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Absolute configuration of pterocidin, a potent inhibitor of tumor cell invasion from a marine-derived *Streptomyces*

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ABSTRACT

During the course of screening natural products for the inhibitors of tumor cell invasion, pterocidin, a linear polyketide with a δ-lactone terminus, was rediscovered from a *Streptomyces* strain of a marine sediment-origin. A series of *J*-based configuration analyses and NOESY analysis, coupled with chemical derivatization and chiral anisotropy analysis, established the absolute stereochemistry of five asymmetric centers in this compound.

Actinomycetes, especially those belonging to the genus *Streptomyces* have been an unparalleled rich source of bioactive polyketides.[1,2] We recently reported the discovery of pterocidin (**1**), a cytotoxic compound produced by *Streptomyces hygroscopicus* TP-A0451 isolated from a stem of bracken *Pteridium aquilinum*.[3] **1** is a linear polyketide featured by an α,β-unsaturated δ-lactone ring on its terminus and four almost contiguous substituents in the center of a highly unsaturated aliphatic chain. Although the former feature is not uncommon in natural products, **1** is the first to have a methoxy substitution on the lactone moiety in this class. In our previous study, stereochemical assignment was hampered by the limited availability of this material and the poor reproducibility of production culture. During the course of our continuing effort toward the discovery of new inhibitors of tumor cell invasion from natural products,[4–8] another *Streptomyces* strain obtained from a marine sediment sample was found to produce a substantial amount of **1** that enabled us to establish the absolute configurations of all five asymmetric centers (C-4, C-5, C-10, C-12, and C-13) present in this molecule (Fig. 1).

The producing strain *Streptomyces* sp. TP-A0879 was isolated from a marine sediment sample collected at a depth −44.5 m in Otsuchi Bay, Iwate, Japan.[9] The strain was cultured in A11M medium[10] (7 L), and the whole culture broth was extracted with 1-butanol. The crude extract (24.6 g) was consecutively fractionated by silica gel and ODS column chromatographies, followed by HPLC purification to yield 36 mg of pterocidin (**1**). Its spectroscopic data were fully consistent with those previously reported.[3]

Stereochemical analysis of **1** was started with MTPA derivatization.[11] Esterification of **1** with (*R*)- and (*S*)-MTPA acids by DCC coupling yielded (*R*)- and (*S*)-MTPA esters (**2a** and **2b**), respectively.[12,13] In the ^{1}H NMR of **2a** and **2b**, positive $\Delta\delta_{S-R}$ values were observed for the protons from H-15 to H_3-19, while negative $\Delta\delta_{S-R}$ values were observed for the protons from H-9 to H-12 and 22-Me (Fig. 2). These data established the absolute configuration at C-13 as *S*.

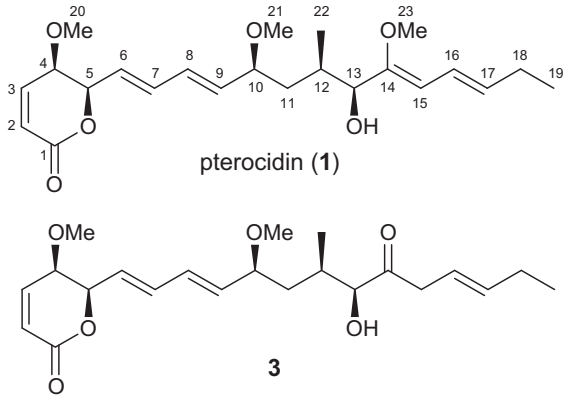

Figure 1. Structures of pterocidin (**1**) and 14-*O*-demethylpterocidin (**3**).

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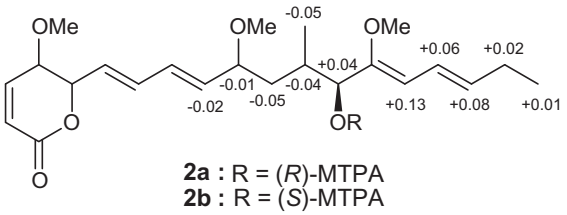

Figure 2. $\Delta\delta_{S-R}$ values for MTPA esters (**2a** and **2b**) of **1**.

