

Vibralactones D—F from Cultures of the Basidiomycete *Boreostereum vibrans*

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Three new metabolites, vibralactones D—F (1—3) were isolated from cultures of the basidiomycete *Boreostereum vibrans*. The relative configuration of 1 was established on the basis of X-ray diffraction analysis, while the absolute configurations of 1—3 were assigned using a modified Mosher's method. Compound 1 showed weak inhibitory activities against isozymes of 11 β -hydroxysteroid dehydrogenases (HSD) with IC₅₀ values of 85.7 μ M (human HSD1), 295.2 μ M (mouse HSD1), and 87.1 μ M (human HSD2).

Key words vibralactone; 11 β -hydroxysteroid dehydrogenase activity; *Boreostereum vibrans*

Our previous study on the secondary metabolites of the basidiomycete *Boreostereum vibrans* resulted in the isolation of a series of vibralactone-related compounds.^{1,2} The main constituent vibralactone showed inhibitory activity against pancreatic lipase with an IC₅₀ value of 0.4 μ g/ml. Our continuing investigation on the chemical constituents of this fungus has led to the isolation of three further new metabolites, vibralactones D—F (1—3). Vibralactone D (1) showed inhibitory activities against two isozymes of 11 β -hydroxysteroid dehydrogenases (11 β -HSD1 and 11 β -HSD2) that catalyze the interconversion of active cortisol and inactive cortisone.

Results and Discussion

Vibralactone D (1) was obtained as colorless crystal. Its molecular formula was established to be C₁₂H₁₈O₃ according to the pseudomolecular molecular ion at *m/z* 211.1330 [M+H]⁺ using high-resolution electrospray ionization mass spectrometry (HR-ESI-MS). The IR spectrum showed the presence of hydroxy (3504 cm⁻¹) and carbonyl (1714 cm⁻¹) groups. ¹³C-NMR and distortionless enhancement by polarization transfer (DEPT) data (Table 1) revealed 12 carbon

signals assigned to one carbonyl, one trisubstituted double bond, one quaternary carbon, two methines (one oxygenated), four methylenes (one oxygenated), and two methyls. The signals at δ_C 17.8 (q, C-11), 25.9 (q, C-12), 30.5 (t, C-8), 119.4 (d, C-9), 135.0 (s, C-10), δ_H 1.60 (s, H-11), 1.68 (s, H-12), and 5.15 (dd, *J*=7.8, 6.9 Hz, H-9) indicated the presence of an isoprenyl group. In the heteronuclear multiple bond connectivity (HMBC) spectrum of 1 (Fig. 1), the correlations of H-8/C-2, -5, and C-7; H-2/C-4, -5, C-13; H-3/C-1, -5; H-4/C-1, -13; H-5/C-3, -7, -8 and H-13/C-2, -4 were observed. The structure of 1 was confirmed in an X-ray experiment (Fig. 2). The absolute stereochemistry of 1 was determined using a modified Mosher's method.³ The (*S*)- and (*R*)- α -methoxy- α -(trifluoromethyl)phenylacetic acid (MTPA) esters of 1 were prepared. Positive $\Delta\delta$ ($\delta_S - \delta_R$) values (Fig. 3) were observed for H-5 (+0.10), H-8 (+0.09, +0.05), H-9 (+0.04), H-11 (+0.05), and H-12 (+0.01), while negative $\Delta\delta$ values were observed for H-3 (-0.04), H-4 (-0.10, -0.18), and H-13 (-0.05, -0.12). These data indicate an *S* configuration for C-5 and therefore, an absolute configuration of 1*R*, 3*S*, 5*S* for vibralactone D (1).

