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A Novel Ascochlorin Glycoside from the Insect Pathogenic Fungus *Verticillium hemipterigenum* BCC 2370

Prapairat Seephonkai,[a] Masahiko Isaka,[b,*] Prasat Kittakoop,[b] Uraiwan Boonudomlap and Yodhathai Thebtaranonth[a,b]

[a] Department of Chemistry, Mahidol University, Rama 6 Road, Bangkok 10400, Thailand
[b] National Center for Genetic Engineering and Biotechnology (BIOTEC), 113 Phaholyothin Road, Klong 1, Klong Luang, Pathumthani 12120, Thailand

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Vertihemipterin A, the ascochlorin glycoside, and its aglycone, 4′,5′-dihydro-4′-hydroxyascochlorin, were isolated from the insect pathogenic fungus *Verticillium hemipterigenum* BCC 2370. A new analog, 8′-hydroxyascochlorin, and five known compounds, ascochlorin, LL-Z1272ζ, 8′,9′-dehydroascochlorin, ascofuranone and ascofuranol, were also isolated from the same fermentation broth. Structures of these compounds were elucidated by spectroscopic methods. Stereochemistry of the known compound, 4′,5′-dihydro-4′-hydroxyascochlorin, was addressed by NMR spectral analyses and the modified Mosher's method. Antiviral (HSV-1) and cytotoxic activities of these ascochlorin analogs were evaluated.

Ascochlorin, first isolated from the fungus *Ascochyta viciae* in 1968,[1,2] is an antibiotic, which consists of a 5-chloroorcylaldehyde substituted at C-3 with a sesquiterpene side chain. Many structurally related compounds have subsequently been isolated from a variety of fungi such as *Ascochyta viciae*,[3,4] *Fusarium* sp. LL-Z1272,[5] *Colletotrichum* sp.,[6] *Cylindrocladium* sp.,[7] *Cylindrocladium ilicicola* MFC-870,[8,9] *Nectria coccinea*,[10] *Acremonium luzulae*,[11] *Cylindrocarpon lucidum*[12] and *Verticillium* sp.[13] Members of this class of compounds have been known to exhibit antiviral[2] and antitumor[2,8] activities. They are also known to be Ras farnesyl-protein transferase (FPTase) inhibitors[12] as well as testosterone 5α-reductase inhibitors.[13] In our continued search for novel bioactive compounds from insect pathogenic fungi,[14~16] we came across a cytotoxic ascochlorins mixture in the extracts of *Verticillium hemipterigenum* BCC 2370. Chromatographic fractionation led to the isolation of a novel analog, vertihemipterin A (**1**), together with its aglycone, 4′,5′-dihydro-4′-hydroxyascochlorin (**2**; designated as 4′-hydroxy-5′-hydroascochlorin in the original literature),[17] a new analog, 8′-hydroxyascochlorin (**3**), and five other known compounds, ascochlorin (**4**),[1] LL-Z1272ζ (8′-acetoxyascochlorin; **5**),[5] 8′,9′-dehydroascochlorin (**6**),[13] ascofuranone (**7**)[3] and ascofuranol (**8**).[4] We report herein the isolation, structure elucidation and biological activities of these compounds.

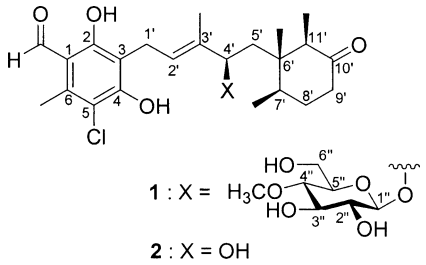
Results and Discussion

Fermentation and Isolation

Verticillium hemipterigenum was collected from Heo Narok waterfall, Khao Yai National Park (Thailand), on *Homoptera cicadellid*, leafhopper, and identified by Dr. Nigel L. Hywel-Jones of the BIOTEC Mycology Research Unit. This fungus was deposited at the Thailand BIOTEC Culture Collection as BCC 2370.

