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Stereochemistry of hydroxy-bearing benzolactones: isolation and structural determination of chrysoarticulins A–C from a marine-derived fungus *Chrysosporium articulatum*

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ABSTRACT

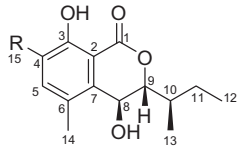
Three new benzolactone metabolites, chrysoarticulins A–C (**1**–**3**), were isolated from the culture broth of *Chrysosporium articulatum*, a marine-derived fungus collected from an unidentified dictyoceratid sponge collected off the coast of Gagu-do, Korea. Structurally, these novel compounds were determined to be benzolactones possessing a branched side chain based on combined spectroscopic analyses. The absolute configurations of the asymmetric carbon centers were determined by extensive chemical and spectroscopic analyses that provided more insights into the stereochemistry of hydroxy-bearing benzolactones. The new compounds exhibited weak cytotoxicity against the K562 and A549 cell lines, and **3** was more active than the other compounds. Compound **3** was also moderately active against sortase A, a bacterial transpeptidase.

Introduction

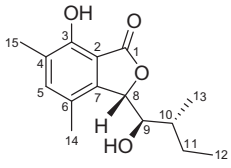
Marine microorganisms, in particular actinomycete bacteria and fungi, produce a wide variety of biologically active and structurally unique metabolites.[1–5] Hundreds of novel compounds are isolated from these organisms annually, and they have become important sources of novel natural products.[6] In our search for bioactive compounds from marine fungi, we isolated a strain of *Chrysosporium articulatum*[7] from an unidentified sponge collected off the coast of Korea whose organic extract was found to contain secondary metabolites in an LC–MS analysis. The present report details the isolation and structural characterization of chrysoarticulins A–C (**1**–**3**), benzolactone metabolites possessing branched side chains. During this process, comprehensive chemical and spectroscopic approaches were used to determine the absolute stereochemistry of these hydroxy-bearing benzolactones.

Results and discussion

The fungal strain *C. articulatum* was isolated from an unidentified marine sponge collected from Gagu-do, Korea, in October 2008. The isolate (strain number F085) was deposited at the Laboratory of Natural Product and Structure Determination, College of Pharmacy, Seoul National University. The isolate was cultured on slants of YPG agar media (5 g yeast extract, 5 g peptone, 10 g glucose, and 18 g agar in 1 L artificial seawater) at 25 °C for 10 days. Spore solutions prepared from the slants (10^7 spores/mL) were inoculated into 250 mL Erlenmeyer flasks, 100 flasks in total, each containing 100 mL of YPG media (5 g yeast extract, 5 g peptone, and 10 g glucose in 1 L artificial seawater). The flask cultures were incubated at 28 °C on a rotary shaker at 150 rpm for 3 weeks.



1 R = CH_3
2 R = CH_2OH



3

The mycelia and culture broth of *C. articulatum* were separated by filtration, and the broth (10 L) was extracted with EtOAc (10 L × 3). The solvent was evaporated to obtain an organic extract (790 mg). The extract was fractionated by C_{18} reversed-phase vacuum flash chromatography using a sequential mixture of MeOH and H_2O as eluents (five fractions eluted with a H_2O–MeOH gradient from 80:20 to 0:100) and finally MeOH–CH_2Cl_2 (50:50). The

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fraction (130 mg) eluted with H_2O–MeOH (20:80) was separated by reversed-phase HPLC (YMC ODS-A column, 10 × 250 mm; H_2O–MeOH, 26:74, 2 mL/min, detected at 254 nm) to afford **1** (15.1 mg) and **3** (6.7 mg). The fraction (70 mg) eluted with H_2O–MeOH (40:60) was separated by reversed-phase HPLC (H_2O–MeOH, gradient (50:50 to 0:100 in 40 min, then 0:100 in 5 min), detected at 254 nm) to yield compound **2** (3.6 mg).