Table 1. ¹H- and ¹³C-NMR Data (500 and 125 MHz, *J* in Hz and δ in ppm) of 1—3

	1		2		3	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	56.8, s		65.9, s		63.4, s	
2	33.9, t	1.87 (dd, 12.4, 2.3) 1.71 (ddd, 12.4, 5.0, 1.8)	127.4, d	5.65 (s)	127.8, d	5.59 (s)
3	32.6, d	2.33 (m)	144.8, s		143.7, s	
4	37.5, t	2.47 (m) 1.61 (m)	41.3, t	2.68 (dd, 16.2, 6.1) 2.31 (dd, 16.2, 2.0)	40.9, t	2.66 (dd, 16.0, 7.0) 2.27 (dd, 16.0, 5.6)
5	77.8, d	4.05 (dd, 10.1, 5.0)	79.0, d	4.19 (dd, 6.1, 2.0)	77.1, d	4.57 (dd, 7.0, 5.6)
7	173.5, s		177.5, s		179.3, s	
8	30.5, t	2.60 (dd, 14.2, 6.9) 2.23 (dd, 14.2, 7.8)	36.0, t	2.55 (dd, 14.0, 6.8) 2.20 (dd, 14.0, 7.8)	31.3, t	2.55 (dd, 14.2, 6.9) 2.36 (dd, 14.2, 7.9)
9	119.4, d	5.15 (dd, 7.8, 6.9)	120.9, d	5.12 (dd, 7.8, 6.8)	121.4, d	5.19 (dd, 7.9, 6.9)
10	135.0, s		135.1, s		134.5, s	
11	17.8, q	1.60 (s)	18.1, q	1.61 (s)	18.0, q	1.62 (s)
12	25.9, q	1.68 (s)	26.0, q	1.69 (s)	26.2, q	1.68 (s)
13	77.0, t	4.31 (dd, 10.5, 1.8) 4.18 (d, 10.5)	61.9, t	4.11 (s)	61.9, t	4.09 (s)

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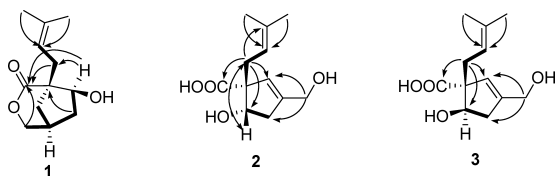


Fig. 1. Key HMBC (\rightarrow), ^1H - ^1H COSY (\longrightarrow) and ROESY (\longleftrightarrow) Correlations of **1**–**3**

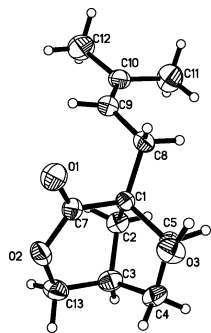


Fig. 2. X-Ray Structure of Vibrallactone D (**1**)

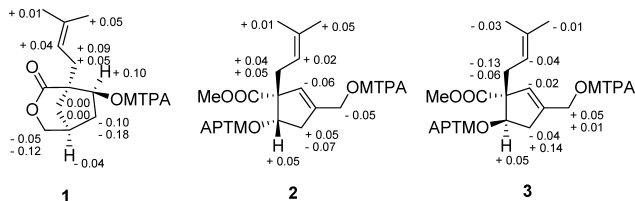


Fig. 3. $\Delta\delta$ Values ($\delta_S - \delta_R$) in ppm of the Two MTPA Esters Derived from **1**–**3**