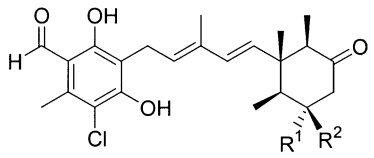
V. hemipterigenum BCC 2370 was maintained on potato dextrose agar at 25°C for 20 days, which was inoculated into 2×1 liter Erlenmeyer flasks each containing 250 ml of potato dextrose broth. After incubation at 25°C for 3 days on a rotary shaker (200 rpm), these primary cultures were transferred into 20×1 liter Erlenmeyer flasks each containing the same liquid medium (total 5 liters), and the fermentation was carried out at 25°C for 39 days under the

* Corresponding author: isaka@biotec.or.th



1 : X = H_3CO (HO, 6", 4", 5", O, 1", HO, 3", 2", OH)

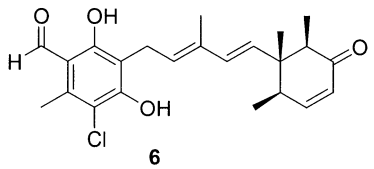
2 : X = OH



3 : R^1 = OH, R^2 = H

4 : R^1 = H, R^2 = H

5 : R^1 = OAc, R^2 = H



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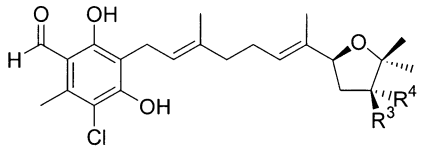


7 : R^3, R^4 = O

8 : R^3 = OH, R^4 = H

stationary condition. The cultures were filtered to separate filtrate and wet mycelia. The filtrate was extracted twice with an equal volume of EtOAc. The EtOAc layer was dried under reduced pressure to obtain a brown solid (338 mg). The wet mycelia were extracted with MeOH (1500 ml, 6 days). To the filtrate was added H_2O (100 ml) and the mixture was washed with hexane (800 ml). The aqueous MeOH layer was concentrated under reduced pressure. The residue was dissolved in EtOAc (300 ml), washed with H_2O (100 ml), and the organic layer was concentrated under reduced pressure to obtain a brown solid (1.13 g). The crude extracts from filtrate and mycelia were separately subjected to chromatographic fractionation.

The extract from filtrate was passed through a Sephadex LH-20 column (3.5×25 cm) with MeOH as eluent to provide three major fractions; Fr-A (24 mg), Fr-B (140 mg) and Fr-C (46 mg). Fr-A was fractionated by silica gel column chromatography (step gradient elution with EtOAc/CH_2Cl_2 to obtain **1** (13 mg). Fr-B was subjected to preparative HPLC using a reversed phase column (MeOH/H_2O=50 : 50) to obtain **2** (40 mg). Fr-C was subjected to silica gel column chromatography (step gradient elution EtOAc/hexane) to obtain **3** (14 mg).

The extract from the mycelia was passed through a Sephadex LH-20 column (3×58 cm) with MeOH as eluent, and two major fractions were collected; Fr-D (340 mg; mainly consisting of compounds **7** and **4**) and Fr-E (200 mg). Fr-D was subjected to silica gel column chromatography (step gradient elution with EtOAc/hexane) to yield **7** (50 mg) and **4** (37 mg). Fr-E was fractionated by silica gel column chromatography (step gradient elution with EtOAc/hexane) to yield **6** (22 mg), **5** (14 mg), **8** (7 mg) and **3** (17 mg).

