New Cytotoxic Steroids from the Indian Ocean Sponge *Axinella* cf. *bidderi*

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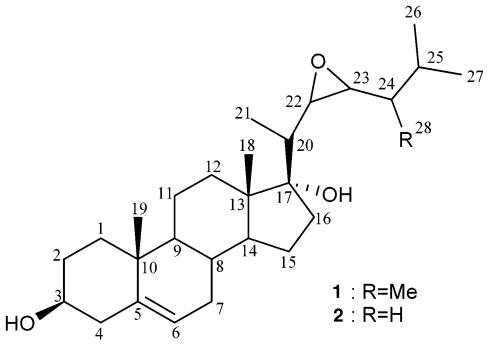
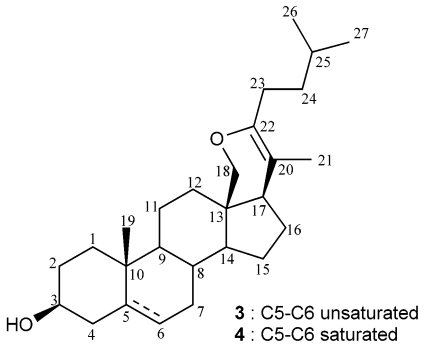
Four new sterols have been isolated from the marine sponge *Axinella* cf. *bidderi*, 17α-hydroxy-22,23-epoxy-24-methylcholest-5-en-3*β*-ol (**1**) and 17α-hydroxy-22,23-epoxycholest-5-en-3*β*-ol (**2**), together with **3** and **4**, which possess respectively the cholestene and the cholestane skeleton with a cyclic enol ether linkage between C-18 and C-22. The structures were elucidated using spectroscopic data. The in vitro activity was evaluated against prostate, ovary, pancreas, colon, and lung cell lines.

Marine sponges are known to be a rich source of steroid metabolites having unusual functionalization and structures,[1,2] and some of them are of great biological interest. In continuation of our studies directed at the discovery of bioactive metabolites from marine organisms,[3−5] we have examined the Indian Ocean sponge *Axinella* cf. *bidderi* (Burton, 1959) (family Axinellidae), whose ethanolic crude extract showed antiproliferative activity in the initial sea-urchin egg bioassay.[6]

In this paper, we describe the isolation and the structure elucidation of four new steroids together with their in vitro inhibition activity against prostate, ovary, pancreas, colon, and lung cell lines. Compounds **1** and **2** are cholest-5-en-3*β*-ol containing an epoxide function on the side chain, and compounds **3** and **4** possess a cyclic enol ether between ring D and the side chain. Moreover, five previously described compounds have been isolated, i.e., sitosterol together with four triterpenes: sodwanone B,[7] sodwanone M,[8] yardenone,[9] and yardenone B.[5]





