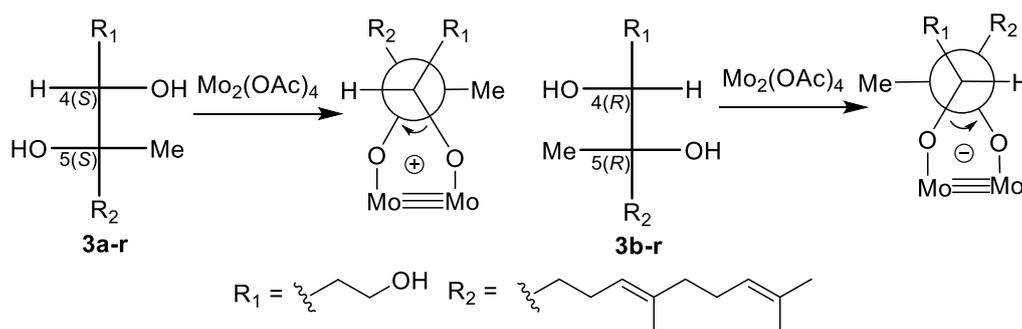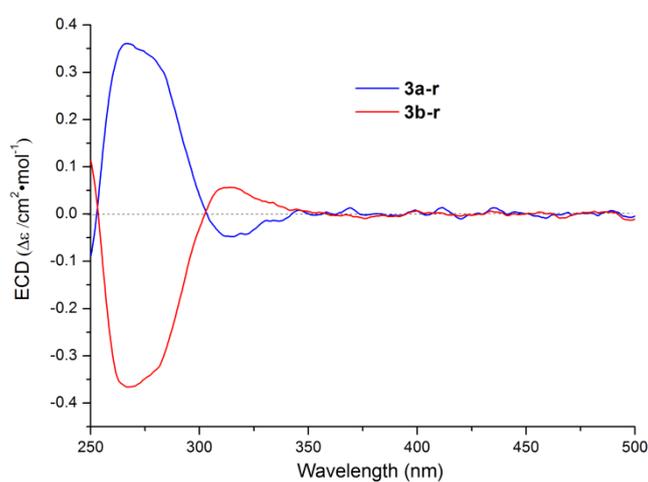**Scheme 1.** LiAlH$_4$ reduction and chiral resolution of compound **3**.

Figure 4. Mo$_2$(OAc)$_4$ complexes ICD of **3a-r** and **3b-r**, and absolute configuration analysis of **3a-r** and **3b-r** by Snatzke's theory.

Compounds **4a/4b** and **5a/5b**, having the same molecular formula of C$_{25}$H$_{40}$O$_4$ as deduced by HR-ESI-MS data (Supplementary Figures S27 and S31), were obtained as two pairs of enantiomers both with an approximate ratio of 1:3 (Supplementary Figure S7). Each pair of isomers possess the same NMR data (Table 2), while their optical rotations ($[\alpha]_D^{20}$ **4a** + 4.1 vs. **4b** − 7.4; **5a** + 3.6 vs. **5b** − 7.7) and ECD curves (Figure 5) are almost opposite. Comparisons of 1D NMR data of **4a/4b** and **5a/5b** (Table 2, Supplementary Figures S28–S30 and S32–S34) with those of literatures revealed their planar structure to be hippolide E and (*E*)-neomanoalide, respectively [28,29]. The absolute configurations

of **4b** [(−)-hippolide E] and **5b** [(−)-(*E*)-neomanoalide] were assigned to be the same as hippolide E and (6*E*)-neomanoalide-24-ol [28,29] by CD spectra comparisons. Their enantiomers **4a** [(+)-hippolide E] and **5a** [(+)-(6*E*)-neomanoalide-24-ol] were both deduced to be 4*S* configuration according to their opposite CD absorptions (Figure 5) and opposite optical rotations comparing with **4b** and **5b**. Thus, **4a** and **5a** were identified as two new enantiomers of hippolide E and (*E*)-neomanoalide-24-ol, respectively. This is the first time to obtain the natural-occurring enantiomers of the well-known manoalide-type sesterterpenoids.
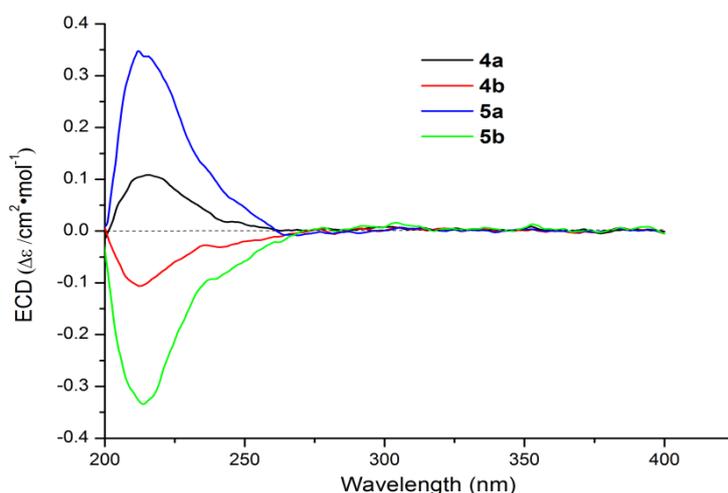

Figure 5. Experimental CD spectra for **4a**/**4b** and **5a**/**5b**.