Structure Elucidation

Vertihemipterin A (**1**), molecular formula of $C_{30}H_{43}ClO_{10}$ (HRMS, ^{13}C NMR), showed UV and IR data similar to those of ascochlorin (**4**). ^1H and ^{13}C NMR spectra of **1** suggested that this compound possessed an ascochlorin skeleton attached to a sugar unit. The presence of a 5-chloroorcylaldehyde moiety was evident by comparison of the ^1H and ^{13}C chemical shifts with those of ascochlorin. The planar structure of the sesquiterpene (C_{15}) side chain was elucidated by 2D-NMR (COSY, HMQC and HMBC) analyses, and HMBC correlations are shown in Table 2. The (*E*)-geometry of C-2′–C-3′ double bond, identical to that of ascochlorin (**4**), was indicated by NOESY correlations of H-1′ to methyl group (δ_H 1.82, 3H, s) on C-3′, and H-2′ to H-4′. The relative configuration of the tetrasubstituted cyclohexanone moiety was determined to be identical to that of ascochlorin (**4**) by analyses of vicinal *J*-values and NOESY spectrum (Fig. 1). One of the C-8′ methylene protons situated at δ_H 1.57 appeared as a double quartet (*J*=5.4, 13.0 Hz), which strongly suggested the chair conformation of the cyclohexanone ring and axial orientation of this proton. Intense NOESY cross signal between this proton (H-8′ax) and a methyl group on C-6′ placed the methyl group (6′-CH_3) in an axial orientation. The *J*-value of 13.0 Hz for H-7′ (δ_H 2.20) and H-8′ax revealed axial orientation of H-7′. NOESY correlation between H-7′ and H-11′ indicated a 1,3-diaxial relationship of these two protons. Therefore, the three methyl groups attached to C-6′, C-7′ and C-11′ were on the same face of the cyclohexanone ring. This assignment was also supported by observation of NOESY correlations from 6′-CH_3 to 7′-CH_3 and 11′-CH_3. On the basis of these spectral data, the structure of the aglycone of vertihemipterin A (**1**) was assigned to 4′,5′-dihydro-4′-hydroxyascochlorin, and

Table 1. Physico-chemical properties of **1** and **3**.

	1	**3**
Appearance	Pale yellow amorphous solid	Pale yellow powder
MP	80~83 °C	148~149 °C
Molecular formula	$C_{30}H_{43}ClO_{10}$	$C_{23}H_{29}ClO_5$
HRMS (ESI-TOF)		
Found (*m/z*)	621.2443 $[M+Na]^+$	419.1624 $[M-H]^-$
Calcd.	621.2442	419.1626
$[\alpha]_D$	+17° (*c* 0.21, MeOH, 25 °C)	–26° (*c* 0.65, MeOH, 25 °C)
UV λ_{max} nm (log ε) in MeOH	228 (3.86), 294 (3.66), 346 (3.53)	238 (4.47), 293 (3.95), 348 (3.71)
IR ν_{max} (KBr) cm^{-1}	3425, 2930, 1701, 1628, 1461, 1423, 1252, 1109, 909	3280, 2976, 1678, 1618, 1455, 1423, 1283, 1111, 971

Table 2. NMR data for compounds **1**~**3** in $CDCl_3$ (400 MHz for ^1H and 100 MHz for ^{13}C).