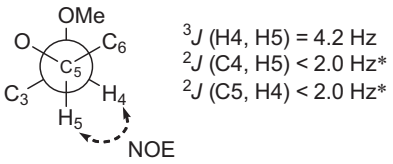

Figure 4. Configuration of C-4 and C-5 and coupling constants for C-4–C-5 in **1**. *Absolute values are given for $^2J_{CH}$ coupling constants.

The relative configuration for the C-12 stereocenter and the 1,3-methine system C-10–C-12 in **1** was investigated using *J*-based configuration analysis.[14] Heteronuclear long-range coupling constants $^2J_{CH}$ and $^3J_{CH}$ were determined by *J*-resolved HMBC experiments.[15–17] However, it was impossible to assign a single conformer around the C-11–C-12 bond because all the $^3J_{CH}$ values between H-11 protons and C-13 and C-22 indicated intermediate values (3.6–3.9 Hz) probably due to the presence of more than two stable rotamers (data not shown). Next, the same method was applied to 14-*O*-demethylpterocidin (**3**)[18] which was obtained as a degradation product after the long storage of **1**. The diastereomeric methylene protons H-11a (δ 1.72) and H-11b (δ 1.61) were stereospecifically assigned with respect to H-10, according to the magnitude of the coupling constants (Fig. 3A). The *gauche* orientations of H-11a and H-11b to C-9 were suggested by the small $^3J_{C9,H11a}$ and $^3J_{C9,H11b}$ values, while the *anti* orientations of H-11a to 10-OMe and H-11b to H-10 were provided by the small $^2J_{H11a,C10}$ and large $^3J_{H10,H11b}$ values. For the C-11–C-12 bond, in this case, only one rotamer was deduced from the observed data (Fig. 3B). The large homonuclear coupling constant between H-11a and H-12 (9.6 Hz) suggested an *anti* relationship between these protons. Small three-bond C–H coupling constants for H-11a/C-22 and H-11b/C-22 indicated that these protons were *gauche* to C-22 methyl group. The *gauche* orientation of H-11a to C-13 was suggested by the small $^3J_{C13,H11a}$ value, while the *anti* orientation of H-11b to C-13 was provided by the large $^3J_{C13,H11b}$ value. On the basis of these data, the *syn* relationship between the C-10 methoxy and the C-22 methyl groups was established, with C-10/C-13 exhibiting *gauche* orientation. Similarly, the *anti* orientation of C-11 and C-14 around the C-12–C-13 bond was confirmed on the basis of large $^3J_{C22,H13}$ and small $^2J_{C13,H12}$ values as well as the small $^3J_{H12,H13}$ value, allowing the assignment of a *syn* relationship between the C-13 hydroxyl and the C-22 methyl groups (Fig. 3C). These configurational assignments were in good agreement with an observed NOE between H-10 and H-13 (Fig. 3D). The 12*R* and 10*S* configurations were thus established on the basis of the relative configuration of C-12 and C-13.

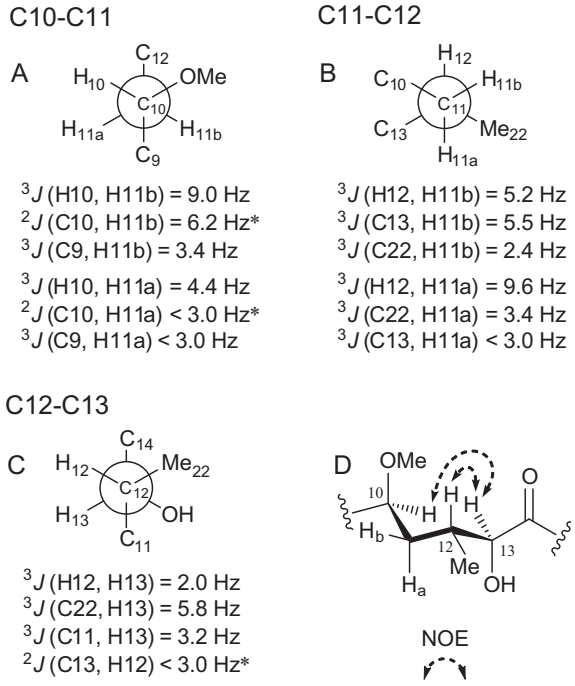

Figure 3. Configuration analysis for C-10–C-13 based on coupling constants and NOE in **3**. *Absolute values are given for $^2J_{CH}$ coupling constants.