Compound **6**, with the molecular formula of $C_{25}H_{40}O_4$, was also considered as a manoalide-type sesterterpenoid according to its similar 1D NMR data with those of **4a**/**4b** and **5a**/**5b** (Table 2, Supplementary Figures S36–S38). Further 1D NMR data comparison of **6** with those of co-isolated luffariolide C (**8**) revealed their structural similarity, except for a slight difference of Me-20 (δ_H 1.68 in **6** vs. δ_H 0.99 in luffariolide C) and Me-21 (1.60 vs. 0.99) [30]. HMBC correlations from H_3-20/H_3-21 to the olefinic carbons of C-18 (δ_C 124.3) and C-19 (δ_C 131.3) (Figure 6, Supplementary Figure S41) suggested that the cyclohexene ring in luffariolide C was cleaved and formed an extra double bound of C-18=C-19 in **6**. Further 2D NMR analysis, such as ^1H-^1H COSY correlations of H_2-16/H_2-17/H-18 and HMBC correlations from H_3-22 to C-14/C-15/C-16, (Figure 6) confirmed the planar structure of **6** to be 14,18-seco luffariolide C. Double bounds of Δ^{10} and Δ^{14} were both deduced to be *E* geometry according to NOE correlations of H_3-23/H_2-9 and H_3-22/H_2-13 (Figure 7). While double bound of Δ^6 was assigned as *Z* geometry by NOE correlation of H_2-24/H_2-5. Additionally, NOE correlations of H-3/H_2-5 and H-4/H_2-25 (Figure 7), as well as the coupling constant between H-3 and H-4 (*J* = 0 Hz) in **6**, suggested it takes the same 3,4-*trans* relative configuration with luffariolide C. Moreover, the similar optical rotations of **6** ($[\alpha]_D^{20} = +3.9$) and luffariolide C ($[\alpha]_D^{20} = +4.4$) revealed the same 3*R*,4*R* absolute configurations. The result was further supported by experimental and theoretical ECD comparisons of the main chiral lactone structure in **6** (Figure 8). Thus, **6** was determined as (3*R*,4*R*)-14,18-secoluffariolide C.
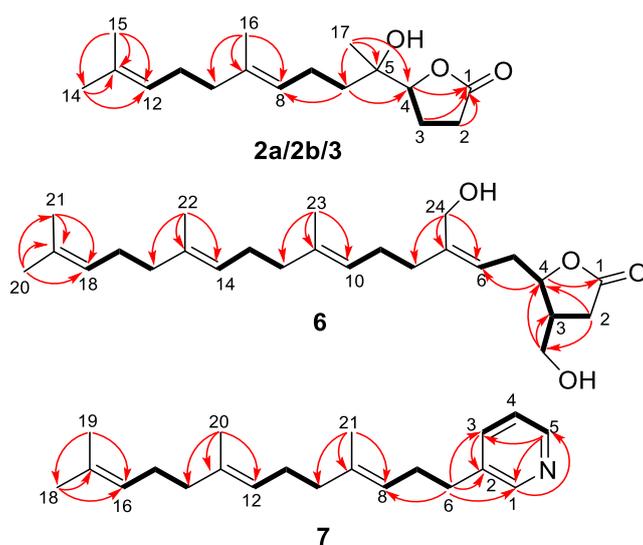
Figure 6. Key COSY (–) and HMBC (→) correlations of **2a**/**2b**, **3**, **6**, and **7**.
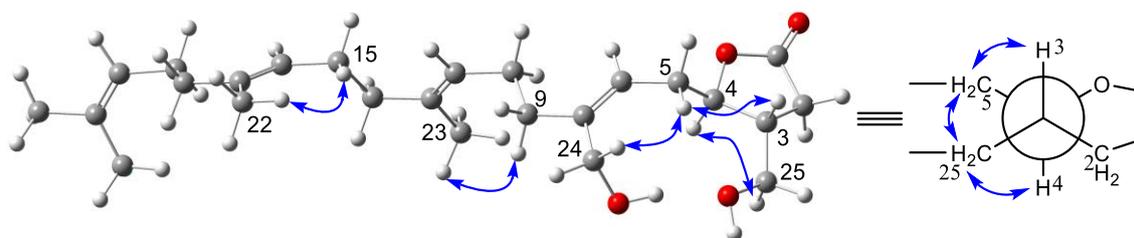

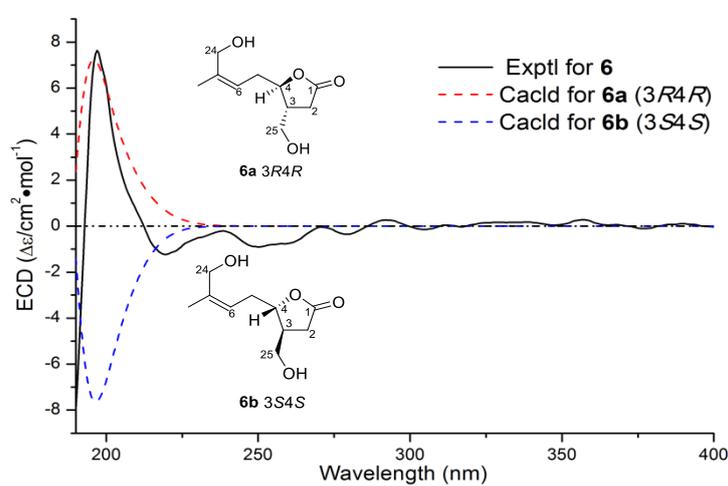**Figure 7.** Key NOESY correlations of compounds **6**.

