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Tritoniopsins A−D, Cladiellane-Based Diterpenes from the South China Sea Nudibranch *Tritoniopsis elegans* and Its Prey *Cladiella krempfi*

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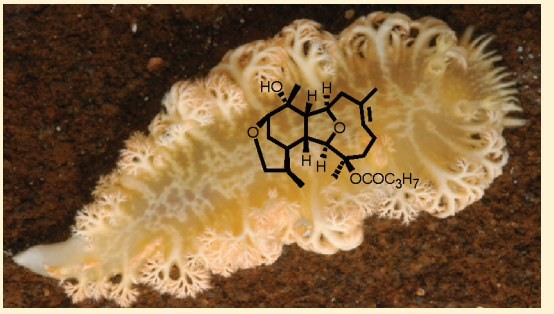
Supporting Information

ABSTRACT: Four diterpenes, tritoniopsins A−D (**1**−**4**), have been isolated from the South China Sea nudibranch *Tritoniopsis elegans* and its prey, the soft coral *Cladiella krempfi*. They display an unprecedented pyran ring in the cladiellane framework, thus representing a novel cladiellane-based diterpene family. Their structures have been mainly characterized by NMR and mass spectrometric techniques, whereas the relative configuration of compound **1** was secured by X-ray analysis. Antiproliferative assays on tumor and nontumor cell lines have been carried out for the main metabolite, tritoniopsin B (**2**).



Nudibranchs are naked mollusks able to escape from the attack of predators by using different kinds of defensive strategies, including the use of chemicals.[1] From the suborder Dendronotina only two families, Tritonidae and Thethydidae, have been chemically studied. Tritoniids accumulate their chemical arsenal from dietary organisms, whereas thetydids are able to synthesize their own defenses. The chemical studies on the first family resulted in the isolation of typical soft coral diterpenoid metabolites,[2] whereas de novo biosynthesized prostaglandin lactones have been reported from species belonging to the second group.[2]

As a part of our ongoing studies on bioactive products from opisthobranch mollusks, we report here the chemical analysis of the nudibranch *Tritoniopsis elegans* and the soft coral *Cladiella krempfi* on which individuals of *T. elegans* were found grazing. The animals were collected in the South China Sea waters. Four diterpenoids, named tritoniopsins A−D (**1**−**4**), were characterized. They belong to the large family of 2,11-cyclized cembranoids, but possess an unprecedented six-membered C12/C20 ether-linked ring, thus representing a new group in the class of cladiellanes in addition to the eunicellins, briarellins, asbestinins, and sarcodictyns.[3] Cladiellanes are typical soft coral diterpene metabolites,[4] and a number of them possess interesting pharmacological activities.[5]

RESULTS AND DISCUSSION

Five individuals of *T. elegans* (4 cm average length) and a sample of the soft coral *C. krempfi* (40 g dry weight) were collected by scuba diving at −10 m off Wei Zhou Island (South China Sea) and immediately frozen. These materials were then transferred to Italy for chemical studies. The soft coral sample was cut into small pieces and extracted with acetone. Nudibranchs were carefully dissected into lateral appendages, mantle, and internal parts and extracted with acetone. A detailed extraction scheme appears in the Experimental Section. The TLC chromatographic comparison of the diethyl ether extracts showed main spots in the nudibranch and its prey at R_f 0.4−0.2 (light petroleum ether/diethyl ether, 4:6), and in particular, tritoniopsin A (**1**) was detected particularly concentrated in the mollusk mantle. The Et_2O extract (1 g) from *C. krempfi* was fractionated on a Sephadex LH-20 column to obtain eight fractions. Two fractions containing the main metabolites were sequentially purified by silica gel and RP-HPLC chromatography, leading to the isolation of pure tritoniopsins A−D (**1**−**4**).

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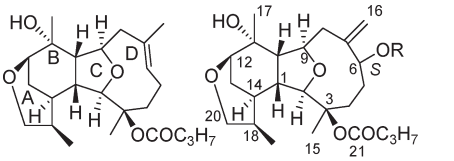