Compound **1** was isolated as a white powder. The molecular formula established as $C_{28}H_{46}O_3$ by HRLSIMS involved six degrees of unsaturation. *J*-modulated and DEPT spectra revealed the presence of six methyl, eight methylene, 10 methine, and four quaternary carbons (Table 1). The ^{1}H NMR ($CDCl_3$) spectrum showed the presence of two methyl singlet resonances at *δ* 0.78 (Me-18) and 1.04 (Me-19) and four methyl doublet at *δ* 0.91 (*J* = 7.0 Hz, Me-28), 0.95 (*J* = 6.8 Hz, Me-26), 1.00 (*J* = 5.9 Hz, Me-27), and 1.01 (*J* = 6.1 Hz, Me-21). The tertiary methyl signal of C19-H resonating at *δ* 1.04 (3H, s), a multiplet at *δ* 3.53 (H-3), and a broad doublet at *δ* 5.36 (H-6, *J* = 5.1 Hz) are representative of a cholest-5-ene-3*β*-ol skeleton. Moreover, 2D experiments confirmed this Δ5-sterol nucleus (see Experimental Section). As five degrees of unsaturation were located in the sterol nucleus (four rings and one olefin), the last unsaturation had to be in the side chain. The ^{1}H NMR spectrum showed two one-proton signals at *δ* 2.85 (dd, *J* = 7.8, 2.3 Hz) and 2.50 (dd, *J* = 8.1, 2.3 Hz) which were coupled to each other in the COSY experiment and were correlated to the methine at *δ* 59.6 (C-22) and 60.5 (C-23), respectively, in the HSQC experiment (^1J). These chemical shifts for protons and carbons suggested the presence of an epoxide function. In the ^{1}H NMR spectrum, the coupling constant of 2.3 Hz between the two epoxide protons implied their trans relationship. Actually, 2.5 and 4.0 are typical values for a trans and cis relationship, respectively. The proton spin system for H-22 and H-23 (two double doublets) required that the functionality of their respective neighbors C-20 and C-24 was a methine. This was corroborated by the ^3J correlations on the COSY spectrum between H-22 and the methine H-20 on one hand and between H-23 and the methine H-24 on the other hand. As shown in the HMBC and COSY experiments, C-24 bore Me-28 (*δ* 12.9) and the chain ended with an isopropyl group (Me-26 and -27 at *δ* 18.9 and 20.5). In addition to C-5 at *δ* 140.9, C-10 at *δ* 36.7, and C-13 at *δ* 47.8, compound **1** possessed a fourth quaternary carbon at *δ* 85.5, suggesting an oxygen-bearing carbon. To be in accordance with the HMBC correlations between this carbon (*δ* 85.5) and H-16b, Me-18, and Me-21, the hydroxyl group had to be located on C-17. Consequently, the chemical shift of Me-18 was shifted downfield from *δ* 11.8 to 14.8, when compared to the cholesterol.[10] The NOESY correlations between Me-18*β* and H-20 suggested the *β* orientation for C-20 and conse-

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Table 1. NMR Data for **1** and **3** in $CDCl_3$

	1		**3**	
no.	δ_C[a]	δ_H[b] (*J* in Hz)	δ_C[a]	δ_H[b] (*J* in Hz)
1	37.5 (CH_2)	1.87 m 1.12 m	37.2 (CH_2)	1.89 m 1.12 m
2	31.9 (CH_2)	1.86 m 1.53 m	31.6 (CH_2)	1.87 m 1.52 m
3	71.9 (CH)	3.53 m	71.7 (CH)	3.55 m
4	42.5 (CH_2)	2.31 m 2.26 m	42.2 (CH_2)	2.31 m 2.25 m
5	140.9 (C)		140.9 (C)	
6	121.8 (CH)	5.36 d (5.1)	121.3 (CH)	5.36 d (5.2)
7	32.1 (CH_2)	2.02 dtd (17.3, 5.0, 2.5) 1.53 m	32.2 (CH_2)	2.03 m 1.56 m
8	32.4 (CH)	1.65 m	31.8 (CH)	1.42 m
9	50.1 (CH)	0.99 m	50.3 (CH)	1.05 m
10	36.7 (C)		36.6 (C)	
11	21.1 (CH_2)	1.61 m 1.51 m	20.6 (CH_2)	1.63 m 2.04 m
12	32.5 (CH_2)	1.65 m	31.6 (CH_2)	0.92 m
13	47.8 (C)		40.9 (C)	
14	51.1 (CH)	1.66 m	54.7 (CH)	1.31 m
15	24.1 (CH_2)	1.76 m 1.18 m	24.3 (CH_2)	1.72 m 1.33 m
16	37.2 (CH_2)	2.24 m 1.75 m	30.5 (CH_2)	2.12 m 1.49 m
17	85.5 (C)		47.7 (CH)	1.59 m
18	14.8 (CH_3)	0.78 s	63.7 (CH_2)	3.97 dd (10.6, 1.7) 3.32 d (10.6)
19	19.5 (CH_3)	1.04 s	19.4 (CH_3)	1.03 s
20	42.6 (CH)	1.58 m	106.0 (C)	
21	11.9 (CH)	1.01 d (6.1)	16.8 (CH_3)	1.62 s
22	59.6 (CH)	2.85 dd (7.8, 2.3)	146.2 (C)	
23	60.5 (CH)	2.50 dd (8.1, 2.3)	27.9 (CH)	2.07 m
24	42.3 (CH)	1.13 m	36.8 (CH)	1.35 m
25	31.4 (CH)	1.78 m	27.9 (CH)	1.55 m
26	18.9 (CH_3)	0.95 d (6.8)	22.5 (CH_3)	0.91 d (6.6)
27	20.5 (CH_3)	1.00 d (5.9)	22.5 (CH_3)	0.91 d (6.6)
28	12.9 (CH_3)	0.91 d (7.0)		