Vibrallactone E (**2**) had the molecular formula $\text{C}_{12}\text{H}_{18}\text{O}_4$ as determined by HR-ESI-MS (Found, 225.1133 $[\text{M}-\text{H}]^-$; Calcd for $\text{C}_{12}\text{H}_{17}\text{O}_4$, 225.1126), indicating four degrees of unsaturation. IR absorptions at 3421 (broad) and 1708 cm^{-1} revealed the presence of hydroxy and carbonyl functionalities. An analysis of the ^1H -NMR spectrum indicated the presence of two olefinic protons at δ 5.65 (1H, s) and 5.12 ppm (1H, dd, $J=7.8, 6.8$ Hz), in addition to two isolated methyl groups at δ 1.69 (3H, s) and 1.61 ppm (3H, s). The ^{13}C -NMR and DEPT spectra of **2** showed 12 carbon resonances, including two methyls (δ 18.1, C-11; 26.0, C-12), three methylenes (δ 36.0, C-8; 41.3, C-4; 61.9, C-13), one oxymethine (δ 79.0, C-5), two trisubstituted double bonds (δ 120.9, d, C-9; 135.1, s, C-10; 127.4, d, C-2; 144.8, s, C-3), a quaternary (δ 65.9, C-1) carbon, and an acid carbonyl (δ 177.5, C-7) carbon. Therefore the remaining one degree of unsaturation identified compound **2** as a one-cyclic metabolite. The connectivity of the protons and C-atoms was established based on the heteronuclear single quantum coherence (HSQC) spectrum. Cross-peaks between H-4 and H-5, and H-8 and H-9 were observed in the ^1H - ^1H correlated spectroscopy (^1H - ^1H COSY) spectrum. ^{13}C , ^1H long-range couplings (3J) observed in the HMBC experiments gave the following correlations from H-2 to C-4, C-5, and C-13, from H-9 to C-1, C-11, and C-12, from H-5 to C-2, C-3, C-7, and C-8, from H-13 to C-2 and C-4, from H-4 to C-2, from H-8 to C-2, C-5, and C-10, from H-12 to C-9 and C-11, and from H-11 to C-9 and C-12. The data of **2** were similar to those of vi-

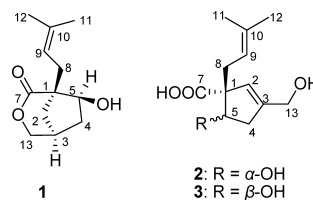


Fig. 4. Structures of **1**–**3**

brallactone,¹⁾ except that **2** had one degree of unsaturation less and chemical shifts at C-1 ($\Delta\delta -9.1$), C-2 ($\Delta\delta +5.2$), C-4 ($\Delta\delta +4.1$), C-7 ($\Delta\delta +4.3$), and C-8 ($\Delta\delta +8.5$) as compared with those of vibrallactone. By combining all this evidences and data, we were able to assign the planar structure of **2** as shown in Fig. 4.

Vibrallactone F (**3**) had the molecular formula $\text{C}_{12}\text{H}_{18}\text{O}_4$ as determined by HR-ESI-MS (225.1124 $[\text{M}-\text{H}]^-$; Calcd for $\text{C}_{12}\text{H}_{17}\text{O}_4$, 225.1126), which required four degrees of unsaturation in the molecule. The IR spectrum showed absorptions at 3424 and 1703 cm^{-1} , revealing the presence of hydroxy and carbonyl groups. The ^{13}C -NMR and DEPT spectra (Table 1) showed two methyl singlets at δ 18.0 and 26.2, three methylenes at δ 31.3, 40.9, and 61.9, two trisubstituted double bonds at δ 121.4, 127.8, 134.5, and 143.7, one oxymethine at δ 77.1, one quaternary carbon at δ 63.4, and an acid carbonyl at δ 179.3. The NMR data of **3** were closely similar to those of vibrallactone E (**2**); the main differences were the chemical shifts at C-1 ($\Delta\delta -2.5$), C-5 ($\Delta\delta -1.9$), C-7 ($\Delta\delta +1.8$), and C-8 ($\Delta\delta -4.7$). By interpretation of HSQC, ^1H - ^1H COSY and HMBC spectra (Fig. 1), **3** was determined to have a planar structure identical to that of **2**.

The absolute configurations of the two metabolites were established on the basis of the modified Mosher method in combination with rotating-frame Overhauser enhancement spectroscopy (ROESY) experiments. Esterification of **2** and **3** yielded the diastereoisomeric MTPA diesters, bis[(*S*)-MTPA] (**2a**, **3a**) and bis[(*R*)-MTPA] (**2b**, **3b**) esters. Diagnostic ^1H -NMR chemical shift differences between the MTPA esters of **2** [$\Delta\delta = \delta_S - \delta_R$] (Fig. 3) revealed the absolute configuration at C-5 to be *S*. This suggested that H-5 took the β orientation. The cross peaks between H-5 and H-8 in the ROESY spectrum suggested that the isoprenyl group also took the β orientation. Thus the absolute configuration of vibrallactone E (**2**) was elucidated to be *1R, 5S*. In the same manner, the absolute configuration of **3** at C-5 was assigned as *R*, indicating that H-5 was α oriented. No NOE correlation peak was observed between H-5 and H-8, indicating that the isoprenyl group was β oriented. From the data above, the *1R, 5R* configuration was assigned to vibrallactone F (**3**). A possible biogenetic pathway of vibrallactones A–F is proposed (Chart 1).