The molecular formula of chrysoarticulin A (**1**) was deduced to be $C_{15}H_{20}O_4$ by HRFABMS analysis.[8] The ^{13}C NMR spectra of this compound showed signals corresponding to a carbonyl (δ_C 170.7), six aromatics or olefinics (δ_C 159.6–108.1), two oxymethines (δ_C 90.3 and 63.4), and six upfield (δ_C 38.3–10.9) carbons (Table 1). The downfield shifts of the six carbon signals in the region of δ_C 159.6–108.1 were indicative of a substituted benzene ring based on the six degrees of unsaturation inherent in the molecular formula. Thus, compound **1** must possess an additional ring structure.

Given this information, the structure of **1** was determined by a combination of 2D NMR experiments. A partial structure accommodating the oxymethines and upfield NMR signals was readily defined to be a 1,2-dioxy-3-methyl-pentyl chain by combined ^1H COSY and gHMBC analyses (Fig. 1). In addition, long-range proton–proton couplings of the aromatic proton at δ_H 7.32 with the methyl protons at δ_H 2.32 and 2.22 in the ^1H COSY data indicated that the benzylic methyls were *meta* to each other, and this arrangement was confirmed by the gHMBC data (Fig. 1). An additional long-range correlation of the methyl proton at δ_H 2.32 placed the quaternary aromatic carbon at δ_C 135.1 at the neighboring C-7 position, which further coupled with the oxymethine protons at δ_H 4.93 and 4.47, thus connecting the pentyl chain and the aromatic ring at this position. A long-range coupling between the other methyl proton at δ_H 2.22 and the downfield carbon at δ_C 159.6 placed a hydroxyl group at C-3, *ortho* to this benzylic methyl group. The aromatic carbon at δ_C 108.1 was determined to be located at C-2 based on its upfield carbon chemical shift and its long-range coupling with the H-8 benzylic proton at δ_H 4.93. Finally, the remaining ester carbon at δ_C 170.7 located at C-1, the only available position, was determined to form a six-membered lactone by its long-range coupling with the H-9 oxymethine at δ_H 4.47 in the gHMBC data. Thus, chrysoarticulin A (**1**) was determined to be a new isocoumarin metabolite possessing a *sec*-butyl side chain. The absolute configurations of **1** at the asymmetric centers at C-8, C-9, and C-10 were assigned based on the results of combined spectroscopic and chemical experiments described below together with the results of congener **3**.

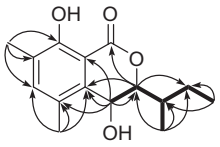


Figure 1. Selected *g*HMBC (arrows) and COSY (bold lines) correlations of compound **1**.

The molecular formula of a related compound, chrysoarticulin B (**2**), was deduced to be $C_{15}H_{20}O_5$ by HRFABMS analysis.[9] The NMR data for this compound were very similar to those for **1**, with the replacement of a methyl with an oxymethylene (δ_H 4.66, 4.64; δ_C 59.6) being the most noticeable difference. These spectroscopic differences were readily identified as the result of the oxidation of the C-15 methyl to a hydroxymethyl group by combined 2D NMR analyses, including the HMBC correlations of the protons and carbons at the oxymethylene and those at the neighboring C-3 and C-5. Thus, chrysoarticulin B (**2**) was determined to be a benzylic oxidant of **1**.

The molecular formula of chrysoarticulin C (**3**) was determined to be $C_{15}H_{20}O_4$, identical to **1**, by HRFABMS analysis.[10] The NMR spectra of this compound were also similar to those of **1**. However, detailed examination of the ^1H and ^{13}C NMR data revealed that the signals for the C-8 and C-9 oxymethines were shifted noticeably (**1**: δ_C 90.3 and 63.4, δ_H 4.47 and 4.93, **3**: δ_C 85.8 and 78.1, δ_H 5.58 and 3.72) (Table 1), suggesting a change in the functional groups at these positions. A combination of ^1H COSY and gHSQC analyses of **3** showed the same aliphatic proton spin systems as found in **1**, adequately assigning the signals of the carbons and protons at these positions (C-8: δ_C 85.8, δ_H 5.58, C-9: δ_C 78.1, δ_H 3.72). The significant shifts of the protons relative to those of **1** suggested a change of the lactone from a six-membered ring to the corresponding five-membered ring. This difference was confirmed by the key carbon-proton correlations at H-8/C-1, C-2, C-7 and H-9/C-7, C-8 in the gHMBC data. Thus, chrysoarticulin C (**3**) was determined to be a new benzolactone metabolite.