[a] 125 MHz, $CDCl_3$. [b] 500 MHz, $CDCl_3$.

quently the α orientation for the hydroxyl group. In addition, this orientation was confirmed on the basis of ^{13}C resonance effects observed between the epimeric pair of 17-androstanols in comparison with the androstane hydrocarbonic skeleton.[11] The presence of the 17α-hydroxyl group in compound **1** and in 17α-androstanol led to a shielding effect at C-12 (−7.3 and −7.4 ppm, respectively) and at C-14 (−5.7 and −5.8 ppm, respectively), whereas for the 17*β*-hydroxyl group, the shielding effect observed was −2.1 (C-12) and −3.4 ppm (C-14). Despite the trans geometry of the epoxide ring and the NOESY correlations evidenced in the NMR data, the relative stereochemistry of the side chain in compound **1** remains undetermined. Configurational assignment of 22,23-epoxides of steroids by carbon-13 NMR spectroscopy has been discussed by Gonzales Sierra et al.,[12] but in the present case, the presence of the 17-hydroxyl group does not permit any reliable correlation.

The complete structure of **1** was confirmed as 17α-hydroxy-22,23-epoxy-24-methylcholest-5-en-3*β*-ol on the basis of COSY and HMBC correlations (see Experimental Section).

Previously, 17-hydroxy steroids have been isolated from the marine worm *Cephalodiscus gilchrist*,[13−17] from the tunicate *Ritterella tokioka*,[18−20] from the sponge *Echinoclathria subhispida*,[21] and from the gorgonian *Lophogorgia punicea*.[22] The analogue of compound **1** without the 17-hydroxyl group was reported from the marine sponge *Hyrtios* sp., as the 22,23-epoxy-24-methylcholest-5-en-3*β*-ol.[23] Biosynthetically, 22,23-epoxy steroids would be intermediates to the 23-oxo function from the 22,23-olefinic linkage,[24] prevalent in marine sterols.

Compound **2** was isolated as a white powder. The molecular formula was determined as $C_{27}H_{44}O_3$ by HRL-SIMS (six degrees of unsaturation). Analysis of *J*-modulated and DEPT spectra proved that compound **2** possessed five methyl, nine methylene, nine methine, and four quaternary carbons (see Experimental Section). Except for the side chain, most of the NMR spectral data of **2** were similar to **1**. Two epoxide signals still appeared in the ^{13}C spectrum (C-22 at *δ* 60.8 and C-23 at 56.0) and in the ^1H spectrum (H-22 at *δ* 2.86 and H-23 at 2.68), but the Me-28 (in **1**) had disappeared and the C-24 methine at *δ* 42.3 was replaced with a methylene at *δ* 41.6. Consequently, the proton spin system of H-23 (double doublet in **1**) became a double triplet ($J = 6.3, 2.2$ Hz) in compound **2**. The coupling constant (2.2 Hz) between H-22 and H-23 implied their trans relationship; however, the relative stereochemistry of the side chain remains undetermined. As for compound **1**, the α orientation for the hydroxyl group was suggested by the NOESY correlation between Me-18*β* and H-20. Thus, compound **2** was unambiguously identified as 17α-hydroxy-22,23-epoxycholest-5-en-3*β*-ol.