Figure 8. Experimental CD spectra of **6** overlaid with calculated spectra for the candidate stereostructures of the main chiral partial structure in **6**.

Table 2. 1H and 13C NMR Data for Compounds **4–7** (500 MHz for 1H and 125 MHz for 13C).

No.	4a/4b [a]		5a/5b [a]		6 [a]		7 [b]	
	δ_C, Type	δ_H (J in Hz)	δ_C, Type	δ_H (J in Hz)	δ_C, Type	δ_H (J in Hz)	δ_C, Type	δ_H (J in Hz)
1	172.2, C		172.2, C		176.0, C		149.6, CH	8.40, s
2	116.3, CH	6.05, s	116.3, CH	6.05 s	31.7, CH$_2$	2.39, dd (8.2, 17.1) 2.62, m	137.0, C	
3	170.8, C		170.9, C		41.4, CH	2.50, m	135.8, CH	7.60, d (,7.6)
4	81.5, CH	5.07, t (5.6)	81.5, CH	5.07, t (5.3)	82.8, CH	4.44, dd (4.9, 10.4)	123.2, CH	7.27, dd (6.7, 5.6)
5	30.5, CH$_2$	2.72, m; 2.46, m	30.5, CH$_2$	2.72, m; 2.47, m	32.5, CH$_2$	2.57, m	147.0, CH	8.38, d (4.1)
6	117.8, CH	5.39, t (7.0)	117.7, CH	5.39 t (7.3)	121.7, CH	5.40, t (7.5)	32.3, CH$_2$	2.60, t (7.6)
7	143.4, C		143.4, C		142.8, C		29.0, CH$_2$	2.25, q (7.3)
8	28.5, CH$_2$	2.12, m	28.5, CH$_2$	2.12, m	35.8, CH$_2$	2.17, m	122.9, CH	5.12, t (6.9)
9	26.6, CH$_2$	2.06, m	27.8, CH$_2$	2.03, m	26.6, CH$_2$	2.06, m	135.5, C	
10	123.3, CH	5.11, m	122.7, CH	5.13, t (6.5)	123.6, CH	5.10, m	39.66, CH$_2$	1.92, m
11	136.2, C		137.1, C		135.8, C		25.99, CH$_2$	2.01, m
12	39.7, CH$_2$	1.98, m	39.8, CH$_2$	2.01, m	39.7, CH$_2$	1.97, m	123.8, CH	5.05, m
13	26.7, CH$_2$	2.06, m	26.7, CH$_2$	2.11, m	26.7, CH$_2$	2.06, m	134.3, C	
14	124.0, CH	5.10, m	137.0, C		124.1, CH	5.10, m	39.6, CH$_2$	1.92, m
15	135.1, C		127.0, C		135.0, C		26.6, CH$_2$	2.01, m
16	39.7, CH$_2$	1.98, m	32.8, CH$_2$	1.90, t (6.1)	39.7, CH$_2$	1.98, m	124.1, CH	5.05, m
17	26.8, CH$_2$	2.06, m	19.5, CH$_2$	1.57, m	26.7, CH$_2$	2.15, m	130.6, C	
18	124.3, CH	5.09, m	40.2, CH$_2$	1.41, m	124.3, CH	5.10, m	25.5, CH$_3$	1.63, s
19	131.3, C		35.0, C		131.3, C		17.5, CH$_3$	1.54, s
20	25.7, CH$_3$	1.68, s	28.6, CH$_3$	0.99, s	25.7, CH$_3$	1.68, s	15.7, CH$_3$	1.54, s
21	17.7, CH$_3$	1.60, s	28.6, CH$_3$	0.99, s	17.7, CH$_3$	1.60, s	15.7, CH$_3$	1.47, s
22	16.1, CH$_3$	1.60, s	19.8, CH$_3$	1.63, s	16.1, CH$_3$	1.60, s		
23	16.0, CH$_3$	1.60, s	16.1, CH$_3$	1.60, s	16.0, CH$_3$	1.60, s		
24	66.4, CH$_2$	4.06, s	66.4, CH$_2$	4.07, s	60.2, CH$_2$	4.08, d (11.9) 4.17, d (12.1)		
25	58.8, CH$_2$	4.54, d (16.7); 4.45, d (16.5)	58.8, CH$_2$	4.55, d (16.8) 4.45, d (16.5)	63.2, CH$_2$	3.65, dd (7.2, 10.1) 3.75, dd (4.5, 10.6)		

[a] Recorded in CDCl$_3$; [b] Recorded in DMSO-d_6.