	vertihemipterin A (**1**)			4′,5′-dihydro-4′-hydroxyascochlorin (**2**)		8′-hydroxyascochlorin (**3**)	
position	δ_C (mult.)	δ_H (mult., *J* in Hz)	HMBC (H to C)	δ_C (mult.)	δ_H (mult., *J* in Hz)	δ_C (mult.)	δ_H (mult., *J* in Hz)
1	113.5 (s)[a]			113.6 (s)[e]		113.7 (s)[b]	
2	162.0 (s)			162.1 (s)		162.2 (s)	
3	113.3 (s)[a]			113.6 (s)[e]		113.6 (s)[b]	
4	156.2 (s)			156.1 (s)		156.2 (s)	
5	113.3 (s)[a]			113.1 (s)		113.2 (s)	
6	138.3 (s)[c]			137.9 (s)		137.8 (s)	
1-*CH*O	193.3 (d)	10.13 (s)	1, 2	193.3 (d)	10.13 (s)	193.2 (d)	10.15 (s)
2-O*H*		12.71 (s)	2		12.70 (s)		12.71 (s)
4-O*H*		6.94 (brs)			not detected		6.45 (brs)
6-CH_3	14.5 (q)	2.60 (s)	6	14.5 (q)	2.60 (s)	14.5 (q)	2.60 (s)
1′	21.5 (t)	3.40 (m)[f], 3.37 (m)[f]	2, 3, 4, 2′, 3′	21.6 (t)	3.40 (2H)(d, 7.2)	22.2 (t)	3.54 (2H)(d, 7.4)
2′	124.4 (d)	5.41 (t, 7.0)	3, 4′, 3′-CH_3	122.8 (d)	5.49 (t, 7.1)	128.0 (d)	5.54 (t, 7.4)
3′	138.3 (s)[c]			139.8 (s)		133.9 (s)[d]	
4′	85.1 (d)	4.04 (dd, 6.1, 4.2)	2′, 3′-CH_3	74.6 (d)	4.21 (dd, 7.2, 4.1)	133.9 (d)[d]	5.91 (d, 16.0)
5′	40.6 (t)	1.85 (dd, 15.6, 6.6)	4′, 6′, 11′	41.4 (t)	1.67 (dd, 15.4, 7.4)	134.7 (d)	5.33 (d, 16.0)
		1.47 (dd, 15.6, 3.9)			1.46 (dd, 15.4, 4.0)		
6′	44.0 (s)			44.1 (s)		45.8 (s)	
7′	36.5 (d)	2.20 (m)		36.7 (d)	2.31 (m)	48.1 (d)	1.78 (dq, 10.2, 6.7)
8′	31.1 (t)	1.80 (m)		31.2 (t)	1.81 (m)	72.4 (d)	3.67 (ddd, 12.1, 10.2, 5.4)
		1.57 (dq, 5.4, 13.0)			1.55 (dq, 5.5, 13.1)		
9′	41.3 (t)	2.28 (ddd, 13.4, 5.4, 2.4)	10′	41.6 (t)	2.27 (m)	50.8 (t)	2.81 (dd, 12.0, 5.4)
		2.21 (m)	8′, 10′		2.23 (m)		2.47 (t, 12.1)
10′	213.8 (s)			214.1 (s)		209.3 (s)	
11′	50.4 (d)	2.53 (q, 6.7)	6′, 10′, 6′-CH_3, 11′-CH_3	50.4 (d)	2.58 (q, 6.6)	53.8 (d)	2.43 (q, 6.7)
3′-CH_3	11.5 (q)	1.82 (s)	2′, 3′, 4′	11.4 (q)	1.83 (s)	12.6 (q)	1.93 (s)
6′-CH_3	15.6 (q)	0.57 (s)	5′, 6′, 7′, 11′	15.5 (q)	0.55 (s)	11.5 (q)	0.67 (s)
7′-CH_3	15.8 (q)	0.98 (d, 6.7)	6′, 7′, 8′	15.8 (q)	0.97 (d, 6.7)	12.5 (q)	0.98 (d, 6.7)
11′-CH_3	8.1 (q)	0.77 (d, 6.7)	6′, 10′, 11′	8.0 (q)	0.82 (d, 6.7)	8.9 (q)	0.85 (d, 6.7)
1″	101.9 (d)	4.22 (d, 7.9)	4′				
2″	74.1 (d)	3.38 (m)[f]	1″				
3″	76.7 (d)	3.56 (t, 8.8)					
4″	79.9 (d)	3.08 (dd, 9.5, 8.7)	3″, 4″-OCH_3				
5″	75.2 (d)	3.14 (ddd, 9.5, 5.7, 2.8)	3″				
6″	62.5 (t)	3.79 (dd, 11.6, 2.7)					
		3.64 (dd, 11.6, 5.7)					
4″-OCH_3	60.7 (q)	3.55 (s)	4″				

[a,b] Assignments can be interchanged. [c-e] The ^{13}C signals are superimposed. [f] The proton signals are overlapped.

this aglycone (**2**) was also isolated from the same culture broth.