Compound **3** was isolated as a white powder and exhibited the molecular formula $C_{27}H_{42}O_2$ suggested by HREIMS (seven degrees of unsaturation). Four methyl, 11 methylene, seven methine, and five quaternary carbons appeared in *J*-modulated and DEPT spectra (Table 1). 2D NMR spectra indicated that **3** featured the same A−C rings and a terminal isopropyl group as **1** and **2**, but significant differences were observed in the *J*-modulated spectrum. Me-18 at *δ* 14.8 in **2** had disappeared and a methyl at *δ* 16.8 appeared. The main difference was the presence of an oxygen-bearing methylene at *δ* 63.7 and two olefinic quaternary carbons at *δ* 106.0 and 146.2. The HSQC spectrum showed the methylene at *δ* 63.7 bore inequivalent protons at *δ* 3.97 (H-18a, dd, $J = 10.6, 1.7$ Hz) and 3.32 (H-18b, d, $J = 10.6$ Hz). HMBC correlations around the D ring and the side chain showed that C-18 was correlated with H-12a,b, H-17, and H-14 and that C-17 was correlated with H-12a, H-15a, H-16a, H-18a,b, and the vinyl methyl-21. In addition, the quaternary olefinic carbon C-22 was correlated with H-21, H-23, and H-18a, which argued for an oxygen bridge between C-18 and C-22. Finally, thanks to the combination of COSY and HMBC experiments (see Experimental Section), the presence of a cyclic enol ether in the side chain of compound **3** was confirmed.

A compound containing this unusual side chain is described here for the first time from a sponge. An enoic A ring steroid was previously isolated from the soft coral *Alcyonium gracillimum*.[25] The ^{13}C chemical assignments of the D ring and of the side chain for **3** were in accordance with the published data except for the inverted positions for carbons 16 and 23. Indeed, chemical shifts found for **3** were δ_C 27.9 (C-23) and 30.5 (C-16), respectively, unlike δ_C 27.9 (C-16) and 30.6 (C-23) reported in the literature. These assignments for compound **3** were corroborated by the HMBC correlations between C-13/H-16a and C-23/H-25, whereas no evidence was mentioned in the literature.

Compound **4** was isolated as a white powder with elemental composition $C_{27}H_{44}O_2$ determined by HREIMS and contained six degrees of unsaturation. From *J*-modulated and DEPT spectra, four methyl, 12 methylene, seven methine, and four quaternary carbons were counted (see Experimental Section). Data of **4** were similar to those of **3** especially for the side chain signals. However, in the *J*-modulated spectrum, the quaternary carbon C-5 at *δ*

Table 2. In Vitro Cytotoxic Activity of Compound **1**−**4** (GI_{50} in μg/mL)

	cell lines				
compound	LN-caP	IGROV-ET	PANC1	LOVO	NSCLC N6-L16
1	6.42	1.68	2.74	4.02	0.60
2	3.09	1.24	2.17	6.23	2.00
3	3.52	7.95	8.92	6.91	inactive
4	2.88	6.66	inactive	8.29	inactive

140.9 and the methine C-6 at δ 121.3 in **3** were respectively replaced with a methine at δ 44.8 and a methylene at δ 28.5 in **4**; the double bond at C-5 had disappeared. The COSY spectrum showed correlations between H-6 and both H-5 and H-7b. The HMBC spectrum showed that C-10 was correlated to H-1a (^2J), H-1b (^2J), H-5 (^2J), Me-19 (^2J), and H-4b (^3J) (see Experimental Section). All together, these results were in line with the proposed structure of compound **4**.