Both compounds **1** and **3** possessed the same asymmetric centers at C-8, C-9, and C-10. A previously published study showed that the configurations of similar lactone compounds can be assigned using data from diverse spectroscopic and crystallographic methods.[11–17] Among these spectroscopic methods, one widely used approach is the analysis of the magnitude of the proton cou-

Table 1
^{13}C NMR (ppm, mult) and ^1H NMR (δ, mult (J in Hz)) assignments for compounds **1–3**[a]

Position	**1**		**2**		**3**	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	170.7, C		170.4, C		173.7, C	
2	108.1, C		109.1, C		112.2, C	
3	159.6, C		158.4, C		154.3, C	
4	128.1, C		131.1, C		125.3, C	
5	140.8, CH	7.32, s	138.0, CH	7.55, s	141.0, CH	7.24, s
6	127.5, C		127.6, C		126.9, C	
7	135.1, C		136.4, C		145.8, C	
8	63.4, CH	4.93, d (1.2)	63.3, CH	4.95, d (1.3)	85.8, CH	5.58, d (5.0)
9	90.3, CH	4.47, dd (9.6, 1.2)	90.2, CH	4.47, dd (9.6, 1.3)	78.1, CH	3.72, dd (5.0, 5.0)
10	38.3, CH	1.43, dddq (9.6, 8.3, 3.5, 6.8)	38.2, CH	1.42, m	38.2, CH	1.57, dddq (9.3, 5.0, 3.2, 6.9)
11	26.6, CH_2	1.65, ddq (13.6, 3.5, 7.5)	26.4, CH_2	1.64, m	25.1, CH_2	1.66, ddq (15.1, 3.2, 7.5)
		1.29, ddq (13.6, 8.3, 7.5)		1.29, m		1.22, ddq (15.1, 9.3, 7.5)
12	10.9, CH_3	0.89, t (7.5)	10.7, CH_3	0.89, t (7.1)	12.0, CH_3	0.87, t (7.5)
13	15.8, CH_3	0.88, d (6.8)	15.7, CH_3	0.87, d (6.1)	16.8, CH_3	0.80, d (6.9)
14	17.2, CH_3	2.32, s	17.2, CH_3	2.36, s	18.6, CH_3	2.31, s
15	15.7, CH_3	2.22, s	59.6, CH_2	4.66, d (17.7)	15.0, CH_3	2.22, s
				4.64, d (17.7)		

[a] Data were obtained in MeOH-d_4 solutions at 150 and 600 MHz (**1**), and 125 and 500 MHz (**2** and **3**).

pling constant between oxymethines as a key indicator of their orientation (typically, *cis* ⩽ 2 Hz, *trans* ⩾ 4 Hz).[11–15] The coupling constant is usually combined with NOE data to assign the relative configurations of the lactone moiety. In our data for compound **1**, the small coupling constant ($J_{8,9}$ = 1.2 Hz) was indicative of the *cis* orientation between H-8 and H-9. However, our initial NOESY experiment showed conspicuous cross-peaks at H-8/H-13 that should not be observed for an ordinary chair-type conformation. Furthermore, in the ORTEP drawing of a crystal structure in the literature, the lactone ring appeared to have a half-chair conformation.[14] These findings prompted us to thoroughly investigate the stereochemistry of these compounds.

First, the relative configurations of **1** were investigated by proton coupling constant analysis. The large coupling constant ($J_{9,10}$ = 9.6 Hz) revealed an *anti*-orientation between these protons, as suggested by Murata and coworkers.[18] In contrast, the small coupling constant ($J_{8,9}$ = 1.2 Hz) suggested a gauche conformation between H-8 and H-9. A molecular modeling study indicated that this conformation could be interpreted as either the *cis*- or *trans*-orientation depending on the conformation of the lactone ring. That is, these protons could be *cis*-oriented in the half chair-type conformation or *trans*-orientation in a boat-type conformation. The NOESY data showed a conspicuous cross-peak at H-8/H-9, supporting this interpretation (Fig. 2). Additional cross-peaks at H-8/H-13 and H-9/H-13 also supported the *anti*-conformation between H-9 and H-10. The absolute configuration at the C-8 oxymethine was determined to be *S* by the Mosher method (Fig. 3).[19,20] Therefore, the absolute configuration was either 8*S*, 9*R*, and 10*R* or 8*S*, 9*S*, and 10*S* depending on the conformation of the lactone ring, but distinguishing these two conformations required additional data.