The remaining seven carbons, including one methoxy group at δ_C 60.7 (δ_H 3.55; 3H, s), were classified as those of a sugar unit. The proton at δ_H 4.22 (H-1″), which was attached to δ_C 101.9 carbon (C-1″), was assigned to the

Fig. 1. Selected NOESY correlations for **1**.

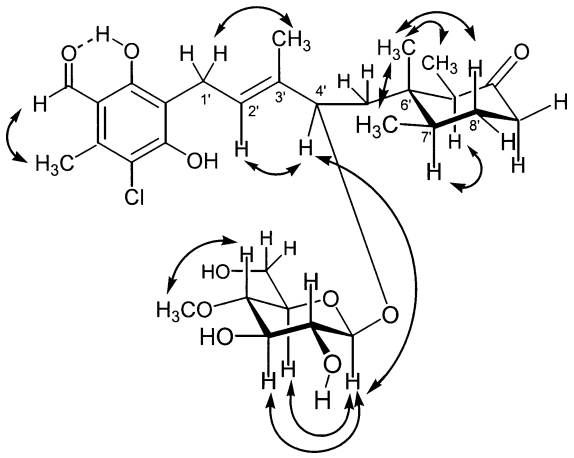
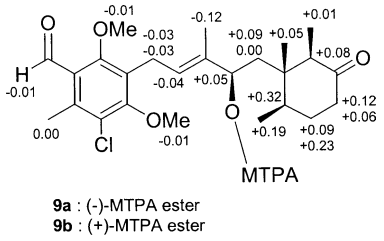


Fig. 2. $\Delta\delta$ Values [$\delta_{(-)}$-$\delta_{(+)}$] for MTPA esters **9a** and **9b**.



anomeric position. The connectivity from C-1″ to C-6″ was revealed by analyses of ^{1}H NMR, COSY and HMQC spectra. HMBC correlation from H-4″ to the methoxy carbon (δ_C 60.7) clearly indicated that the hydroxyl group on C-4″ was methylated. Vicinal coupling constants of $J_{1'',2''}$=7.9, $J_{2'',3''}$=8.8, $J_{3'',4''}$=8.7, and $J_{4'',5''}$=9.5 Hz, placed all these protons in axial orientation on a pyranose ring. This structural assignment was strongly supported by the NOESY correlations from H-1″ to H-3″ and H-5″, hence, the sugar unit was identified as 4-*O*-methyl-β-glucopyranose. An intense HMBC correlation from H-1″ to C-4′ clearly indicated the connectivity between the sugar moiety and the aglycone, which was also supported by the observation of a NOESY cross signal between H-1″and H-4′.