The coupling constant between H-4 and H-5 (J = 4.2 Hz) as well as an NOE between these protons in **1** suggested their *syn* relationship. This was supported by the small $^2J_{CH}$ values for H-4/C-5 (<2 Hz) and H-5/C-4 (<2 Hz) indicating *anti* orientations of H-4 to O-5 and H-5 to 4-OMe (Fig. 4). In order to determine the absolute configurations at these stereocenters, it became necessary to apply the ^{1}H NMR anisotropy method. **1** was subjected to $Ca(BH_4)_2$ reduction[19,20] in MeOH to afford diol **4** along with the C-2–C-3-unsaturated diol in a ratio of 5:2.[21,22] **4** was then treated with (*R*)- or (*S*)-MPA (methoxyphenylacetic acid)[23,24] in the presence of DIC and DMAP, yielding tris MPA esters **5a** and **5b**.[25,26] Analysis of ^{1}H NMR data for these MPA esters allowed the assignment of the $\Delta\delta_{R-S}$ values, which were positive for the protons from H-6 to H-9, while those for the protons from H-1 to H-4, except for one of the diastereomeric H-3 methylene protons, were negative (Fig. 5). This is sufficiently consistent to assign the absolute configuration of C-5 as *R*. On the basis of the relative configuration of C-4 and C-5, the absolute configuration of C-4 was assigned as *R*. The absolute configurations of all five asymmetric centers in **1** were determined as 4*R*, 5*R*, 10*S*, 12*R*, and 13*S*.

Invasion is a key feature of metastasis because it promotes tumorigenesis by enabling tumor cells to migrate through the connective tissue surrounding a tumor and enter the circulatory system and also supporting endothelial cell migration and angiogenesis.[27] In addition to antiproliferative property,[3] pterocidin (**1**) was found to exhibit potent antiinvasive activity at non-cytotoxic concentrations. The invasion of murine colon 26-L5 carcinoma cells across the Matrigel-fibronectin membrane[28] was inhibited by **1** with an IC_{50} value of 0.25 μM, whereas the cytotoxicity was not apparent up to 7 μM. The demethyl derivative **3** showed slightly weaker activity with an IC_{50} value of 1.6 μM with no cytotoxic effect up to 7 μM.

In summary, prerocidin (**1**), a linear polyketide with a δ-lactone terminus, was rediscovered from a marine-derived *Streptomyces*

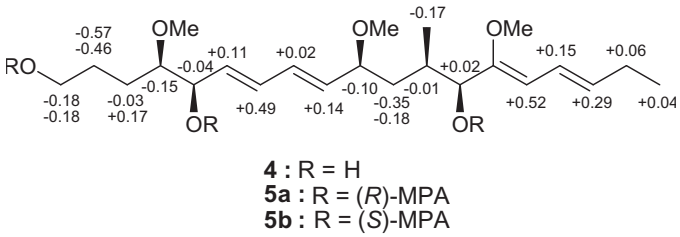

Figure 5. $\Delta\delta_{R-S}$ values for MPA esters (**5a** and **5b**) of **4**.

strain as an inhibitor of tumor cell invasion, and the absolute configurations of all five asymmetric centers in this molecule were established on the basis of *J*-based configuration analyses and NOESY analysis, coupled with chemical derivatization and chiral anisotropy analysis. Linear polyketides with a δ-lactone terminus have been reported from various organisms including actinomycetes,[29] myxobacteria,[30] fungi,[31] slime molds,[32] marine sponges,[33] and colonial ascidians.[34] Although a wide range of bioactivity was reported for this class of natural products, pterocidin (**1**) represents the first example of invasion inhibitor, providing a new template for the development of antiinvasive agents.