1

2 R= OH; **3** R= H; **4** R= Ac
3a R= *S*-MTPA
3b R= *R*-MTPA

Tritoniopsin A (**1**) was isolated as a white powder and had a molecular formula of $C_{24}H_{38}O_5$, as suggested by the sodiated molecular ion peak at m/z 429.2593 in the HRESIMS spectrum. The ^{1}H NMR spectrum of **1** revealed a typical terpene pattern exhibiting methyl signals at δ 0.90 (d, J = 6.8 Hz, H_3-19), 1.28 (s, H_3-17), 1.78 (br s, H_3-16), and 1.85 (s, H_3-15), multiplets at δ 3.41−4.40, which were due to five protons on oxygenated carbons, and a vinyl signal at δ 5.44 (app t, J = 8.8 Hz, H-6). The ^{13}C NMR spectrum of **1** (Table 2) showed 24 resolved resonances that included signals attributed to two sp^2 carbons of a double bond [δ 128.5 (d, C-6) and 126.8 (s, C-7)] and an ester carbonyl (δ 172.2, C-21), with the remaining signals being due to sp^3 carbons. This accounted for two of the six degrees of unsaturation required by the molecular formula, indicating that tritoniopsin A (**1**) had to be tetracyclic. The DEPT and HSQC data identified 20 carbons linked to hydrogen atoms (5 × CH_3, 7 × CH_2, 8 × CH), and four of them were oxygenated [δ 67.9 (t, C-20), 74.7 (d, C-12), 78.3 (d, C-9), and 87.5 (d, C-2)]. Detailed analysis of the ^{1}H−^{1}H COSY spectrum identified three partial structures, **a**−**c** (Figure 1). The first spin system (partial structure **a**) started with an oxymethine at δ 3.45 (H-12) and continued in sequence with a methylene at δ 2.40 and 1.55 (H_2-13), which was connected to a methine at δ 2.04 (H-14). This latter proton had cross-peaks with both methines H-18 (δ 2.13) and H-1 (δ 2.35). The proton H-18 was in turn linked to a methyl doublet at δ 0.90 (H_3-19) and a diastereotopic geminal oxymethylene resonating at δ 3.70 and 3.41 (H_2-20), whereas H-1 was correlated with both an oxymethine at δ 4.20 (H-2) and a methine at δ 2.15 (H-10). Finally, H-10 was connected to an oxymethine at δ 4.40 (H-9), which was in turn coupled with a methylene at δ 2.54 and 1.91 (H_2-8). Fragment **b** consisted of an olefinic proton at δ 5.44 (H-6) showing allylic correlations with both a methyl at δ 1.78 (Me-16) and a methylene at δ 2.42 and 2.16 (H_2-5), which was further linked to another methylene at δ 2.40 and 1.90 (H_2-4). Partial structure **c**, which was part of a butanoyl residue, consisted of a methyl triplet at δ 0.92 (H_3-24) coupled with a methylene at δ 1.58 (H_2-23), which was in turn connected to a methylene at δ 2.12 (H_2-22).

These data supported the presence of a functionalized cyclic diterpene framework bearing a C_4 acyl residue. HMBC analysis allowed us to link fragments **a**−**c** by closing the required four rings. Diagnostic HMBC correlations were observed between the tertiary methyl H_3-15 (δ 1.85) and the carbon resonances at δ 30.6 (C-4), 87.5 (C-2), and 90.0 (C-3) (Table 1). This implied the connection of C-2 in fragment **a** with C-4 in fragment **b** through a nonprotonated oxygenated C-3 bearing the methyl C-15 (Figure 1). The other end of the partial structure **b**, the trisubstituted double bond, was attached to C-8 by the observation of HMBC correlations of C-6 (δ 128.5) with H_2-8 (δ 1.91 and 2.54). The connection of the remaining carbons with unsatisfied valences in fragment **a** (C-10 and C-12) with a second oxygenated tertiary carbon at δ 71.5 (C-11) was supported by the HMBC correlations of the tertiary methyl H_3-17 attached to

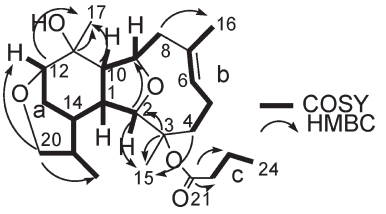

Figure 1. COSY and selected HMBC for tritoniopsin A (**1**).

C-11 with C-10 (δ 53.3) and C-12 (δ 74.7). This led to the formation of the ring B (Figure 1). At this point, it remained to define the oxygenated functions at C-2, C-3, C-9, C-11, C-12, and C-20 and to locate the *n*-butanoyl residue. Bearing in mind the molecular formula of **1**, only four oxygen atoms had to be positioned on the carbon skeleton. Consequently, two of them were suggested to be involved in ether linkages. Diagnostic HMBC correlations were observed between C-2 (δ 87.5) and H-9 (δ 4.40) and between C-20 (δ_C 67.9) and H-12 (δ 3.45), thus implying the connections through oxygen bridges between C-2 and C-9 and between C-20 and C-12, respectively. Finally, the *n*-butanoyl group was assumed to esterify the hydroxy function at C-3 on the basis of both the downfield shift of the C-3 carbon value and the upfield shift of the H_3-15 resonance with respect to the corresponding values in eunicellin model compounds[6] with a free alcoholic group in the same position.