Biological Activity. Antitumoral assays were performed at PharmaMar. A colorimetric type of assay using sulforhodamine B (SRB) reaction has been adapted for a quantitative measurement of cell growth and viability, following the technique described in the literature.[26] The in vitro activity of the compounds was evaluated against four human tumoral cell lines: prostate (LN-caP), ovary (IGROV-ET), pancreas (PANC1), and colon (LOVO). The activity against lung cell lines (NSCLC N6-L16) was carried out at University of Nantes: cell growth was estimated using a colorimetric assay based on the conversion of tetrazolium dye (MTT) due to blue formazan product using live mitochondria.[27] The results, presented in Table 2, indicated a moderate cytotoxicity of the compounds screened and showed that compounds **1** and **2** displayed the strongest activity against IGROV-ET, PANC1, and NSCLC N6-L16, while compound **3** and **4** were more active against LN-caP.

Experimental Section

General Experimental Procedures. Optical rotations were determined with a Bellingham ADP 220 polarimeter, and IR spectra were recorded on a Perkin-Elmer Paragon 1000 FT-IR spectrophotometer. NMR spectra were acquired on a Bruker Avance 500 and on a Bruker DRX 500 and referenced to residual solvent signal. HPLC was performed with a Waters model 600 pump, diode array and light scattering detectors, and Si Interchrom (250 × 10 mm, 10 μm) and Diol Hypersil Lichrosorb (250 × 10 mm, 5 μm) columns. HRMS were determined at Service Central d'Analyses du CNRS (Solaize, France).

Animal Material. The sponge *Axinella* cf. *bidderi* (Burton, 1959) was collected in November 1997 off the Socotra Islands (Yemen) at a depth of 25 m. The identification of the sample was realized by Dr. Jean Vacelet (Station Marine d'Endoume, Marseille, France), and a voucher specimen (MHNM2000Im161) is deposited at the Museum d'Histoire Naturelle (Marseille, France).

Extraction and Isolation. The freshly collected sponge (4.7 kg, wet weight) was stored in ethanol until extraction. A portion of the ethanolic extract was evaporated under reduced pressure, and the resulting crude extract (160 g) was partitioned between dichloromethane and water. The organic residue (49 g) was partitioned between heptane and methanol. The heptanic fraction (9.9 g) was found to be the most active, with a GI_{50} value of 5 μg/mL against NSCLC cells. The heptanic fraction was subjected to flash chromatography (Si 60 gel) eluting with *n*-hexane/EtOAc mixtures of increasing polarity from 100% *n*-hexane to 100% EtOAc. One of the active fractions (eluting with *n*-hexane/EtOAc, 8:2) was rechromatographed on Si 60 gel with *n*-hexane/EtOAc mixtures of increasing polarity to yield four crude compounds and five pure known compounds: sodwanone B (26 mg, 0.016%), sodwanone M (74 mg, 0.046%), yardenone (140 mg, 0.088%), yardenone B (283 mg, 0.177%), and sitosterol (13 mg, 0.008%). Final purification by HPLC on Diol Hypersil Lichrosorb 250 × 4.6 mm, 5 μm (using *n*-hexane/EtOAc, 79:21 as eluent) and Si Interchrom 250 × 10 mm, 10 μm (using *n*-hexane/EtOAc, 87:13 as eluent) columns afforded compounds **1**, **2**, **3**, and **4**, respectively.