Acknowledgments

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9. The bacterial strain TP-A0879 was isolated from a sediment sample collected at a depth −44.5 m in Otsuchi Bay, Iwate, Japan in 2006 by using Smith–McIntyre grab. The strain was identified as a member of the genus *Streptomyces* on the basis of 98.1% 16S rRNA gene sequence (1409 nucleotides; DDBJ accession number AB666472) identity with *Streptomyces hygroscopicus* subsp. *crystallogenes* NBRC 16551 (accession number AB184723).
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12. *(R)-MTPA ester of* ***1*** *(****2a****)*: To a solution of **1** (5.0 mg, 12 μmol) in dry CH_2Cl_2 (200 μL) was add (*R*)-MTPA acid (5.6 mg, 24 μmol), DCC (5.0 mg, 24 μmol), and DMAP (0.7 mg, 6 μmol) at room temperature. After standing for 4 h, the reaction mixture was concentrated under reduced pressure, and the residue was purified by silica gel column chromatography (*n*-hexane/EtOAc = 1:0–1:1) to give (*R*)-MTPA ester **2a** (7.3 mg, 95% yield): ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.93 (3H, d, *J* = 7.0 Hz, H-22), 1.02 (3H, t, *J* = 7.5 Hz, H-19), 1.52 (1H, m, H-11), 1.61 (1H, m, H-11), 2.07 (1H, m, H-12), 2.09 (2H, m, H-18), 3.25 (3H, s), 3.43 (3H, s), 3.57 (3H, s), 3.65 (1H, m, H-10), 4.03 (1H, dd, *J* = 4.3, 3.8 Hz, H-4), 4.99 (1H, dd, *J* = 6.7, 3.8 Hz, H-5), 5.29 (1H, d, *J* = 10.8 Hz, H-15), 5.36 (1H, d, *J* = 5.6 Hz, H-13), 5.54 (1H, dd, *J* = 15.4, 7.9 Hz, H-9), 5.54 (1H, dt, *J* = 15.5, 7.6 Hz, H-17), 5.89 (1H, dd, *J* = 15.4, 6.7 Hz, H-6), 6.17 (1H, d, *J* = 9.9 Hz, H-2), 6.20 (1H, dd, *J* = 15.4, 10.7 Hz, H-8), 6.23 (1H, dd, *J* = 15.5, 10.8 Hz, H-16), 6.43 (1H, dd, *J* = 15.4, 10.7 Hz, H-7), 6.95 (1H, dd, *J* = 9.9, 4.3 Hz, H-3); HR-ESITOFMS *m*/*z* 645. 2640 $[M+Na]^+$ (calcd for $C_{33}H_{41}F_3Na_1O_8$ 645.2646).
13. *(S)-MTPA ester of* ***1*** *(****2b****)*: In the same manner as described for **2a**, **2b** was prepared from pterocidin and (*S*)-MTPA acid: ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.88 (3H, d, *J* = 6.8 Hz, H-22), 1.03 (3H, t, *J* = 7.5 Hz, H-19), 1.60 (1H, m, H-11), 1.70 (1H, m, H-11), 2.05 (1H, m, H-12), 2.12 (2H, m, H-18), 3.24 (3H, s), 3.43 (3H, s), 3.64 (1H, m, H-10), 3.67 (3H, s), 4.03 (1H, dd, *J* = 4.3, 4.1 Hz, H-4), 4.99 (1H, dd, *J* = 6.3, 4.1 Hz, H-5), 5.40 (1H, d, *J* = 6.0 Hz, H-13), 5.42 (1H, d, *J* = 10.7 Hz, H-15), 5.52 (1H, dd, *J* = 15.2, 8.0 Hz, H-9), 5.62 (1H, dd, *J* = 15.5, 6.5 Hz, H-17), 5.89 (1H, dd, *J* = 15.5, 6.3 Hz, H-6), 6.12 (1H, d, *J* = 9.9 Hz, H-2), 6.22 (1H, dd, *J* = 15.2, 10.5 Hz, H-8), 6.29 (1H, ddt, *J* = 15.5, 10.7, 1.6 Hz, H-16), 6.43 (1H, dd, *J* = 15.5, 10.5 Hz, H-7), 6.94 (1H, dd, *J* = 9.9, 4.3 Hz, H-3); HR-ESITOFMS *m*/*z* 645.2646 $[M+Na]^+$ (calcd for $C_{33}H_{41}F_3O_8Na$ 645.2646).