The structure of compound **2**, molecular formula $C_{23}H_{31}ClO_5$ (HRMS, ^{13}C NMR), was assigned as 4′,5′-dihydro-4′-hydroxyascochlorin based on the spectroscopic analyses, and physico-chemical data were identical in all respects to those reported in the literature.[17] Although relative stereochemistry and ^{13}C NMR data for this compound have not been previously presented, we have successfully assigned all protons and carbons by analyses of 2D-NMR spectra (Table 2). The relative configuration of the cyclohexanone moiety of **2** was identical to those of **1** and **4**, as confirmed by the analyses of NOESY correlations and ^{1}H-^{1}H coupling constant data as described above for **1**. The absolute configuration of C-4′ was assigned by a modified Mosher's method.[18,19] Compound **2** was converted into its 2,4-*O*-dimethyl derivative (MeI, K_2CO_3, 2-butanone). Subsequent treatment with (−)- and (+)-MTPACl in pyridine followed by purification (silica gel column) gave the corresponding esters **9a** and **9b**. Based on the $\Delta\delta$ [$\delta_{(-)}$-$\delta_{(+)}$] values shown in Fig. 2, the (4′*R*)-configuration was established for compound **2**. Syrindrol A, a closely related analog which could also be designated as 5-dechloro-4′,5′-dihydro-4′-propanoyloxyascochlorin, was also reported to possess the (4′*R*)-configuration.[12] Unfortunately, attempted hydrolysis of vertihemipterin A (**1**) had met with failure, for example, treatment of **1** with aqueous hydrochloric acid in MeOH or dioxane resulted in slow conversion of **1**, giving complex mixture of products. However, the co-occurrence of **1** and **2** suggested that they are likely to possess the same configuration at C-4′. The specific rotation value of ascochlorin (**4**), $[\alpha]_D^{25}$ −26° (*c* 0.67, MeOH), isolated from the same culture broth (BCC 2370), was in good agreement with the literature, wherein the absolute configuration of **4** was unambiguously established by an X-ray analysis of its 4-*O*-*p*-bromobenzenesulfonyl derivative.[20] Therefore, it is not unreasonable to assume that the absolute configuration of the cyclohexanone moiety of the co-metabolites **1** and **2** likely to be identical to that of **4**.

Another new analog, **3**, possessed the molecular formula $C_{23}H_{29}ClO_5$ as determined by HRMS and ^{13}C NMR. The ^{1}H and ^{13}C NMR spectra of **3** were similar to those of LL-Z1272ζ (8′-acetoxyascochlorin; **5**), except for the lack of an acetyl group and the upfield shift of H-8′ in **3** (δ_H 3.67) as compared to that in **5** (δ_H 4.89). These data suggested that compound **3** was likely to be an 8′-hydroxy analog of ascochlorin (**4**). Coupling constants between H-8′ and three vicinal protons, 10.2 Hz ($J_{8',7'}$), 12.1 Hz ($J_{8'\text{-}9'ax}$) and 5.4 Hz ($J_{8'\text{-}9'eq}$), clearly indicated the axial orientation of H-8′, which, in turn, placed the hydroxyl group in an equatorial position. This assignment was strongly supported by a NOESY correlation between H-8′ and 6′-CH_3. NOESY

correlations of H-7′ to H-9′ax and H-11′ revealed axial orientations of these three protons on the same face of the six-membered ring. H-7′ was also correlated to H-5′, which confirmed the configuration of C-6′. From these spectral data, the relative configuration of the asymmetric carbon centers on the cyclohexanone ring of **3** (C-6′, C-7′ and C-11′) was shown to be identical to that of **4** and **5**. Final structural confirmation of 8′-hydroxyascochlorin (**3**) by either conversion of **5** to **3** (by deacetylation) or **3** to **5** (by poly-acetylation and subsequent selective deacetylation) have been unsuccessful due to the competing β-elimination of AcOH under the slightly basic conditions to give a cyclohexenone moiety. That compound **3** was not an artifact resulting from Michael addition of H_2O to the enone **6** during the course of isolation was proven by HPLC-UV analysis of the crude extracts from culture filtrate and mycelia, wherein the peak corresponding to **3** was detected. Co-injection experiments using an authentic sample also supported this conclusion.

Structures of five known compounds, ascochlorin (**4**),[11,13] LL-Z1272ζ (**5**),[5] 8′,9′-dehydroascochlorin (**6**),[13] ascofuranone (**7**)[3,21] and ascofuranol (**8**)[4,21] were elucidated by analyses of NMR, MS, UV and IR spectral data and comparison with those reported in the literature.