Compound 1: white powder; 2 mg, 0.001%; IR (film) 3437, 1691, 890, 839 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1; COSY correlations (in a geminal pair, Ha is the lower-field proton and Hb is the higher-field proton) (H/H) H-1a/H-2b; H-1b/H-2a, H-2b; H-2a/H-3; H-2b/H-3; H-3/H-4a, H-4b; H-4b/H-6; H-6/H-7a, H-8; H-7a/H-7b, H-8; H-15a/H-15b; H-15b/H-16a; H-16a/H-16b; H-20/H-21, H-22; H-22/H-23; H-23/H-24; H-25/H-26, H-27; HMBC correlations (H/C) H-1a/C-5, C-10; H-4a/C-2, C-3, C-5, C-6; H-4b/C-2, C-3, C-5, C-6; H-6/C-8, C-10; H-8/C-5, C-6; H-12/C-13, C-18; H-14/C-15; H-16b/C-13, C-14, C-15, C-17; H-18/C-12, C-13, C-14, C-17; H-19/C-1, C-5, C-9, C-10; H-20/C-21, C-22; H-21/C-17, C-22; H-22/C-20; H-23/C-22, C-24, C-25; H-24/C-23, C-25; H-25/C-24, C-26, C-27, C-28; H-26/C-24, C-27; H-27/C-26; H-28/C-23, C-24, C-25; HRLSIMS *m*/*z* 437.3619 [M + Li]$^+$(calcd for $C_{28}H_{46}O_3Li$ 437.3607).

Compound 2: white powder; 2 mg, 0.001%; IR (film) 3437, 1690, 890, 837 cm^{-1}; δ_H (500 MHz, $CDCl_3$) 5.36 (1H, d, J = 5.2 Hz, H-6), 3.53 (1H, m, H-3), 2.68 (1H, td, J = 6.3, 2.2 Hz, H-23), 2.86 (1H, dd, J = 7.7, 2.2 Hz, H-22), 2.31 (1H, m, H-4a), 2.26 (1H, m, H-4b), 2.23 (1H, m, H-16a), 2.02 (1H, dtd, J = 17.5, 5.2, 2.6 Hz, H-7a), 1.86 (1H, m, H-1a), 1.86 (1H, m, H-2a), 1.82 (1H, m, H-25), 1.76 (1H, m, H-15a), 1.75 (1H, m, H-16b), 1.66 (1H, m, H-14), 1.65 (1H, m, H-8), 1.65 (2H, m, H-12), 1.62 (1H, m, H-11a), 1.59 (1H, m, H-20), 1.53 (1H, m, H-2b), 1.53 (1H, m, H-7b), 1.51 (1H, m, H-11b), 1.44 (1H, m, H-24), 1.18 (1H, m, H-15b), 1.11 (1H, m, H-1b), 1.04 (3H, s, H-19), 1.00 (3H, d, J = 7.0 Hz, H-21), 0.99 (1H, m, H-9), 0.97 (3H, d, J = 6.8 Hz, H-27), 0.96 (3H, d, J = 6.7 Hz, H-26), 0.75 (3H, s, H-18); δ_C (125 MHz, $CDCl_3$) 140.8 (s, C-5), 121.8 (d, C-6), 85.5 (s, C-17), 71.9 (d, C-3), 60.8 (d, C-22), 56.0 (d, C-23), 50.9 (d, C-14), 50.0 (d, C-9), 47.8 (s, C-13), 42.6 (d, C-20), 42.4 (t, C-4), 41.6 (t, C-24), 37.4 (t, C-1), 36.9 (t, C-16), 36.6 (s, C-10), 32.4 (t, C-12), 32.3 (d, C-8), 32.0 (t, C-7), 31.8 (t, C-2), 26.6 (d, C-25), 24.1 (t, C-15), 23.1 (q, C-27)*, 22.8 (q, C-26)*, 21.0 (t, C-11), 19.5 (q, C-19), 14.8 (q, C-18), 11.9 (q, C-21) (*, these assignments may be interchanged); COSY correlations (in a geminal pair, Ha is the lower-field proton and Hb is the higher-field proton) (H/H) H-1a/H-1b; H-1b/H-2b; H-2a/H-3; H-2b/H-3; H-3/H-4a, H-4b; H-4b/H-6; H-6/H-7a, H-8; H-7a/H-7b, H-8; H-8/H-11b; H-11b/H-12; H-15a/H-15b, H-16a, H-16b; H-20/H-21, H-22; H-23/H-24; H-25/H-26, H-27; HMBC correlations (H/C) H-1a/C-5, C-10; H-4a/C-2, C-3, C-5, C-6; H-4b/C-3, C-5, C-6, C-7; H-6/C-4, C-8, C-10; H-7b/C-8; H-8/C-5, C-6, C-15; H-12a/C-18; H-12b/C-18; H-14/C-15; H-15a/C-13; H-16b/C-15, C-17; H-18/C-12, C-13, C-14, C-17; H-19/C-21, C-5, C-10, C-9; H-20/C-21, C-22, C-23; H-21/C-17, C-20, C-22; H-22/C-20; H-23/C-22, C-24; H-24/C-22, C-23, C-25; H-25/C-23; H-26/C-24, C-25, C-27; H-27/C-24, C-25, C-26; HRLSIMS *m*/*z* 423.3440 [M + Li]$^+$ (calcd for $C_{27}H_{44}O_3Li$ 423.3451).