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17. *J*-Resolved HMBC spectra were measured on a Varian INOVA-500 spectrometer at 20 °C using a microtube (Shigemi Inc., Japan) in $CDCl_3$ (9.0 mg of **1** in 0.25 mL; 7.9 mg of **3** in 0.25 mL). In the *J*-resolved HMBC spectra, $^2J_{CH}$ and $^3J_{CH}$ values are obtained as absolute values.
18. *14-O-Demethylpterocidin (****3****)*: $[\alpha]_{29}^{D}$ −17 (*c* 0.18, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.70 (3H, d, *J* = 6.9 Hz, H-22), 1.00 (3H, t, *J* = 7.4 Hz, H-19), 1.61 (1H, ddd, *J* = 14.0, 9.0, 5.2 Hz, H-11b), 1.72 (1H, ddd, *J* = 14.0, 9.3, 4.4 Hz, H-11a), 2.07 (2H, m, H-18), 2.32 (1H, m, H-12), 3.19 (2H, d, *J* = 6.7 Hz, H-15), 3.31 (3H, s, H-21), 3.43 (3H, s, H-20), 3.79 (1H, ddd, *J* = 9.0, 8.3, 4.4 Hz, H-10), 4.02 (1H, dd, *J* = 4.3, 4.2 Hz, H-4), 4.29 (1H, d, *J* = 2.0 Hz, H-13), 4.99 (1H, dd, *J* = 6.7, 4.2 Hz, H-5), 5.52 (1H, dtt, *J* = 15.4, 6.7, 1.3 Hz, H-16), 5.63 (1H, m, H-17), 5.65 (1H, dd, *J* = 15.2, 8.3 Hz, H-9), 5.90 (1H, dd, *J* = 15.2, 6.7 Hz, H-6), 6.17 (1H, d, *J* = 9.9 Hz, H-2), 6.28 (1H, dd, *J* = 15.2, 10.5 Hz, H-8), 6.46 (1H, dd, *J* = 15.2, 10.5 Hz, H-7), 6.95 (1H, dd, *J* = 9.9, 4.3 Hz, H-3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 13.6 (C-19), 13.7 (C-22), 25.8 (C-18), 31.8 (C-12), 39.6 (C-11), 42.2 (C-15), 56.6 (C-21), 57.4 (C-20), 71.4 (C-4), 77.4 (C-13), 79.4 (C-10), 79.9 (C-5), 119.9 (C-16), 123.5 (C-2), 126.0 (C-6), 131.4 (C-8), 134.2 (C-7), 136.2 (C-9), 137.6 (C-17), 143.2 (C-3), 162.7 (C-1), 211.1 (C-14); HR-ESITOFMS *m*/*z* 391.2127 $[M-H]^-$ (calcd for $C_{22}H_{31}O_6$ 391.2126).
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21. Calcium borohydride is used to synthesize diols from α,β-unsaturated lactones without reducing the conjugated C–C double bond.[19,20] However, in case of pterocidin, reduction with $Ca(BH_4)_2$ gave the C-2–C-3-reduced diol (**4**) as a major product. The ratio of **4** to C-2–C-3-unsaturated diol varied depending on the reaction solvent. The ratio was 5:2 in MeOH, 2:1 in 2-propanol, and 1:1 in THF.
22. *Reduction of* ***1*** *to yield* ***4***: Calcium borohydride bis(tetrahydrofuran) (1.6 mg, 7.4 μmol) was added to a solution of **1** (1.5 mg, 3.7 μmol) in MeOH (200 μl) at room temperature, and the reaction mixture was stirred for 3 h. The reaction was quenched by the addition of several drops of water, and the mixture was extracted with EtOAc. The EtOAc layer was concentrated under reduced pressure to give a mixture of **4** and C-2–C-3-unsaturated diol in a ratio of 5:2 (1.1 mg, 72% yield). This mixture was employed to the next reaction without further purification. **4**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.90 (3H, d, *J* = 7.0 Hz, H-22), 1.02 (3H, t, *J* = 7.4 Hz , H-19), 1.57 (1H, m, H-3), 1.58 (1H, m, H-11), 1.66 (2H, m, H-2), 1.68 (1H, m, H-11), 1.71 (1H, m, H-3), 1.96 (1H, m, H-12), 2.13 (2H, m, H-18), 3.17 (1H, m, H-4), 3.27 (3H, s), 3.47 (3H, s), 3.67 (2H, m, H-1), 3.70 (3H, s), 3.73 (1H, m, H-10), 4.08 (1H, m, H-13), 4.13 (1H, m, H-5), 5.54 (1H, dd, *J* = 15.3, 8.2 Hz , H-9), 5.59 (1H, d, *J* = 10.9 Hz , H-15), 5.67 (1H, dt, *J* = 15.2, 6.6 Hz, H-17), 5.69 (1H, dd, *J* = 15.2, 7.2 Hz, H-6), 6.21 (1H, dd, *J* = 15.4, 10.5 Hz, H-8), 6.35 (1H, dd, *J* = 15.2, 10.8 Hz, H-16), 6.35 (1H, dd, *J* = 15.2, 10.6 Hz, H-7); HR-ESITOFMS *m*/*z* 435.2722 $[M+Na]^+$ (calcd for $C_{23}H_{40}O_6Na$ 435.2717).
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25. *Tris-(R)-MPA ester of* ***4*** *(****5a****)*: To a solution of **4** (1.1 mg, 2.7 μmol) in dry CH_2Cl_2 (100 μl) was added (*R*)-MPA acid (1.8 mg, 11 μmol), DIC (1.7 mg, 14 μmol), and DMAP (0.1 mg, 0.8 μmol) at room temperature. After standing for 3 h, the reaction mixture was concentrated under reduced pressure, and the residue was purified by silica gel column chromatography (*n*-hexane/EtOAc = 1:0–1:1) to give tris-(*R*)-MPA ester **5a** (0.5 mg, 21% yield): ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.66 (3H, d, *J* = 6.8 Hz, H-22), 1.01 (3H, t, *J* = 7.5 Hz, H-19), 1.07 (1H, m, H-11), 1.07 (1H, m, H-2), 1.15 (1H, m, H-2), 1.23 (1H, m, H-11), 1.33 (1H, m, H-3), 1.52 (1H, m, H-3), 1.96 (1H, m, H-12), 2.10 (2H, m, H-18), 2.99 (1H, m, H-4), 3.44 (1H, m, H-10), 3.90 (1H, dt, *J* = 13.4, 6.9 Hz, H-1), 3.94 (1H, dt, *J* = 13.4, 6.5 Hz, H-1), 5.24 (1H, d, *J* = 5.3 Hz, H-13), 5.30 (1H, dd, *J* = 15.3, 7.9 Hz, H-9), 5.37 (1H, dd, *J* = 7.0, 5.6 Hz, H-5), 5.39 (1H, d, *J* = 11 Hz, H-15), 5.56 (1H, dd, *J* = 15.3, 7.0 Hz, H-6), 5.61 (1H, dt, *J* = 15.4, 6.6 Hz, H-17), 5.98 (1H, dd, *J* = 15.3, 10.5 Hz, H-8), 6.16 (1H, dd, *J* = 15.3, 10.5 Hz, H-7), 6.27 (1H, dd, *J* = 15.4, 11 Hz, H-16); HR-ESITOFMS *m*/*z* 855.4319 $[M-H]^-$ (calcd for $C_{50}H_{63}O_{12}$ 855.4325).
26. *Tris-(S)-MPA ester of* ***4*** *(****5b****)*: In the same manner as described for **5a**, **5b** was prepared from **4** and (*S*)-MPA acid: ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.83 (3H, d, *J* = 6.8 Hz, H-22), 0.97 (3H, t, *J* = 7.5 Hz, H-19), 1.35 (2H, m, H-3), 1.41 (2H, m, H-11), 1.61 (1H, m, H-2), 1.71 (1H, m, H-2), 1.97 (1H, m, H-12), 2.04 (2H, m, H-18), 3.16 (1H, m, H-4), 3.54 (1H, m, H-10), 4.08 (1H, dt, *J* = 11.0, 6.8 Hz, H-1), 4.12 (1H, dt, *J* = 11.0, 6.6 Hz, H-1), 4.88 (1H, d, *J* = 10.9 Hz, H-15), 5.16 (1H, dd, *J* = 15.3, 8.0 Hz, H-9), 5.22 (1H, d, *J* = 5.4 Hz, H-13), 5.30 (1H, dt, *J* = 15.4, 6.5 Hz, H-17), 5.42 (1H, dd, *J* = 6.0, 5.8 Hz, H-5), 5.45 (1H, dt, *J* = 14.6, 6.0 Hz, H-6), 5.67 (1H, dd, *J* = 14.6, 10.5 Hz, H-7), 5.96 (1H, dd, *J* = 15.3, 10.5 Hz, H-8), 6.12 (1H, dd, *J* = 15.4, 10.9 Hz, H-16); HR-ESITOFMS *m*/*z* 879.4301 $[M+Na]^+$ (calcd for $C_{50}H_{64}O_{12}Na$ 879.4290).
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