In contrast, the NOESY data of **3** showed cross-peaks at H-8/H-9 and H-9/H-13, as observed for **1**. Although the mid-range coupling constants ($J_{8,9}$ = $J_{9,10}$ = 5.0 Hz) were attributed to the five-membered lactone ring, these NOESY data were in good agreement with the biosynthetic relationships between these compounds, in which **3** is formed from **1** by the attack of the lactone carbonyl by the C-8 hydroxyl group, resulting in ring opening. Therefore, the absolute configurations of these two compounds must be identical. Because the results of the Mosher analysis indicated that C-9 of **3** has the *R* configuration (Fig. 3), the absolute configuration was assigned to be 8*S*, 9*R*, and 10*R* for both **1** and **3**.

The stereochemical relationship between **1** and **3** was further studied by both spectroscopic and chemical methods. The CD profiles of these compounds differ significantly, and this difference could be attributed to differences in either the sizes of the lactone rings or the configurations of the asymmetric centers (Fig. 4). Subsequently, both compounds were chemically converted by basic hydrolysis (Scheme 1).[21] The formation of ring-opened carboxylic acids, as, was detected by HPLC-ESI-MS analysis (YMC ODS-A column, 5 μm, 4.6 × 250 mm, H_2O–MeOH gradient (70:30 to 0:100 in 30 min, v/v), 0.7 ml/min flow rate, UV detector, 254 nm, 13.8 min, *m*/*z* 281 $[M\text{-}H]^-$). However, these unstable intermediates readily re-cyclized to form the five-membered lactone compound **3**, as a result. The ^{1}H NMR data, optical rotations, and CD profiles of these lactone compounds were virtually identical to those of **3**. Moreover, MTPA esterification product of hydrolysate of **1** at C-9 yielded the same product as that derived from **3**. Thus, the absolute configurations of these compounds were unambiguously assigned.

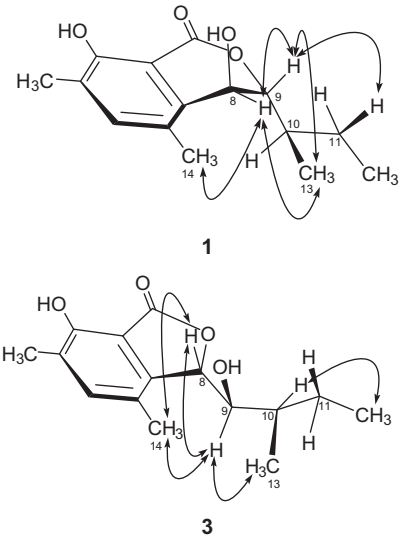
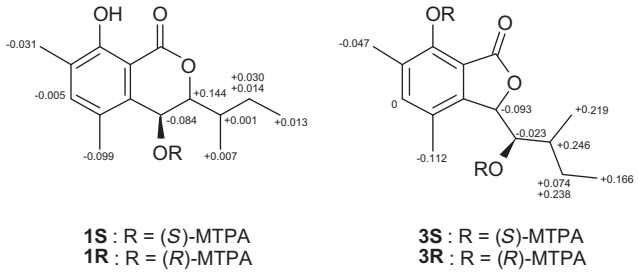


Figure 2. Selected NOESY correlations of compounds **1** and **3**.



1S : R = (*S*)-MTPA
1R : R = (*R*)-MTPA
3S : R = (*S*)-MTPA
3R : R = (*R*)-MTPA

Figure 3. $\Delta\delta_{S\text{-}R}$ values for MTPA esters of compounds **1** (**1S** and **1R**) and **3** (**3S** and **3R**).