The geometry of the double bond was suggested to be *E* by the value of both C-16 and C-8 with respect to model compounds exhibiting the *Z* double-bond geometry.[6] The relative configuration of the stereogenic centers of the molecule was determined by ^{1}H NMR coupling constant analysis and ^{13}C NMR and NOE data. First, H-12 and H-14 in the cyclohexane ring B were observed to resonate as broad singlets (Table 1), suggesting that both protons were equatorial. Consequently, the substituents on the bridgeheads C-12 and C-14 had to be axially oriented, as drawn in **1**.

Diagnostic steric interactions were observed between H_3-19 and H-1 and between H-20b and H-10, thus indicating that all of these protons were directed toward the same side of the molecule. Irradiation of H_3-17, the orientation of which was suggested to be equatorial by the carbon value (δ 27.3), showed enhancement of H-9, H-10, and H-12, thus implying the configuration at C-9 and C-11 as depicted in formula 1. The configuration at C-2 was consequently defined due to the presence of the tetrahydrofuran ring, whereas that at C-3 was tentatively assigned by a strong NOE interaction between H-2 and H_3-15.

The proposed structure was secured by X-ray diffraction analysis of suitable single crystals of tritoniopsin A (**1**).[7] The relative configuration was established as 1*R**, 2*R**, 3*R**, 9*R**, 10*S**, 11*R**, 12*R**, 14*R**, and 18*R** (Figure 2). The absolute configuration could not be reliably determined in the absence of atoms with a strong anomalous scattering contribution. However, all related metabolites so far reported with known absolute configuration[8] display 1*R*, 2*R*, 9*R*, and 10*S* configuration.

Tritoniopsin B (**2**) was the main metabolite of the soft coral extract. The molecular formula $C_{24}H_{38}O_7$ as deduced from the sodiated molecular peak at m/z 461.2503 in the HRESIMS spectrum contained two additional oxygen atoms with respect to **1**. Comparison of ^{1}H and ^{13}C NMR data of **2** with those of **1** showed several similaritites (Tables 1 and 2). The main differences were an exocyclic double bond (C-16, δ_H 5.30 and 5.21, δ_C 115.5; C-7, δ_C 144.3) replacing the trisubstituted double bond at C-6/C-7 and an additional oxygenated methine

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Table 1. ^{1}H NMR Data for Tritoniopsins A−D (**1**−**4**)[a] in $CDCL_3$

	1[b]	**2**[b]	**3**[c]	**4**[c]
proton	δ_H (J in Hz)	δ_H (J in Hz)	δ_H (J in Hz)	δ_H (J in Hz)
1	2.35, m	2.65, app t (9.1)	2.68, app t (8.8)	2.68, app t (9.2)
2	4.20, d (10.4)	4.11, d (9.1)	4.13, d (10.2)	4.14, d (9.7)
4a	2.40, m	2.22, m	2.38, m	2.14, m
4b	1.90, m	1.88, m	1.85, m	2.25, m
5a	2.42, m	2.16, m	1.88, m	1.95, m
5b	2.16, m	−, m	2.38, m	
6	5.44, app t (8.8)	4.50, br dd (3.9, 3.1)	4.28, br s	5.35, br s
8a	2.54, dd (13.1, 6.2)	2.31, m	2.30, m	2.38, m
8b	1.91, br d (13.1)		2.25, m	2.24, −
9	4.40, d (6.2)	4.48, br t (5.1)	4.49, br s	4.50, br t (5.1)
10	2.15, m	2.25, m	2.33, m	2.32, m
12	3.45, br s ($W_{1/2}$ = 7.5)	3.45, br s ($W_{1/2}$ = 7.5)	3.45, overlapped	3.46, br s ($W_{1/2}$ = 7.2)
13a	2.40, dd (13.3, 6.5)	2.35, m	2.36, m	2.32, m
13b	1.55, m	1.57, m	1.57, m	1.60, m
14	2.04, br s ($W_{1/2}$ = 9.0)	2.01, br s ($W_{1/2}$ = 9.0)	1.98, br s ($W_{1/2}$ = 9.0)	1.99, br s ($W_{1/2}$ = 9.0)
15	1.85, s	1.72, s	1.73, s	1.73, s
16	1.78, brs	5.30, s	5.40, s	5.21, s
		5.21, br s	5.14, s	5.11, s
17	1.28, s	1.30, br s	1.30, s	1.30, s
18	2.13, m	2.13, m	2.12, m	2.15, m
19	0.90, d (6.8)	0.83, d (6.9)	0.83, d (6.8)	0.85, d (6.9)
20a	3.70, dd (11.9, 6.5)	3.71, dd (11.7, 6.5)	3.71, dd (11.9, 6.5)	3.73, dd (11.7, 6.5)
20b	3.41, dd (11.9, 11.9)	3.42, dd (11.7, 11.7)	3.43, dd (11.9, 11.9)	3.42, dd (11.7, 11.7)
22	2.12, m	2.13, m	2.12, m	2.12, m
23	1.58, m	1.58, m	1.57, m	1.52, m
24	0.92, t (7.2)	0.92, t (7.4)	0.91, t (7.2)	0.93, t (7.4)
OAc				2.12, s

[a] Assignments based on ^{1}H−^{1}H COSY, HSQC, and HMBC. [b] Bruker 400 MHz. [c] Bruker 600 MHz.