Biological Activities

Ascochlorins **1**~**8** were tested for their cytotoxic activities toward three cancer cell-lines, KB, BC-1 and NCI-H187, as well as Vero cells. The assay employed the colorimetric method as described by SKEHAN and co-workers.[22] These compounds exhibited significant cytotoxicities to all cell lines, except for **2** and **8** (Table 3). In our antiviral assay using herpes simplex virus type 1 (HSV-1),[22] compound **6** exhibited potent activity with an IC_{50} value of 0.19 μg/ml; however, this value was close to that of its cytotoxicity against the host cells (Vero cells; IC_{50} 0.36 μg/ml). Ascochlorin (**4**) is known to be active against Newcastle disease virus.[2]

Table 3. Cytotoxic and antiviral activities of compounds **1**~**8**.

	cytotoxicity (IC_{50}, μg/ml)				anti-HSV-1
cpd	KB[a]	BC-1[b]	NCI-H187[c]	Vero[d]	(IC_{50} μg/ml)[e]
1	19	8.4	7.9	6.9	inactive[f]
2	>20	>20	>20	38	inactive[f]
3	2.7	1.4	2.2	3.4	inactive[f]
4	16	13	4.2	8.5	inactive[f]
5	1.9	2.0	1.3	0.69	inactive[f]
6	2.4	0.53	1.3	0.36	0.19
7	6.9	4.4	0.40	0.47	inactive[f]
8	>20	>20	>20	65	23

[a] Oral human epidermoid carcinoma. [b] Human breast cancer. [c] Human small cell lung cancer. [d] African green monkey kidney fibroblast. [e] The IC_{50} values for the standard compound, acyclovir, were 2~5 μg/ml. [f] Inactive (<25% inhibition) at concentrations non-cytotoxic to the host cells (Vero).

Experimental

General Experimental Procedures

Melting points were measured with an Electrothermal IA9100 digital melting point apparatus and are uncorrected. Optical rotations were measured with a JASCO DIP-370 digital polarimeter. UV spectra were recorded on a Varian CARY 1E UV-Visible spectrophotometer. FT-IR spectra were taken on a Perkin-Elmer 2000 spectrometer. ESI-TOF mass spectra were measured with a Micromass LCT mass spectrometer. NMR spectra were taken on Bruker DRX400 spectrometer.

Preparation of MTPA Esters **9a** and **9b**

To a solution of 4′,5′-dihydro-4′-hydroxyascochlorin (**2**, 10.0 mg) in 2-butanone (0.4 ml) were added MeI (50 μl) and K_2CO_3 (s) (40 mg). After stirring at room temperature for 5 hours, the reaction mixture was extracted with EtOAc. Standard aqueous workup and concentration under reduced pressure gave the 2,4-*O*-dimethyl derivative (8.7 mg): MS (ESI-TOF) *m/z* 473 $[M+Na]^+$; ^1H NMR ($CDCl_3$, 400 MHz) δ 10.41 (1H, s), 5.44 (1H, t, *J*=7.0 Hz), 4.22 (1H, dd, *J*=8.0, 3.4 Hz), 3.87 (3H, s), 3.82 (3H, s), 3.41 (2H, dd, *J*=9.1, 7.0 Hz), 2.64 (3H, s), 2.63 (1H, q, *J*=6.6 Hz), 2.36~2.29 (1H, m), 2.36~2.29 (2H, m), 1.84 (3H, s), 1.81 (1H, m), 1.70 (1H, dd, *J*=15.5, 8.1 Hz), 1.55 (1H, m), 1.43 (1H, dd, *J*=15.4, 3.4 Hz), 0.98 (3H, d, *J*=6.6 Hz), 0.86 (3H, d, *J*=6.7 Hz), 0.57 (3H, s). A 4.3 mg portion of this compound was treated with (−)-(*R*)-MTPACl (20 mg) in pyridine (0.2 ml) at room temperature for 40 hours. After standard aqueous workup, the crude oil was purified by silica gel column chromatography (60% CH_2Cl_2/hexane) to obtain a (−)-(*S*)-MTPA ester **9a** (4.5 mg). (+)-(*R*)-MTPA ester **9b** was prepared in the same manner employing (+)-(*S*)-MTPACl. Full assignments of protons of **9a** and **9b** were achieved by combined analyses of ^1H NMR, COSY

and NOESY spectra.