Our results clearly demonstrate that the spectroscopic features of hydroxy-bearing benzolactones are significantly influenced by the conformation of the lactone moiety. Accordingly, the configurations cannot be determined by simple proton–proton coupling constants and NOE analysis alone. A more comprehensive approach combining diverse chemical and spectroscopic methods is required to determine the stereochemistry of hydroxy-bearing benzolactones.

Isocoumarins and structurally related benzolactones are widely distributed among fungi. However, to the best of our knowledge, the carbon framework of chrysoarticulins is unique. Compounds structurally similar to chrysoarticulins may include melleins,[11–13,15] pestaphthalides,[16] sclerolide,[22] and sclerotinins[23] as well as the synthetic and biosynthetic intermediates of ajudazols,[14] psymberin (irciniastatin A),[24] and ascochitine.[25]

However, chrysoarticulins differ structurally from these compounds because chrysoarticulins possess the combination of a 2-hydroxy-3,5-dimethyl benzolactone and a 3-methyl-pentyl chain.

In our measurement of bioactivities, these compounds were weakly active against the K562 and A549 cancer cell lines.

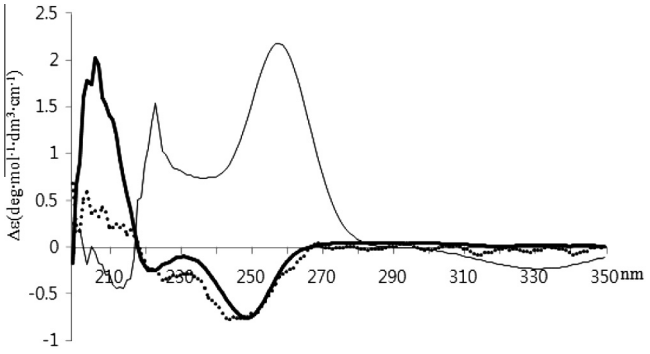
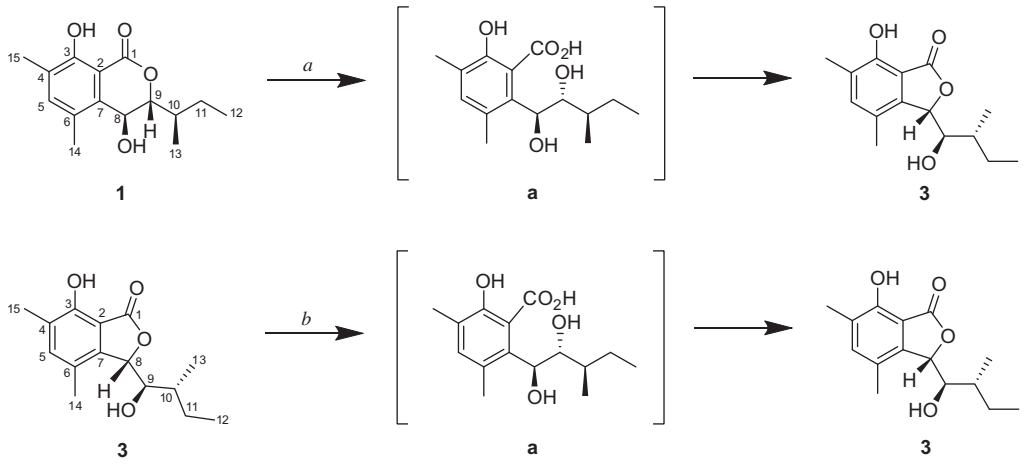


Figure 4. Experimental CD spectra of compounds **1** (solid line), hydrolysate of **1** (dotted line), and **3** (bold line).



Scheme 1. Reagents and conditions: (a) 1 M aqueous KOH, 0 °C, 30 min; (b) 1 M aqueous KOH, 0 °C, 22 h

Table 2
Results of bioactivity tests

Compound	LC_{50} (μM)		IC_{50} (μM)	
	K562	A549	Sortase A	ICL
1	164.0	147.3	>300	166.7
2	63.0	63.2	>300	>300
3	25.4	34.5	95.1	236.4
Doxorubicin	4.8	2.8		
p-HMB[a]			104.4	
3-NP[b]				12.6

[a] *para*-hydroxymercuribenzoic acid.
[b] 3-Nitropropionic acid.