(δ_H 4.50, δ_C 88.1). Analysis of the ^{1}H−^{1}H COSY spectrum indicated that this oxygenated methine was in ring D, whereas the rest of the molecule was the same as tritoniopsin A (**1**). In particular, a spin system encompassing H-6 and two contiguous methylene groups at δ 2.16 (H_2-5) and 2.22 and 1.88 (H_2-4) was identified by HMBC and COSY data. Finally, an allylic COSY correlation between H-6 (δ 4.50) and H-16a (δ 5.30) led us to locate the oxygenated functionality at C-6. Bearing in mind the molecular formula and according to the high-field carbon value of C-6 (δ 88.1), this functionality was assumed to be a hydroperoxide group.[9] All spectroscopic data were consistent with the proposed structure **2** (see Tables and Experimental Section). The relative configuration of the molecule with the exception of the C-6 asymmetric center was suggested to be the same as tritoniopsin A (**1**) on the basis of NOESY data.

Tritoniopsin C (**3**) had the molecular formula $C_{24}H_{38}O_6$, containing one oxygen less than **2**. The ^{1}H and ^{13}C NMR spectra of **3** exhibited strong similarities with tritoniopsin B (**2**). The main differences were in the proton and carbon values of C-6 (δ_H 4.28, δ_C 75.0 in **3** vs δ_H 4.50, δ_C 88.1 in **2**) and in the proton chemical shifts of exomethylene H_2-16 (δ_H 5.40 and 5.14 in **3** vs δ_H 5.30 and 5.21 in **2**). This clearly indicated that tritoniopsin C (**3**) was the corresponding hydroxy derivative of hydroperoxide **2**. A series of NOE difference experiments carried out on tritoniopsin C (**3**), the analysis of the ^{1}H−^{1}H vicinal coupling constants, and the comparison of ^{1}H and ^{13}C NMR data with co-occurring **1** and **2** suggested the same relative configuration. In order to confirm the structural relationship between **2** and **3**, an aliquot of tritoniopsin B (**2**) was treated with $NaBH_4$.[10] The corresponding alcohol derivative obtained by this reaction was identical to tritoniopsin C (**3**), confirming the proposed structures. The absolute configuration at C-6 of **3**, and consequently of **2**, was determined by the modified Mosher method.[11] Due to the scarce amounts of natural **3**, the method was applied on the alcohol derivative obtained by the reduction of **2**. Two aliquots of this compound were treated with (*R*)- and (*S*)-MTPA chlorides to obtain the (*S*)- and (*R*)-esters, **3a** and **3b**, respectively. Both Mosher esters were characterized by 2D-NMR experiments. The $\Delta\delta$ values (δ_S ester − δ_R ester) observed for the signals of protons close to the hydroxy group at C-6 indicated the *S* configuration at this carbon.

Analysis of the HRESIMS data of tritoniopsin D (**4**) provided a molecular formula of $C_{26}H_{40}O_7$, indicating that it was closely related to compound **3**. The ^{1}H and ^{13}C NMR spectra of **4** differed from those of **3** only in the presence of additional signals due to an acetyl moiety [δ_H 2.12 (3H, s); δ_C 21.2 (CH_3), 169.9 (C)]. The typical acylation shift observed for the proton and carbon values of C-6 (δ_H 5.35, δ_C 76.5) clearly indicated that compound **4** was the 6-acetyl derivative of tritoniopsin C (**3**). All NMR resonances assigned by 2D NMR experiments were

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Table 2. ^{13}C NMR Data for Tritoniopsins A−D (1−4) in $CDC1_3$