Compound 3: white powder; 8 mg, 0.005%; $[\alpha]_D^{25}$ +178.6° (*c* 0.056 g/100 mL, CH_2Cl_2); IR (film) 3390, 1632, 1264, 885 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1; COSY correlations (in a geminal pair, Ha is the lower-field proton and Hb is the higher-field proton) (H/H) H-1a/H-1b, H-2b; H-1b/H-2a, H-2b; H-2a/H-2b, H-3, H-4a; H-2b/H-3; H-3/H-4a, H-4b; H-4b/H-6, H-7a, H-7b; H-6/H-7a, H-7b; H-7a/H-7b; H-9/H-11; H-11/H-12a, H-12b; H-12a/H-12b; H-15a/H-15b, H-16a; H-15b/H-16a; H-16a/H-16b, H-17; H-17/H-18a; H-18a/H-18b; H-23/H-24; H-25/H-26, H-27; HMBC correlations (H/C) H-1a/C-2, C-3, C-5, C-6, C-10, C-19; H-1b/C-2, C-3, C-5, C-9, C-10, C-19; H-2a/C-3; H-2b/C-3; H-4a/C-2, C-3, C-5, C-6, C-10; H-4b/C-3, C-5, C-6; H-6/C-4, C-7, C-10; H-7a/C-5, C-6; H-7b/C-5, C-6; H-8/C-9, C-14, C-15; H-11/C-8, C-9, C-10, C-12, C-13; H-12a/C-9, C-11, C-13, C-14, C-17, C-18; H-12b/C-11, C-13, C-18; H-14/C-13, C-15 C-18; H-15a/C-13, C-14, C-16, C-17; H-15b/C-8, C-14, C-16; H-16a/C-13, C-15, C-17; H-16b/C-14, C-20; H-17/C-18, C-21; H-18a/C-12, C-13, C-17, C-22; H-18b/C-12, C-17; H-19/ C-1,

C-5, C-9, C-10; H-21/ C-16, C-17, C-20, C-22, C-23; H-23/ C-20, C-22, C-24, C-25; H-24/C-22, C-23, C-25, C-26, C-27; H-25/ C-23, C-24, C-26, C-27; H-26/C-24, C-25, C-27; H-27/ C-24, C-25, C-26; HREIMS *m/z* 398.3198 (calcd for $C_{27}H_{42}O_2$ 398.3185).