We investigated the inhibitory effects of the compounds on human and mouse 11β -HSD1 and 11β -HSD2. All experiments were done in triplicate with glycyrrhizinic acid as a positive control. IC_{50} (\pm S.D., $n=3$) values were calculated using Prism Version 4 (GraphPad Software, San Diego, CA, U.S.A.). IC_{50} values of glycyrrhizinic acid (positive control) are 29.5, 18.6, and 0.71 nM for mouse 11β -HSD1, human 11β -HSD1, and human 11β -HSD2, respectively. Vibrallactone D (**1**) showed inhibitory activities against 11β -HSD1 (human $\text{IC}_{50}=85.7 \mu\text{M}$; mouse $\text{IC}_{50}=295.2 \mu\text{M}$) and 11β -HSD2 (human $\text{IC}_{50}=87.1 \mu\text{M}$). Vibrallactones E and F (**2** and

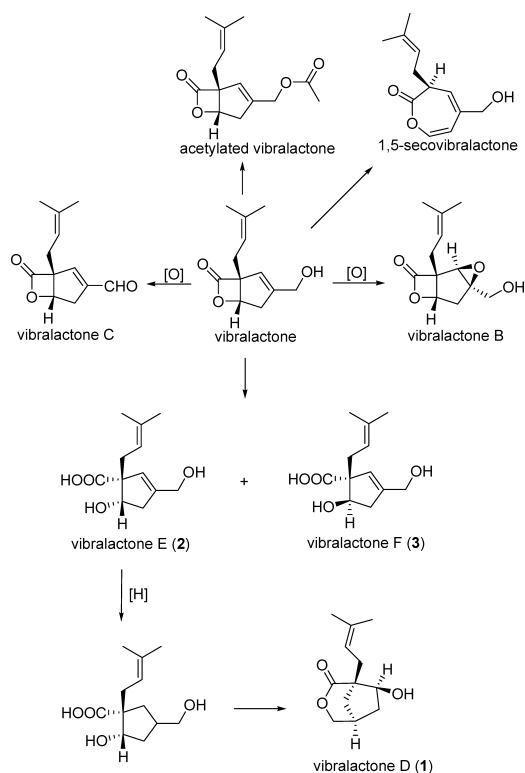


Chart 1. Plausible Biogenetic Synthetic Pathway of Vibralactones A–F

3) showed weak activity (43.6% and 31.2% inhibition at 150 $\mu\text{g/ml}$) against human $11\beta\text{-HSD1}$ and (37.7% and 24.8% inhibition at 150 $\mu\text{g/ml}$) mouse $11\beta\text{-HSD1}$.

Experimental

General Experimental Procedures Melting points were measured on an XRC-1 apparatus (Sichuan University, Sichuan, P. R. China). Optical rotations were obtained with a Horiba SEPA-300 digital polarimeter. IR spectra were recorded on a Bruker Tensor-27 spectrometer. NMR spectra were recorded on Bruker AV-400 and DRX-500 instruments. Mass spectra were recorded on a VG Autospec-3000 mass spectrometer and an API QSTAR pulsar 1 spectrometer. Column chromatography and TLC were carried out on Silica gel (200–300 mesh) and precoated silica gel GF254 plates (Qingdao Marine Chemical Inc., P. R. China).

Fungal Material and Cultivation *B. vibrans* was provided and fermented by Da-Gan Ji, Kunming Institute of Botany. A voucher specimen was deposited in the Herbarium of Kunming Institute of Botany, Chinese Academy of Sciences. The culture medium consisted of: potato (peeled) 200.0 g; glucose 20.0 g; KH_2PO_4 3.0 g; MgSO_4 1.5 g; citric acid 0.1 g; and thiamin hydrochloride 10 mg in 1 l of deionized water (pH 6.5 before autoclaving). The fungus was grown in Erlenmeyer flasks (500 ml with 300 ml of medium). Fermentation was carried out in a rotary shaker at 22 °C and 150 rpm for 14 d.