	1[a,c,d]	2[a,c,d]	3[b−d]	4[b−d]
position	δ_C mult.	δ_C mult.	δ_C mult.	δ_C mult.
1	35.8, CH	35.7, CH	35.5, CH	35.6, CH
2	87.5, CH	88.7, CH	86.3, CH	86.2, CH
3	90.0, C	85.4, C	85.4, C	85.2, C
4	30.6, CH_2	24.0, CH_2	24.0, CH_2	24.6, CH_2
5	21.9, CH_2	24.0, CH_2	23.3, CH_2	24.6, CH_2
6	128.5, CH	88.1, CH	75.0 CH,	76.5, CH
7	126.8, C	144.3, C	148.7, C	143.9, C
8	44.8, CH_2	40.0, CH_2	39.7, CH_2	40.1, CH_2
9	78.3, CH	80.0, CH	79.6, CH	79.4, CH
10	53.3, CH	51.7, CH	51.0, CH	51.0, CH
11	71.5, C	71.6, C	71.7, C	71.6, C
12	74.7, CH	74.7, CH	74.6, CH	74.7, CH
13	25.0, CH_2	25.0, CH_2	25.0, CH_2	25.0, CH_2
14	29.8, CH	30.8, CH	30.8, CH	30.8, CH
15	21.3, CH_3	23.6, CH_3	23.3, CH_3	23.5, CH_3
16	18.6, CH_3	115.5, CH_2	113.2, CH_2	113.8, CH_2
17	27.3, CH_3	27.4, CH_3	27.4, CH_3	27.4, CH_3
18	35.2, CH	34.7, CH	34.7, CH	34.7, CH
19	15.4, CH_3	15.2, CH_3	15.3, CH_3	15.4, CH_3
20	67.9, CH_2	68.0, CH_2	68.1, CH_2	68.0, CH_2
21	172.2, C	172.3, C	172.2, C	172.3, C
22	38.1, CH_2	37.5, CH_2	37.5, CH_2	37.5, CH_2
23	18.5, CH_2	18.4, CH_2	18.2, CH_2	18.4, CH_2
24	13.5, CH_3	13.6, CH_3	13.6, CH_3	13.6, CH_3
OAc				21.2, CH_3
				169.9, C

[a] 75 MHz for ^{13}C spectra. [b] 150 MHz for ^{13}C spectra. [c] Multiplicity deduced by DEPT. [d] Assignments aided by HSQC and HMBC correlations, constant optimized for J = 10 Hz.

consistent with the proposed structure. The chemical correlation between tritoniopsins C and D was confirmed by submitting an aliquot of compound **3** to the acetylation reaction. After the usual workup, a compound identical in all respects with tritoniopsin D (**4**) was obtained. The presence of these unique metabolites in both the nudibranch and the soft coral clearly indicated the trophic relationship between the two organisms.

Considering the wide range of biological activities exhibited by diterpenoids belonging to the cladiellane class, we performed in vitro experiments on a panel of tumor and nontumor cell lines in order to investigate the bioactivity of tritoniopsin B (**2**), the main metabolite. The results showed moderate to weak cytotoxicity toward embryonic H9c2 rat cardiac myoblasts, 3T3-L1 murine fibroblasts, and Caco-2 human epithelial colorectal adenocarcinoma cells, with IC_{50} values ranging from 40 to 65 μM. Under the same experimental conditions no cytotoxicity was observed against C6 rat glioma cells and HeLa cervical cancer cells.

This is the first chemical report on the nudibranch *T. elegans*. The tritoniopsins display an unprecedented ether ring bridging C-12 and C-20 in addition to the five-membered 2,9-cyclized ether, which is also present in eunicellin, briarellin, and asbestinin diterpene frameworks.

Comparative chemical analysis of the nudibranch and the soft coral indicated that tritoniopsins were present in both organisms,

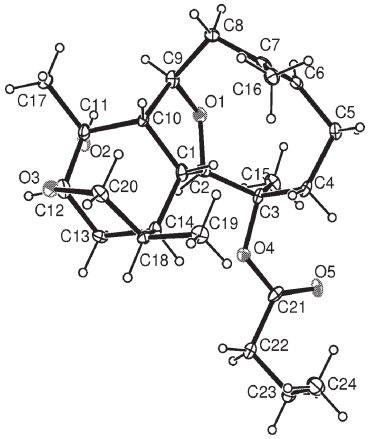

Figure 2. Molecular structure of tritoniopsin A (**1**). Thermal ellipsoids are drawn at the 20% probability level.

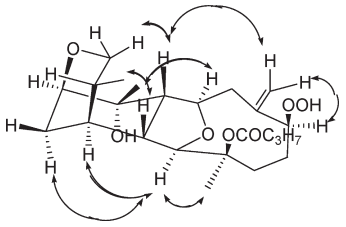

Figure 3. NOE effects for tritoniopsin B (**2**).