　　　The molecular formula of compound **7** was deduced as $C_{21}H_{31}N$ by HR-ESI-MS at *m/z* 298.2532 ([M + H]$^+$ calcd 298.2529, Supplementary Figure S43). ^{1}H NMR spectrum of **7** showed four aromatic proton signals (δ_H 8.40, s; 8.38, d, *J* = 4.1 Hz; 7.60, d, *J* = 7.6 Hz; 7.27, dd, *J* = 6.7, 5.6 Hz). The remaining proton signals in upfield were similar with those of **6**, especially the four olefinic methyls of H$_3$-18 (δ_H 1.63 in **7** vs. 1.68 in **6**), H$_3$-19 (1.54 vs. 1.60), H$_3$-20 (1.54 vs. 1.60) and H$_3$-21 (1.47 vs. 1.60). ^{1}H-^{1}H COSY correlations of H$_2$-6/H$_2$-7/H-8, H$_2$-10/H$_2$-11/H-12 and H$_2$-14/H$_2$-15/H-16, together with HMBC correlations from H$_3$-18/ H$_3$-19 to C-16/C-17, from H$_3$-20 to C-12/C-13/C-14, and from H$_3$-21 to C-8/C-9/C-10, indicated a C$_{16}$ prenyl chain (C-6-C-18). Additionally, a monosubstituted pyridine ring was constructed based on ^{1}H-^{1}H COSY correlations of H-3/H-4/H-5 and HMBC correlations from H-5 to C-1/C-3, from H-3 to C-2 and from H-1 to C-5 (Figure 6, Supplementary Figures S48 and S49). The C$_{16}$ linear chain was connected with the pyridine ring at C-2 by HMBC correlations from H$_2$-6 (δ_H 2.60, t, *J* = 7.6 Hz) to C-1 (δ_C 149.6), C-2 (δ_C 137.0) and C-3 (δ_C 135.8) (Figure 6). The *E* geometry of double bounds Δ^8 and Δ^{12} were both deduced from the chemical shifts of C-21 and C-20 (δ_C both at 15.7, < 20 ppm) [24]. Thus compound **7** was determined as a C$_{21}$ pyridine terpenoid and was named as cacospongine A.

　　　The structures of the known analogs of luffariolide C (**8**) [30], (*Z*)-neomanoalide (**9**) [29] and hippolide J (**10**) [31] were determined by comparison of their 1D/2D NMR data, ESI-MS data and optical rotations with those of reported values.

　　　The cytotoxicity of the isolates against human tumor cell lines of K562, HCT116, Hep3B, A-549, and Jurkat was evaluated, using MTT method [32] with adriamycin as positive control. However, all the tested compounds were inactive (IC$_{50}$ > 10 μM).

3. Experimental Section

3.1. General Methods

　　　Optical rotations were measured on a JASCO P-1020 digital polarimeter (JASCO Corporation, Tokyo, Japan). UV spectra were measured on a Beckman DU640 spectrophotometer. ECD spectra were obtained on a Jasco J-815 CD spectrometer. IR spectra were recorded on a Nicolet Nexus 470 (FT-IR) spectrophotometer (Thermo Electron Co., Madison, WI, USA), KBr pellets. NMR spectra were recorded on a Bruker DRX-500MHz instrument (Bruker BioSpin GmbH Co., Rheinstetten, Germany), 500 MHz for ^{1}H NMR and 125 MHz for ^{13}C NMR in CDCl$_3$; chemical shifts δ in ppm referred to the solvent peaks at δ_H 7.26 and δ_C 77.0 for CDCl$_3$, δ_H 2.50 and $_C$ 39.5 for DMSO-d$_6$ and δ_H 7.16 for C$_6$D$_6$, and coupling constant *J* in Hz. HR-ESI-MS were obtained from a Micromass Q-Tof Ultima GLOBAL GAA076 LC-mass spectrometer (Waters Corporation, Milford, MA, USA). HPLC separation was performed on an Agilent 1100 series instrument with DAD detector (Agilent technologies, Santa Clara, CA, USA), equipped with a semi-preparative reversed-phased column (YMC-packed C18, 5 μm, 250 × 10 mm, 1.5 mL/min) or an analytic chiral column DAICEL IC-3 (DAICEL chiral technologies, Shanghai, China). Precoated silica gel plates (GF$_{254}$, Qingdao Marine Chemical Inc., Qingdao, China) were used for TLC analyses. Silica gel (200–300 mesh, Qingdao Marine Chemical Inc., Qingdao, China) was used for column chromatography (CC).

3.2. Animal Material

　　　The sponge *Cacospongia* sp. is in irregular shape, with black-brownish color, and covered with a tight encrusting crust. There are irregularity distributed holes on the surface, wihch is thick and solid, and is in the dimensions of around 4 × 5 cm ∼ 6 × 8 cm (see Supplementary I. Photos of sponge specimen). The specimen was collected from the coral-reef regions of Yong Xing Island (16°50′ N, 112°20′ E) in the South China Sea, at a depth ranging from 18 to 25 m, in November 2010. The specimen, with a voucher of no. XS-2009-34, was frozen immediately at −20 °C until it was examined. The voucher was deposited at the School of Medicine and Pharmacy, Ocean University

of China, China. The sponge was identified by Dr. N.J.d.V. (Naturalis Biodiversity Centre, Leiden, The Netherlands).