(−)-(*S*)-MTPA ester **9a**: Colorless oil; HRMS (ESI-TOF) *m/z* 689.2474 $[M+Na]^+$ (calcd for $C_{35}H_{42}ClF_3O_7$ Na, 689.2469); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 10.38 (1H, s, 1-C*H*O), 7.47 (2H, m, phenyl), 7.44～7.36 (3H, m. phenyl), 5.62 (1H, dd, *J*=6.7, 6.7 Hz, H-2′), 5.49 (1H, dd, *J*=7.3, 4.6 Hz, H-4′), 3.84 (3H, s, 4-OCH_3), 3.78 (3H, s, 2-OCH_3), 3.43 (1H, dd, *J*=15.0, 6.7 Hz, H-1′a), 3.40 (3H, brs, OCH_3 of MTPA), 3.35 (1H, dd, *J*=14.8, 6.8 Hz, H-1′b), 2.64 (3H, s, 6-CH_3), 2.47 (1H, q, *J*=6.6 Hz, H-11′), 2.25 (1H, ddd, *J*=13.4, 4.7, 1.7 Hz, H-9′a), 2.13 (1H, ddd, *J*=13.5, 13.5, 7.0 Hz, H-9′b), 1.90 (1H, dd, *J*=15.8, 7.5 Hz, H-5′a), 1.74 (1H, m, H-7′), 1.73 (3H, s, 3′-CH_3), 1.65 (1H, m, H-8′a), 1.57 (1H, dd, *J*=15.1, 4.7 Hz, H-5′b), 1.47 (1H, dq, *J*=4.9, 13.1 Hz, H-8′b), 0.84 (3H, d, *J*=6.7 Hz, 11′-CH_3), 0.78 (3H, d, *J*=6.6 Hz, 7′-CH_3), 0.54 (3H, s, 6′-CH_3).

(+)-(*R*)-MTPA ester **9b**: Colorless oil; HRMS (ESI-TOF) *m/z* 689.2473 $[M+Na]^+$ (calcd for $C_{35}H_{42}ClF_3O_7$ Na, 689.2469); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 10.39 (1H, s, 1-C*H*O), 7.54 (2H, m, phenyl), 7.40～7.35 (3H, m. phenyl), 5.66 (1H, brt, *J*=6.5 Hz, H-2′), 5.44 (1H, dd, *J*=8.3, 3.5 Hz, H-4′), 3.85 (3H, s, 4-OCH_3), 3.79 (3H, s, 2-OCH_3), 3.55 (3H, brs, OCH_3 of MTPA), 3.46 (1H, dd, *J*=14.3, 7.2 Hz, H-1′a), 3.38 (1H, dd, *J*=14.9, 6.5 Hz, H-1′b), 2.64 (3H, s, 6-CH_3), 2.39 (1H, q, *J*=6.7 Hz, H-11′), 2.19 (1H, m, H-9′a), 2.01 (1H, m, H-9′b), 1.90 (1H, dd, *J*=15.9, 8.3 Hz, H-5′a), 1.85 (3H, s, 3′-CH_3), 1.48 (1H, dd, *J*=15.9, 3.6 Hz, H-5′b), 1.42 (2H, m, H-7′ and H-8′a), 1.39 (1H, dq, *J*=4.7, 13.3 Hz, H-8′b), 0.83 (3H, d, *J*=6.7 Hz, 11′-CH_3), 0.59 (3H, d, *J*=5.9 Hz, 7′-CH_3), 0.49 (3H, s, 6′-CH_3).

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