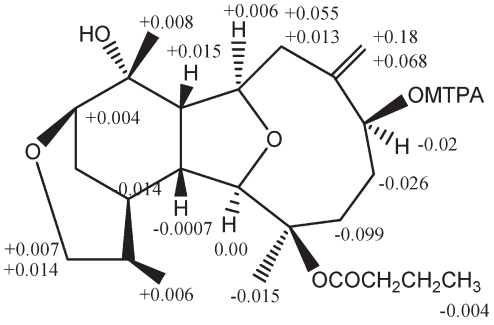

Figure 4. $\Delta\delta$ values ($\Delta\delta = \delta_S - \delta_R$ in ppm) obtained for MTPA esters **3a**/**3b**.

thus confirming the trophic relationship between the two animals. Tritoniopsins A (**1**) and B (**2**) were the major secondary metabolites. It is worthy of note that the relative ratio between compounds **1** and **2** appeared inverted from the soft coral (**2** > **1**) in the nudibranch (**1** > **2**). This could be due to the ability of the nudibranch to accumulate selectively dietary compounds useful for its own protection.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured using a Jasco DIP 370 digital spectropolarimeter. IR spectra were measured on a Biorad FTS 155 FTIR spectrophotometer. 1D- and 2D-NMR spectra were recorded on a Bruker Avance-400 (400.13 MHz) and on a Bruker DRX-600 equipped with a TXI CryoProbeTM in $CDCl_3$ and C_6D_6 (δ values are reported with reference to $C\underline{H}Cl_3$ at 7.26 ppm and to $C_6\underline{H}_6$ at 7.15 ppm), and ^{13}C NMR spectra were

recorded on Bruker DPX-300 (75 MHz) and Bruker DRX-600 (150 MHz) spectrometers (δ values are reported with reference to $CDCl_3$ at 77.0 ppm and to C_6D_6 at 128.0 ppm). HRESIMS measurements were carried out on a Micromass Q-TOF micro; a HPLC Waters 501 pump with a refractometer detector was used, equipped with a reverse-phase Kromasil C-18, 5 μ column (250 × 4.60 mm, Phenomenex). TLC plates (KieselGel 60 F254) were from Merck, silica gel powder (Kieselgel 60 0.063−0.200 mm) was from Merck, and Sephadex LH-20 was from Amersham Bioscience. Solvents for chromatography were HPLC grade and were used without further purification.

Collection of the Animal Material. Five specimens of *Tritoniopsis elegans* were collected by E. Mollo using scuba at a depth of 10 m off Weizhou Island, South China Sea, China, in May 2007. The soft coral *Cladiella krempfi*, on which the nudibranchs were found grazing, was collected in the same site. Voucher specimens of *T. elegans* and *C. krempfi* are deposited at the Istituto di Chimica Biomolecolare (ICB) with the codes WZ77 and WZ77C, respectively.

*Extraction of the Soft Coral and Isolation of Tritoniopsins A−D (**1**−**4**).* The frozen sample of *C. krempfi* (40 g, dry weight) was extracted with acetone (3 × 200 mL). The acetone extracts were concentrated in vacuo, and the aqueous residue was fractionated between H_2O and Et_2O (4 × 250 mL). After evaporation under reduced pressure we obtained 1 g of ethereal extract. A portion (800 mg) of the soft coral extract was subjected to Sephadex LH-20 chromatography with $CHCl_3$/MeOH (1:1) to afford five fractions, from A to E. Tritoniopsins A−D (**1**−**4**) were obtained from fractions C (55 mg) and D (85 mg) by purification on silica gel column chromatography using light petroleum ether and an increasing amount of Et_2O, and subsequently by C18 reversed-phase HPLC using a 5 μ Kromasil column (250 × 4.60 mm, Phenomenex) with MeOH/H_2O (8:2) as the eluent system over 50 min (flow rate 1 mL/min). The purification of these fractions afforded 6 mg of compound **1**, 12 mg of **2**, 1.5 mg of **3**, and 1.0 mg of **4**.

Extraction of the Tritoniopsis elegans. The frozen specimens of *T. elegans* were carefully dissected into lateral appendages (63.7 mg dry weight), mantle (268 mg dry weight), and glands (109 mg dry weight) and extracted separately with acetone (3 × 5 mL) using ultrasound. The extracts were concentrated in vacuo, and the aqueous residues were fractionated between H_2O and Et_2O (3 × 50 mL). The Et_2O portions were evaporated under reduced pressure to give 8.4 mg from lateral appendages, 31.4 mg from the mantle, and 29.7 mg from the glands. Comparison of these extracts by TLC with that of *C. krempfi*, in different system solvents, revealed the presence of similar spots in both animals. Tritoniopsins A−D were isolated from the mantle and glands of the nudibranchs using the same chromatographic procedure as described for the soft coral. The amounts of the four purified diterpenes **1**−**4** were 2.0, 0.5, 0.3, and 0.2 mg, respectively.

*Tritoniopsin A (**1**):* white, amorphous solid; $[\alpha]^{25}_D$ −31.7 (*c* 0.18, $CHCl_3$); IR (liquid film) 3447, 2969, 2874, 1727, 1462, 1184, 1072 cm^{-1}; ^1H NMR, see Table 1; ^{13}C NMR, see Table 2; HRESIMS $[M + Na]^+$ *m/z* 429.2593 (calcd for $C_{24}H_{38}O_5Na$, 429.2617).