Compound 4: white powder; 15 mg, 0.009%; $[\alpha]_D^{25}$ +101.7° (*c* 0.118 g/100 mL, CH_2Cl_2); IR (film) 3390, 1630, 1264, 885 cm^{-1}; δ_H (500 MHz, $CDCl_3$) 3.92 (1H, dd, J = 11.1, 4.8 Hz, H-18a), 3.61 (1H, m, H-3), 3.30 (1H, d, J = 10.5 Hz, H-18b), 2.11 (1H, m, H-16a), 2.06 (2H, m, H-23), 2.00 (1H, m, H-12a), 1.82 (1H, m, H-2a), 1.76 (1H, dt, J = 13.2, 3.5 Hz, H-1a), 1.70 (1H, m, H-7a), 1.69 (1H, m, H-15a), 1.61 (1H, m, H-11a), 1.61 (3H, s, H-21), 1.59 (1H, m, H-4a), 1.57 (1H, m, H-17), 1.54 (1H, m, H-25), 1.46 (1H, m, H-16b), 1.45 (1H, m, H-11b), 1.42 (1H, m, H-2b), 1.34 (2H, m, H-24), 1.31 (1H, m, H-4b), 1.29 (1H, m, H-8), 1.29 (1H, m, H-9)*, 1.29 (1H, m, H-15b), 1.28 (1H, m, H-6), 1.13 (1H, m, H-5), 1.01 (1H, td, J = 13.4, 3.6 Hz, H-1b), 0.91 (3H, d, J = 6.6 Hz, H-27)#, 0.90 (1H, m, H-7b), 0.90 (3H, d, J = 6.6 Hz, H-26)#, 0.86 (1H, m, H-12b), 0.82 (3H, s, H-19), 0.74 (1H, m, H-14)* (*,#, these assignments may be interchanged); δ_C (125 MHz, $CDCl_3$) 146.0 (s, C-22), 106.0 (s, C-20), 71.3 (d, C-3), 63.9 (t, C-18), 54.4 (d, C-9), 54.4 (d, C-14), 47.7 (d, C-17), 44.8 (d, C-5), 41.0 (s, C-13), 38.1 (t, C-4), 37.0 (t, C-1), 36.8 (t, C-24), 35.6 (s, C-10), 35.3 (d, C-8), 32.4 (t, C-7), 31.5 (t, C-2), 31.7 (t, C-12), 30.5 (t, C-16), 28.5 (t, C-6), 27.9 (t, C-23), 27.9 (d, C-25), 24.3 (t, C-15), 22.5 (q, C-26), 22.5 (q, C-27), 20.8 (t, C-11), 16.8 (q, C-21), 12.2 (q, C-19); COSY correlations (in a geminal pair, Ha is the lower-field proton and Hb is the higher-field proton) (H/H) H1a/H-1b, H-2a, H-2b; H-1b/H-2a, H-2b, H-19; H2a/H-2b, H-3, H-4a; H-2b/H-3; H-3/H-4a, H-4b; H-5/H-6; H-7a/H-7b; H-7b/H-6 and/or H-8; H11a/H-11b, H-12a, H-12b; H-11b/H-12a, H-12b; H-12a/H-12b; H-12b/H-18; H-15a/H-15b, H-16a; H-16a/H-15a, H-16b, H-17; H-17/H-18a; H-18a/H-18b; H-23/H-24; H-25/H-26, H-27; HMBC correlations (H/C) H-1a/C-2, C-3, C-5, C-6, C-10, C-19; H-1b/C-2, C-3, C-5, C-9, C-10, C-19; H-2b/C-3, C-4; H-4a/C-3, C-5; H-4b/C-2, C-3, C-5, C-10; H-5/C-1, C-3, C-4, C-10, C-19; H-7a/C-8; H-7b/C-8; H-8/C-9; H-9/C-8; H-11a/C-8, C-9, C-12, C-13; H-11b/C-9, C-12; H-12a/C-11, C-13, C-18; H-12b/C-11, C-13, C-17, C-18; H-14/C-8, C-15; H-16a/C-13, C-15, C-17; H-16b/C-15; H-17/C-20; H-18a/C-12, C-13, C-17, C-22; H-18b/C-12, C-17; H-19/C-1, C-5, C-9, C-10; H-21/C-16, C-17, C-20, C-22, C-23, C-24; H-23/C-20, C-22, C-24, C-25; H-24/C-22, C-23, C-25, C-26, C-27; H-25/C-23, C-24, C-26, C-27; H-26/C-24, C-25, C-27; H-27/C-24, C-25, C-26; HREIMS *m/z* 400.3338 (calcd for $C_{27}H_{44}O_2$ 400.3341).

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