3.3. Extraction and Isolation

The frozen sponge (2.6 kg, wet weight) was minced and extracted with MeOH for three times (each time for one day) at room temperature (5 L × 3). The combined solution was evaporated in vacuum and desalinated for three times to yield a residue (56.0 g). The crude extract was then subjected to a reduced pressure silica gel column eluting with a step-by-step gradient elution of acetone-petroleum ether (from 0:1 to 1:0, *v/v*), to give six fractions. Fraction 2 was then further separated by silica gel column eluting with petroleum ether/acetone (2:1, 1:1, 0:1, *v/v*) to give subfractions F2-1–F2-5. F2-2 was further purified by semi-preparative HPLC with a mobile phase of MeOH/H$_2$O (70:30, *v/v*) to give three pairs of isomer mixtures **1** (6.0 mg; t_R 53.0 min), **2** (30.1 mg; t_R 59.0 min) and **3** (4.1 mg; t_R 56.0 min). These isomers were finally separated by chiral HPLC with a mobile phase of n-hexane/ isopropanol (93:7, *v/v*), and yielded **1a** (2.3 mg; t_R 15.0 min), **1b** (2.4mg; t_R 28.0 min), **2a** (12.1 mg; t_R 15.0 min), **2b** (12.0 mg; t_R 24.5 min), **3a/3b** (enantiomeric mixture, 4.1 mg; t_R 17.5 min). HPLC purification (85:15, MeOH/H$_2$O, *v/v*) of F2-3 afforded compound **7** (6.1 mg; t_R 37.0 min). Fraction 3 was chromatographed on another silica gel column to give two subfractions F3-1 and F3-2, which were found to contain terpenoid components by TLC analyses. Then F3-1 was further separated by HPLC (85:15, MeOH/H$_2$O, *v/v*) to give **6** (2.4 mg; t_R 42.0 min), **8** (1.5 mg; t_R 37.0 min), **9** (4.6 mg; t_R 56.0 min) and **10** (5.0 mg; t_R 61.0 min). The two pairs of isomers of **4a** (1.6 mg; t_R 47.0 min)/**4b** (4.2 mg; t_R 42.0 min) and **5a** (1.5 mg; t_R 49.0 min) /and **5b** (4.3 mg; t_R 44.0 min) were ultimately separated by chiral HPLC with a mobile phase of n-hexane/ethanol (94:6, *v/v*) from F3-2.

Cavernosine (**1**): colorless oil; *(+)-cavernosine* (**1a**), $[\alpha]_D^{20}$ + 7.5 (*c* 0.1, MeOH); *(−)-cavernosine* (**1b**), $[\alpha]_D^{20}$ − 10.1 (*c* 0.1, MeOH); IR (KBr) v_{max} 3479, 2926, 1779, 1450, 1374, 1191 cm^{-1}; ^{1}H NMR (CDCl$_3$, 500 MHz) and ^{13}C NMR (CDCl$_3$, 125 MHz), see Table 1; HRESIMS *m/z* 303.1936 [M + Na]$^+$ (calcd for C$_{17}$H$_{28}$O$_3$Na, 303.1931).

8,13-secocavernosine (**2**): colorless oil; *(+)-8,13-secocavernosine* (**2a**): $[\alpha]_D^{20}$ = +3.9 (*c* 0.1, MeOH); *(−)-8,13-secocavernosine* (**2b**), $[\alpha]_D^{20}$ − 10.4 (*c* 0.1, MeOH); IR (KBr) v_{max} 3469, 2928, 1774, 1453, 1377, 1191 cm^{-1}; ^{1}H NMR (CDCl$_3$, 500 MHz) and ^{13}C NMR (CDCl$_3$, 125 MHz), see Table 1; ^{1}H NMR in C$_6$D$_6$ (500 MHz) δ 5.22 (t, *J* = 6.9 Hz, 1H), 5.16 (t, *J* = 6.9 Hz, 1H), 3.64 (t, *J* = 7.5 Hz, 1H), 1.68 (s, 3H), 1.57 (s, 3H), 1.57 (s, 3H), 1.02 (s, 3H); HRESIMS *m/z* 303.1938 [M + Na]$^+$ (calcd for C$_{17}$H$_{28}$O$_3$Na, 303.1931).

(±)-8,13-secoepicavernosine (**3a/3b**, unseparated): colorless oil (MeOH); $[\alpha]_D^{20}$ ∼ 0 (*c* 0.1, MeOH); IR (KBr) v_{max} 3473, 2919, 1774, 1649, 1456, 1379, 1190, 1004 cm^{-1}; ^{1}H NMR (CDCl$_3$, 500 MHz) and ^{13}C NMR (CDCl$_3$, 125 MHz), see Table 1; ^{1}H NMR in C$_6$D$_6$ (500 MHz) δ 5.25 (t, *J* = 6.9 Hz, 1H)), 5.20 (t, *J* = 7.0 Hz, 1H), 3.62 (t, *J* = 7.5 Hz, 1H), 1.70 (s, 3H), 1.61 (s, 3H), 1.59 (s, 3H), 0.74 (s, 3H); HRESIMS *m/z* 303.1936 [M + Na]$^+$ (calcd for C$_{17}$H$_{28}$O$_3$Na, 303.1931).

(+)-hippolide E (**4a**): colorless oil (MeOH); $[\alpha]_D^{20}$ = +4.1 (*c* 0.1, MeOH); IR (KBr) v_{max} 3410, 2925, 2360, 2338, 1746, 1700, 1650, 1540, 1455, 1381 cm^{-1}; ^{1}H NMR (CDCl$_3$, 500 MHz) and ^{13}C NMR (CDCl$_3$, 125 MHz), see Table 2; HRESIMS *m/z* 425.2671 [M + Na]$^+$ (calcd for C$_{25}$H$_{38}$O$_4$Na, 425.2662).

(+)-(6E)-neomanoalide (**5a**): colorless oil (MeOH); $[\alpha]_D^{20}$ = +3.6 (*c* 0.1, MeOH); IR (KBr) v_{max} 3415, 2926, 2860, 2359, 2338, 1744, 1647, 1455, 1380, 1143, 1063 cm^{-1}; ^{1}H NMR (CDCl$_3$, 500 MHz) and ^{13}C NMR (CDCl$_3$, 125 MHz), see Table 2; HRESIMS *m/z* 425.2674 [M + Na]$^+$ (calcd for C$_{25}$H$_{38}$O$_4$Na, 425.2662).