Although compound **3** was more active than the six-membered congeners **1** and **2**, its cytotoxicity was far weaker than that of doxorubicin. Compound **3** moderately inhibited sortase A, a key enzyme involved in the adhesion and invasion of Gram-positive bacteria. The inhibition of isocitrate lyase (ICL), another key enzyme in microbial biosynthesis, was also very weak Table 2). These compounds were also inactive (MIC > 100 μg/mL) against diverse pathogenic bacteria and fungi.

In summary, we isolated three new benzolactone metabolites, chrysoarticulins A–C (**1**-**3**), from the marine-derived fungus *C. articulatum*. Their structures and stereochemistry were determined using diverse spectroscopic and chemical approaches. Compounds **1**-**3** possess a unique branched side chain and have moderate or no bioactivity.

Acknowledgments

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Supplementary data

Supplementary data (^{1}H and ^{13}C NMR spectra of compounds **1**–**3**, and specific information of general experimental procedure, biological assays, and hydrolysates' features) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2013.04.006. References [26–28] are cited in the Supplementary data.

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7. The fungal strain (F085) was identified using standard molecular biological protocols including DNA amplification and sequencing of the ITS region. Genomic DNA extraction was performed using Intron's i-genomic BYF DNA Extraction Mini Kit according to the manufacturer's protocol. The nucleotide sequence of F085 has been deposited in the GenBank database under the accession number JF901807. The 18S rDNA sequence of this strain showed 100% identity with *Chrysosporium articulatum* (GenBank accession number AJ007841).
8. *Chrysoarticulin A* (***1***): brown, amorphous solid, $[\alpha]_D^{25}$ +56 (*c* 0.40, MeOH); UV (MeOH) λ_{max} (log ε) 212 (4.16), 250 (3.48), 328 (3.42) nm; IR (ZnSe) ν_{max} 3349 (br), 2925, 1745, 1464 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; HRFABMS *m*/*z* 265.1442 $[M+H]^+$ (calcd for $C_{15}H_{21}O_4$, 265.1440).
9. *Chrysoarticulin B* (***2***): brown, amorphous solid, $[\alpha]_D^{25}$ +43 (*c* 0.25, MeOH); UV (MeOH) λ_{max} (log ε) 212 (4.38), 250 (3.62), 328 (3.49) nm; IR (ZnSe) ν_{max} 3344 (br), 2925, 1730, 1455 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; HRFABMS *m*/*z* 281.1386 $[M+H]^+$ (calcd for $C_{15}H_{21}O_5$, 281.1389).
10. *Chrysoarticulin C* (***3***): brown, amorphous solid, $[\alpha]_D^{25}$ +28 (*c* 0.25, MeOH); UV (MeOH) λ_{max} (log ε) 210 (4.46), 276 (3.42), 312 (3.53) nm; IR (ZnSe) ν_{max} 3423 (br), 2929, 1730, 1461 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; HRFABMS *m*/*z* 265.1443 $[M+H]^+$ (calcd for $C_{15}H_{21}O_4$, 265.1440).
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20. The esterification of compounds **1**, hydrolysate of **1**, and **3** was performed as follows. A total of 30 μL of (−)-(R)-MTPA chloride was added to a solution containing 1.6, 0.5, and 1.2 mg of the alcoholic compound in 300 μL of dry pyridine. The mixture was allowed to stand under N_2 at room temperature for 2 h. After the consumption of the starting material was confirmed by thin-layer chromatography, 1 mL of H_2O–MeOH (10:90) was added. The solvent was removed under vacuum, and the residue was separated by reversed-phase HPLC (YMC-ODS column, 4.6 × 250 mm; H_2O–MeOH, 15:85) to give 1.0, 0.4, and 1.1 mg of (S)-MTPA esters (**1S**, hydrolysate of **1S**, and **3S**). The corresponding (*R*)-MTPA esters (**1R** and **3R**) were also obtained from the same esterification reactions with (+)-(S)-MTPA chloride. ^{1}H NMR (MeOH-d_4,