*Tritoniopsin B (**2**):* white, amorphous solid; $[\alpha]^{25}_D$ −47.6 (*c* 0.27, $CHCl_3$); IR (liquid film) 3380, 2969, 2928, 2874, 1718, 1443, 1180, 1078, 756 cm^{-1}; ^1H NMR, see Table 1; ^{13}C NMR, see Table 2; ^1H NMR (600 MHz, C_6D_6) δ 5.31 (1H, s, H-16a), 4.97 (1H, s, H-16b), 4.35 (1H, overlapped, H-9), 4.34 (1H, overlapped, H-2), 4.28 (1H, br s $J_{(W1/2)}$ = 10 Hz, H-6), 3.65 (1H, dd, *J* = 11.7, 6.5 Hz, H-20a), 3.42 (1H, dd, *J* = 11.7, 11.7 Hz, H-20b), 3.40 (1H, br s, $J_{(W1/2)}$ = 8.3 Hz, H-12), 2.65 (1H, app. t, *J* = 9.0 Hz, H-1), 2.45 (1H, m, H-13a), 2.25 (2H, m, H_2-5), 2.11 (1H, d, *J* = 8.5 Hz, H-10), 2.05 (1H, br s, $J_{(W1/2)}$ = 9.0 Hz, H-14), 2.02 (2H, m, H_2-8), 2.00 (2H, m, H_2-4), 1.95 (1H, m, H-18), 1.92, (2H, m, H_2-22), 1.88 (3H, s, H_3-15), 1.57 (1H, m, H-13b), 1.48 (2H, m, H_2-23), 1.10 (3H, br s, H_3-17), 0.76 (3H, t, *J* = 7.4 Hz, H_3-24), 0.66 (3H, d, *J* = 6.7 Hz, H_3-19); ^{13}C NMR (150 MHz, C_6D_6) δ 171.7 (C, C-21), 144.8 (C, C-7), 114,9 (CH_2, C-16), 87.7 (CH, C-6), 86.6 (CH, C-2), 85.4 (C, C-3), 79.7 (CH, C-9), 74.8 (CH, C-12), 71.4 (C, C-11), 68.1 (CH_2, C-20), 51.9 (CH, C-10), 40.5 (CH_2, C-8), 37.4 (CH_2, C-22), 36.0 (CH, C-1), 35.2 (CH, C-18), 31.4 (CH, C-14), 27.4 (CH_3, C-17), 25.6 (CH_2, C-13), 24.8 (CH_2, C-5), 23.6 (CH_2, C-4), 23.6 (CH_3, C-15), 18.7 (CH_2, C-23), 15.4 (CH_3, C-19), 13.6 (CH_3, C-24); selected proton signals in DMSO-d_6, δ 11.39 (1H, s, OOH-6), 4.34 (1H, s, OH-11); HRESIMS $[M + Na]^+$ *m/z* 461.2503 (calcd for $C_{24}H_{38}O_7Na$, 461.2515).

*Tritoniopsin C (**3**):* white, amorphous solid; $[\alpha]^{25}_D$ −32.7 (*c* 0.15, $CHCl_3$); IR (liquid film) 3445, 2962, 2931, 2878, 1731, 1455, 1383, 1185, 1078 cm^{-1}; ^1H NMR, see Table 1; ^{13}C NMR, see Table 2; HRESIMS $[M + Na]^+$ *m/z* 445.2558 (calcd for $C_{24}H_{38}O_6Na$, 445.2566).

*Tritoniopsin D (**4**):* white, amorphous solid; $[\alpha]^{25}_D$ −31.7 (*c* 0.10, $CHCl_3$); IR (liquid film) 3447, 2969, 2874, 1727, 1462, 1184, 1072 cm^{-1}; ^1H NMR, see Table 1; ^{13}C NMR, see Table 2; HRESIMS $[M+ Na]^+$ *m/z* 487.2671 (calcd for $C_{26}H_{40}O_7Na$, 487.2672).

*Reduction of Tritoniopsin B (**2**).* $NaBH_4$ (4 mg, 0.106 mmol) was added to a solution of **2** (9 mg, 0.021 mmol) in anhydrous MeOH (2 mL). The mixture was stirred at rt for 80 min. The reaction was neutralized with 2 M HCl and extracted with $CHCl_3$. Removal of the solvent under reduced pressure gave a compound that was identical to tritoniopsin C (**3**).