(3R,4R)-14,18-secoluffariolide C (**6**): colorless oil (MeOH); $[\alpha]_D^{20}$ = +3.9 (*c* 0.1, MeOH); IR (KBr) v_{max} 3384, 2922, 2856, 2359, 2337, 1750, 1451, 1375, 1265, 1196, 1018 cm^{-1}; ^{1}H NMR (CDCl$_3$, 500 MHz) and ^{13}C NMR (CDCl$_3$, 125 MHz), see Table 2; HRESIMS *m/z* 405.2995 [M + H]$^+$ (calcd for C$_{25}$H$_{41}$O$_4$, 405.2999).

Cacospongine A (**7**): colorless oil (MeOH); IR (KBr) v_{max} 2924, 2854, 2361, 2339, 1718, 1453, 1379, 1190 cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz) and ^{13}C NMR (DMSO-d_6, 125 MHz), see Table 2; HRESIMS *m/z* 298.2532 [M + H]$^+$ (calcd for $C_{21}H_{32}N$, 298.2529).

3.4. LiAlH$_4$ Reduction of **3**

The solution of **3** (3 mg) in dry THF (2 mL) containing LiAlH$_4$ (3 mg) were stirred for 6 h at room temperature, under the protection of argon atmosphere. The reduction reaction was quenched by addition of 2 mL of 10% KOH aqueous solution, and the mixture was then extracted by EtOAc to yielded crude products (2.7 mg) [11]. The isomer mixtures were further separated by chiral HPLC (n-hexane/ isopropanol, 93:7, *v/v*) to give **3a-r** (1.3 mg; t_R 11.7 min) and **3b-r** (1.2 mg; t_R 12.7 min).

Compounds **3a-r** *and* **3b-r**: colorless oil from MeOH; $[\alpha]_D^{20}$ − 7.8 for **3a-r** and +11.1 for **3b-r** (*c* 0.1, MeOH); ^{1}H NMR (CDCl$_3$, 500 MHz) δ 5.14 (1H, t, *J* = 6.7 Hz, H-8), 5.08 (1H, t, *J* = 6.7 Hz, H-12), 3.70 (2H, m, H$_2$-1), 3.46 (1H, d, *J* = 10.9 Hz, H-4), 2.11 (1H, m, H-7a), 2.06 (1H, m, H-7b), 2.06 (2H, m, H$_2$-11), 1.98 (2H, m, H$_2$-10), 1.75 (2H, m, H$_2$-2), 1.68 (3H, s, H$_3$-14), 1.62 (3H, s, H$_3$-16), 1.60 (3H, s, H$_3$-15), 1.56 (1H, m, H-6a), 1.50 (each 1H, m, H-6b, H-3a), 1.40 (1H, m, H$_2$-3b), 1.12 (3H, s, H$_3$-17); ^{13}C NMR (CDCl$_3$, 125 MHz) δ 135.8 (C, C-9), 131.5 (C, C-13), 124.2 (CH, C-12), 124.1 (CH, C-8), 77.2 (CH, C-4), 75.1 (C, C-5), 62.9 (CH$_2$, C-1), 39.7 (CH$_2$, C-10), 38.8 (CH$_2$, C-6), 30.1 (CH$_2$, C-2), 28.6 (CH$_2$, C-3), 26.6 (CH$_2$, C-11), 25.7 (CH$_3$, C-14), 20.8 (CH$_3$, C-17), 22.0 (CH$_2$, C-7), 17.7 (CH$_3$, C-15), 16.0 (CH$_3$, C-16).

3.5. Determination of the Absolute Configuration of the Diol Moiety in **3a-r** and **3b-r** by Snatzke's Method

ICD spectra of Mo-complexes of **3a-r** and **3b-r** were obtained according to reported procedures [26,27]. The reduced products (each 0.5 mg) and Mo$_2$(OAc)$_4$ (1.0 mg) were dissolved in 1.5 mL of dry DMSO to give a solution, with the ligand to metal molar ratio being around 1:1.2. The electronic transitions of the metal complexes in DMSO were monitored by a Jasco J-815 CD spectrometer in the UV–vis region of 250–500 nm, and stationary ICD spectra were obtained after 50 min at 15 °C. Because there were no inherent absorptions for the reduced products, the observed ICD spectra could be directly used to analyze the absolute configurations of diol fragments in the ligands with the characteristic cotton effects around 310 nm, according to Snatzke's theory [26,27].

3.6. Calculating Section

The quantum chemical calculations were performed using the density functional theory (DFT) by Gaussian 09 [33]. The initial key chiral structures in compounds **1a**, **1b**, **2a**, **2b** and **6** were built with Spartan 10 software, and all trial structures were first minimized based on molecular mechanics calculations. Conformational search was performed by Spartan 10 software using MMFF force filed, and conformers occurring within a 10 kcal/mol energy window from the global minimum were chosen for geometry optimization in the gas phase with the DFT method at the B3LYP/DGDZVP level. The B3LYP/DGDZVP harmonic vibrational frequencies were further calculated to confirm their stability. The spin-allowed excitation energies and rotatory (*R*n) and oscillator strengths (*f*n) of the lowest excited states of stable conformers were calculated for ECD spectra using TD-DFT method with the basis set RB3LYP/DGDZVP. Solvent effects of methanol solution were evaluated at the same DFT level by using the SCRF/PCM method in agreement with the experiment condition. Electronic transitions were expanded as Gaussian curves with a FQHM (full width at half maximum) for each peak of 0.32 eV. The ECD spectra were combined after Boltzmann weighting according to their population contribution.