400 MHz) of **1S**: δ_H 7.412−7.322 (5H, m, H-MTPA-Ar), 7.391 (1H, s, H-5), 6.382 (1H, d, J = 1.8 Hz, H-8), 4.642 (1H, dd, J = 9.7, 1.8 Hz, H-9), 3.530 (3H, s, H-MTPA-OMe), 2.221 (3H, s, H-14), 2.219 (3H, s, H-15), 1.708 (1H, m, H-11a), 1.500 (1H, m, H-10), 1.318 (1H, m, H-11b), 0.978 (3H, d, J = 6.7 Hz, H-13), 0.891 (3H, t, J = 7.4 Hz, H-12); LR-FABMS m/z 481.3, $[M+H]^+$ (calcd for $C_{25}H_{28}F_3O_6$, 481.2). ^{1}H NMR (MeOH-d_4, 400 MHz) of **1R**: δ_H 7.413−7.347 (5H, m, H-MTPA-Ar), 7.396 (1H, s, H-5), 6.466 (1H, d, J = 1.4 Hz, H-8), 4.498 (1H, dd, J = 9.6, 1.4 Hz, H-9), 3.389 (3H, s, H-MTPA-OMe), 2.320 (3H, s, H-14), 2.250 (3H, s, H-15), 1.678 (1H, m, H-11a), 1.499 (1H, m, H-10), 1.304 (1H, m, H-11b), 0.965 (3H, d, J = 6.7 Hz, H-13), 0.884 (3H, t, J = 7.5 Hz, H-12); LR-FABMS m/z 481.3, $[M+H]^+$ (calcd for $C_{25}H_{28}F_3O_6$, 481.2). ^{1}H NMR (MeOH-d_4, 400 MHz) of **3S**: δ_H 7.766−7.382 (10H, m, H-MTPA-Ar), 7.510 (1H, s, H-5), 5.882 (1H, d, J = 2.7 Hz, H-8), 5.494 (1H, dd, J = 5.7, 2.7 Hz, H-9), 3.548 (3H, s, H-14), 3.339 (6H, s, H-MTPA-OMe), 2.484 (3H, s, H-15), 1.663 (1H, m, H-11a), 1.610 (1H, m, H-10), 1.145 (1H, m, H-11b), 0.822 (3H, t, J = 7.5 Hz, H-12), 0.786 (3H, d, J = 6.9 Hz, H-13); LR-ESIMS m/z 719.0, $[M+Na]^+$ (calcd for $C_{35}H_{34}F_6O_8Na$, 719.2). ^{1}H NMR (MeOH-d_4, 400 MHz) of **3R**: δH 7.751–7.435 (10H, m, H-MTPA-Ar), 7.510 (1H, s, H-5), 5.975 (1H, br s, H-8), 5.517 (1H, dd, J = 6.1, 2.1 Hz, H-9), 3.660 (3H, s, H-14), 3.339 (6H, s, H-MTPA-OMe), 2.532 (3H, s, H-15), 1.588 (1H, m, H-11a), 1.364 (1H, m, H-10), 0.907 (1H, m, H-11b), 0.656 (3H, t, J = 7.5 Hz, H-12), 0.567 (3H, d, J = 6.9 Hz, H-13); LR-ESIMS m/z 719.0, $[M+Na]^+$ (calcd for $C_{35}H_{34}F_6O_8Na$, 719.2). ^{1}H NMR (MeOH-d_4, 400 MHz) data of hydrolysate of **1S** were superimposed with **3S**.

21. The hydrolysis of compounds **1** and **3** was performed as follows. An aliquot of **1** (0.8 mg) and 1 M aqueous KOH (0.5 mL) was stirred at 0 °C for 30 min. Then, the reaction mixture was neutralized by adding 1 M aqueous HCl (0.5 mL). After cooling, the solution was concentrated under a stream of N_2, and then the residue was purified by reversed-phase HPLC (YMC-ODS column, 4.6 × 250 mm; H_2O–MeOH, 30:70) to obtain **3**. A solution of **3** (0.7 mg) was prepared in a similar manner (reaction time, 22 h). Detailed spectroscopic data of corresponding hydrolysates are available in Supplementary data (S8).
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