*Compound **3a** (S-MTPA ester).* The *S*-MTPA ester was prepared by treating 2 mg of **3**, obtained by reduction of compound **2**, with 0.005 mL of *R*-(−)-MTPA chloride and a catalytic amount of DMAP in dry CH_2Cl_2 (0.5 mL) with stirring for 16 h at rt. The ester was purified by HPLC on a RP-18 column (MeOH/H_2O, 72:25, flow rate 1 mL/min). Selected ^1H NMR values (400 MHz, $CDCl_3$): δ 5.5861 (H-6), 5.2495 (H-16a), 5.1119 (H-16b), 4.4910 (H-9), 3.7017 (H-20a), 3.3789 (H-20b), 2.5757 (H-1), 2.3664 (H-8a), 1.7250 (H_2-4), 2.2703 (H-8b), 2.2515 (H-10), 2.0923 (H_2-5), 1.9553 (H-14), 1.7173 (H_3-15), 1.2959 (H_3-17), 0.7696 (H_3-19), 0.9095 (H_3-24); ESIMS $[M+ Na]^+$ *m/z* 661.

*Compound **3b** (R-MTPA ester).* The *R*-MTPA ester of **3** was prepared by the same procedure except that 0.005 mL of *S*-(+)-MTPA chloride was used. The *R*-MTPA ester was purified by HPLC on a RP-18 column (MeOH/H_2O, 72:25, flow rate 1 mL/min). Selected ^1H NMR values (600 MHz, $CDCl_3$): δ 5.6058 (H-6), 5.0700 (H-16a), 5.0437 (H-16b), 4.4848 (H-9), 3.6946 (H-20a), 3.3653 (H-20b), 2.5764 (H-1), 2.3534 (H-8a), 1.8240 (H_2-4), 2.2152 (H-8b), 2.2362 (H-10), 2.1191 (H_2-5), 1.9696 (H-14), 1.7320 (H_3-15), 1.2874 (H_3-17), 0.7634 (H_3-19), 0.9135 (H_3-24); ESIMS $[M + Na]^+$ *m/z* 661.

*Acetylation of Tritoniopsin C (**3**).* To 0.5 mg of compound **3** was added 0.3 mL of acetic anhydride in dry pyridine, and the solution was stirred overnight at rt. After removing the solvent under vacuum, the reaction product was checked by ^1H and ^{13}C NMR and was determined to be the same as tritoniopsin D (**4**).

X-ray Crystallographic Procedure. Suitable crystals of tritoniopsin A (**1**), obtained by slow evaporation of *n*-hexane, were selected and glued onto a glass fiber and measured at rt with a Rigaku AFC11K diffractometer equipped with a Saturn944 CCD detector using Cu Kα radiation. Data reduction was performed with the crystallographic package CrystalClear.[12] Data have been corrected for Lorentz, polarization, and absorption. The structures were solved by direct methods using the program SIR2002[13] and refined by means of full matrix least-squares based on F^2 using the program SHELXL97.[14]

All non-hydrogen atoms were refined anisotropically, and hydrogen atoms were positioned geometrically and included in structure factor calculations but not refined.

A total of 262 refinable parameters were finally considered. Final disagreement indices are R1 = 0.069 (2213 reflections $F^2 > 2\sigma F^2$), wR2 = 0.28 (all 2460 independent reflections). The ORTEP plot was obtained by means of the program ORTEP32.[15]

Crystal data: $C_{24}H_{38}O_5$, MW = 406.54, orthorhombic, space group $P2_12_12_1$, *Z* = 4, *a* = 9.873(2) Å, *b* = 14.759(4) Å, *c* = 15.008(3) Å, *V* = 2186.9(9) $Å^3$, D_x = 1.235 g cm^{-3}, μ_{calc} = 0.677 mm^{-1}.

Cell Cultures and Evaluation of Cell Growth and Viability. Biological activity was investigated on tumor C6 rat glioma cells, HeLa human cervical cancer cells, Caco-2 human epithelial colorectal adenocarcinoma cells, and nontumor H9c2 rat embryonic cardiac myoblasts and 3T3-L1 murine embryonic fibroblasts. Cells were purchased from ATCC (American Type Culture Collection). The calculation of the concentration required to inhibit the net increase in the 48 h cell count and viability by 50% (IC_{50}) is based on plots of data carried out in triplicate and repeated three times.[16,17] IC_{50} values were obtained using a dose−response curve by nonlinear regression using a curve fitting program, GraphPad Prism 5.0, and are expressed as mean ± SEM (see Supporting Information).

■ ASSOCIATED CONTENT

Supporting Information. Description of X-ray crystal data for **1** and ^{1}H and ^{13}C NMR, $^{1}H-^{1}H$ COSY, HSQC, HMBC, NOESY, and NOEdiff spectra of compounds **1**−**4** are available free of charge via the Internet at http://pubs.acs.org.

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