3.7. Cytotoxicity

In vitro cytotoxicity was determined by MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] colorimetric assay against K562, HCT116, Hep3B, A-549 and Jurkat cell lines by reported

procedures [32]. All the cell lines were purchased from Shanghai Institute of Cell Biology (Shanghai, China). Adriamycin (doxorubicin, ADM) was used as a positive control.

4. Conclusions

In summary, the present study of sponge *Cacospongia* sp. led to the identification of 15 optically pure terpenoids. This is the first time to encounter a series of stereoisomers of the rare linear C_{17} terpenoid and well-known manoalide-type sesterterpenes from an individual sponge species. Furthermore, there is only one C_{17} γ-lactone terpenoid named cavernosine and no C_{21} pyridine terpenoid had been reported before [10]. The structural diversity of manoalides was usually derived from the multi-site cyclization andoxidation of the linear prenyl chain, such as compounds **5**, **8–10**. However, two pairs of enantiotopic manoalide-type sesterterpenoids (**4a/4b** and **5a/5b**) expanded the chemical diversity of manoalides by a stereoisomer manner. The present results showed a remarkable structural diversity of terpenoids in the sponge *Cacospongia* sp. The unique C_{17} and C_{21} meroterpenoids showed chemotaxonomy significance for the unidentified species of *Cacospongia*.

References

1. Liu, Y.; Zhang, S.; Abreu, P.J. Heterocyclic terpenes: Linear furano- and pyrroloterpenoids. *Nat. Prod. Rep.* **2006**, *23*, 630–651. [CrossRef] [PubMed]
2. Wang, B.; Wang, L.; Li, Y.; Liu, Y. Heterocyclic terpenes: Linear furano- and pyrroloterpenoids. *RSC Adv.* **2014**, *4*, 12216–12234. [CrossRef]
3. Hanson, J.R. Sesterterpenoids. *Nat. Prod. Rep.* **1986**, *3*, 123–132. [CrossRef]
4. Hanson, J.R. The sesterterpenoids. *Nat. Prod. Rep.* **1992**, *9*, 481–489. [CrossRef]
5. Hanson, J.R. The sesterterpenoids. *Nat. Prod. Rep.* **1996**, *13*, 529–535. [CrossRef]
6. Liu, Y.; Wang, L.; Jung, J.H.; Zhang, S. Sesterterpenoids. *Nat. Prod. Rep.* **2007**, *24*, 1401–1429. [CrossRef] [PubMed]
7. Wang, L.; Yang, B.; Lin, X.P.; Zhou, X.F.; Liu, Y. Sesterterpenoids. *Nat. Prod. Rep.* **2013**, *30*, 455–473. [CrossRef]
8. Ebada, S.S.; Lin, W.H.; Proksch, P. Bioactive Sesterterpenes and Triterpenes from marine sponges: Occurrence and pharmacological significance. *Mar. Drugs* **2010**, *8*, 313–346. [CrossRef]
9. Gross, H.; König, G.M. Terpenoids from marine organisms: Unique structures and their pharmacological potential. *Phytochem. Rev.* **2006**, *5*, 115–141. [CrossRef]
10. Cen-Pacheco, F.; Nordström, L.; Souto, M.L.; Martín, M.N.; Fernández, J.J.; Daranas, A.H. Studies on polyethers produced by red algae. *Mar. Drugs* **2010**, *8*, 1178–1188. [CrossRef]
11. Braekman, J.C.; Daloze, D.; Bertau, R.; Macedo de Abreu, P. Cavernosine, a novel ichthyotoxic terpenoid lactone from the sponge *Fasciospongia cavernosa*. *Bull. Soc. Chim. Belg.* **1982**, *91*, 791–796. [CrossRef]
12. Wang, L.J.; Xiong, J.; Lau, C.; Pan, L.L.; Hu, J.F. Sesquiterpenoids and further diterpenoids from the rare Chloranthaceae plant *Chloranthus sessilifolius*. *J. Asian Nat. Prod. Res.* **2015**, *17*, 1220–1230. [CrossRef] [PubMed]

13. Mehbub, M.F.; Perkins, M.V.; Zhang, W.; Franco, C.M.M. New marine natural products from sponges (Porifera) of the order Dictyoceratida (2001 to 2012); a promising source for drug discovery, exploration and future prospects. *Biotechnol. Adv.* **2016**, *34*, 473–491. [CrossRef] [PubMed]

14. Guella, G.; Amade, P.; Pietra, F. Cacospongione A, cacospongienone A, and cacospongienone B, new C$_{21}$ difuran terpenoids from the marine sponge *Cacospongia scalaris* SCHMIDT of the Côte d'Azur. *Helv. Chim. Acta.* **1986**, *69*, 726–733. [CrossRef]

15. Derosa, S.; Destefano, S. Cacospongionolide. A new antitumoral sesterterpene, from the marine sponge *Cacospongia mollior*. *J. Org. Chem.* **1988**, *53*, 5020–5023. [CrossRef]

16. Fattorusso, E.; Magno, S.; Santacroce, C.; Sica, D. Scalarin, a new pentacyclic C-25 terpenoid from the sponge *Cacospongia scalaris*. *Tetrahedron* **1972**, *28*, 5993–5997. [CrossRef]

17. Tanaka, J.; Marriott, G.; Higa, T.; Higa, T. Cacofurans A and B, new furanoditerpenes from a marine sponge. *J. Nat. Prod.* **2001**, *64*, 1468–1470. [CrossRef]

18. Rubio, B.K.; van Soest, R.W.M.; Crews, P. Extending the record of meroditerpenes from *Cacospongia* marine sponges. *J. Nat. Prod.* **2007**, *70*, 628–631. [CrossRef]

19. Wang, Q.; Tang, X.L.; Luo, X.C.; de Voogd, N.J.; Li, P.L.; Li, G.Q. (+)-and (−)-Spiroreticulatine, A pair of unusual spiro bisheterocyclic quinoline-imidazole alkaloids from the South China Sea sponge *Fascaplysinopsis reticulate*. *Org. Lett.* **2015**, *17*, 3458–3461. [CrossRef]

20. Liu, C.X.; Tang, X.L.; Li, P.L.; Li, G.Q. Suberitine A–D, four new cytotoxic dimeric aaptamine alkaloids from the marine sponge *Aaptos suberitoides*. *Org. Lett.* **2012**, *14*, 1994–1997. [CrossRef]

21. Jefford, C.W.; Jaggi, D.; Bernardinelli, G.; Boukouvalas, J. The synthesis of (±)-cavernosine. *Tetrahedron Lett.* **1987**, *28*, 4041–4044. [CrossRef]

22. Nishida, Y.; Konno, T.; Ohrui, H.; Meguro, H. Circular Dichroism (CD) of marmelo lactones and the effect of the unsaturation at C-5 on the cotton effect of γ-lactone. *Agr. Biol. Chem. Tokyo* **1983**, *47*, 2683–2684. [CrossRef]

23. Underwood, B.S.; Tanuwidjaja, J.; Ng, S.S.; Jamison, T.F. Total syntheses of the squalene-derived halogenated polyethers ent-dioxepandehydrothyrsiferol and armatol A via bromonium-and Lewis acid-initiated epoxide-opening cascades. *Tetrahedron* **2013**, *69*, 5205–5220. [CrossRef] [PubMed]

24. Lange, G.L.; Lee, M. ^{13}C NMR determination of the configuration of methyl-substituted double bonds in medium-and large-ring terpenoids. *Magn. Reson. Chem.* **1986**, *24*, 656–658. [CrossRef]

25. Mehl, F.; Bombarda, I.; Vanthuyne, N.; Faure, R.; Gaydou, E.M. Hemisynthesis and odour properties of δ-hydroxy-γ-lactones and precursors derived from linalool. *Food Chem.* **2010**, *121*, 98–104. [CrossRef]

26. Frelek, J.; Ikekawa, N.; Takatsuto, S.; Snatzke, G. Application of [Mo$_2$(OAc)$_4$] for determination of absolute configuration of brassinosteroid vic-diols by circular dichroism. *Chirality* **1997**, *9*, 578–582. [CrossRef]

27. Di Bari, L.; Pescitelli, G.; Pratelli, C.; Pini, D.; Salvadori, P. Determination of absolute configuration of acyclic 1, 2-diols with Mo$_2$(OAc)$_4$. 1. Snatzke's method revisited. *J. Org. Chem.* **2001**, *66*, 4819–4825. [CrossRef]

28. Piao, S.J.; Zhang, H.J.; Lu, H.Y.; Yang, F.; Jiao, W.H.; Yi, Y.H.; Chen, W.S.; Lin, H.W. Hippolides A–H, acyclic manoalide derivatives from the marine sponge *Hippospongia lachne*. *J. Nat. Prod.* **2011**, *74*, 1248–1254. [CrossRef]

29. Desilva, E.D.; Scheuer, P.J. Three new sesterterpenoid antibiotics from the marine sponge *Luffariella variabilis* (Polejaff). *Tetrahedron Lett.* **1981**, *22*, 3147–3150. [CrossRef]

30. Tsuda, M.; Shigemori, H.; Ishibashi, M.; Sasaki, T.; Kobayashi, J. Luffariolides A–E, new cytotoxic sesterterpenes from the Okinawan marine sponge Luffariella sp. *J. Org. Chem.* **1992**, *57*, 3503–3507. [CrossRef]

31. Jiao, W.H.; Hong, L.L.; Sun, J.B.; Piao, S.J.; Chen, G.D.; Deng, H.; Wang, S.P.; Yang, F.; Lin, H.W. (±)-Hippolide J–A Pair of unusual antifungal enantiomeric sesterterpenoids from the marine sponge *Hippospongia lachne*. *Euro. J. Org. Chem.* **2017**, *24*, 3421–3426. [CrossRef]

32. Alley, M.C.; Scudiero, D.A.; Monks, A.; Hursey, M.L.; Czerwinski, M.J.; Fine, D.L.; Abbott, B.J.; Mayo, J.G.; Shoemaker, R.H.; Boyd, M.R. Feasibility of drug screening with panels of human tumor cell lines using a microculture tetrazolium assay. *Cancer Res.* **1988**, *48*, 589–601. [PubMed]

33. Frisch, M.J.; Trucks, G.W.; Schlegel, H.B.; Scuseria, G.E.; Robb, M.A.; Cheeseman, J.R.; Scalmani, G.; Barone, V.; Mennucci, B.; Petersson, G.A.; et al. *Gaussian 09*; Revision A.1; Gaussian, Inc.: Wallingford, CT, USA, 2009.