Extraction and Isolation The whole culture broth (21 l) of *B. vibrans* was extracted three times with EtOAc (20 l) after filtration. The organic layer was concentrated under reduced pressure to give a crude extract (6.0 g). The residue was subjected to column chromatography over silica gel (200–300 mesh, 3×45 cm), eluted with a petroleum ether/EtOAc gradient, to afford fractions A–F. Fraction D eluted with petroleum ether/EtOAc (4:1) was further purified on a Sephadex LH-20 ($\text{CHCl}_3/\text{MeOH}$, 1:1) and a silica gel column ($\text{CHCl}_3/\text{MeOH}$, 8:1) to give **1** (17.0 mg). Fraction F eluted with EtOAc was further separated on Sephadex LH-20 (MeOH) and repeated reversed-phased RP₁₈ (MeOH/ H_2O) column chromatography. Subsequently, **3** (33.0 mg) and **2** (35.6 mg) were obtained from 25% and 30% MeOH/ H_2O , respectively.

Biological Testing The inhibition activity of compounds on human or mouse $11\beta\text{-HSD1}$ and $11\beta\text{-HSD2}$ enzymatic activities was determined in the scintillation proximity assay (SPA) using microsomes containing $11\beta\text{-HSD1}$ or $11\beta\text{-HSD2}$ as described in previous studies.⁴⁾ Briefly, the full-

length cDNAs of human or murine $11\beta\text{-HSD1}$ and $11\beta\text{-HSD2}$ were isolated from the cDNA libraries provided by the NIH Mammalian Gene Collection and cloned into a pcDNA3 expression vector. HEK-293 cells were transfected with the pcDNA3-derived expression plasmid and selected after cultivation in the presence of 700 $\mu\text{g/ml}$ of G418. The microsomal fraction over-expressing $11\beta\text{-HSD1}$ or $11\beta\text{-HSD2}$ was prepared from the HEK-293 cells stably transfected with either $11\beta\text{-HSD1}$ or $11\beta\text{-HSD2}$ and used as the enzyme source for Scintillation Proximity Assay (SPA). Microsomes containing human or mouse $11\beta\text{-HSD1}$ were incubated with NADPH and [^3H]cortisone, and then the product [^3H]cortisol was specifically captured by a monoclonal antibody coupled to protein A-coated SPA beads. $11\beta\text{-HSD2}$ screening was performed by incubating $11\beta\text{-HSD2}$ microsomes with [^3H]cortisol and NAD⁺ and monitoring substrate disappearance. IC_{50} (\pm S.D., $n=3$) values were calculated using Prism Version 4 (GraphPad Software) with glycyrrhizinic acid as a positive control.

Vibrallactone D (1): Colorless crystal; mp 131–132 °C; $[\alpha]_{\text{D}}^{21} +24.2$ ($c=0.11$, CHCl_3). IR (KBr) cm^{-1} : 3504, 2957, 2904, 1714, 1639, 1450, 1401, 1290, 1195. $^1\text{H-NMR}$ ($\text{CDCl}_3/\text{CD}_3\text{OD}$ 5:1, 500 MHz) and $^{13}\text{C-NMR}$ ($\text{CDCl}_3/\text{CD}_3\text{OD}$ 5:1, 125 MHz): see Table 1. Positive FAB-MS m/z : 211 $[\text{M}+\text{H}]^+$. HR-ESI-MS m/z : 211.1330 $[\text{M}+\text{H}]^+$ (Calcd for $\text{C}_{12}\text{H}_{19}\text{O}_3$, 211.1334).

X-Ray Crystallography of 1 $\text{C}_{12}\text{H}_{18}\text{O}_3$, MW=210, monoclinic, space group $P2_1$ (1), $a=7.9353(16)$ Å, $b=6.1933(12)$ Å, $c=11.6715(2)$ Å, $V=573.3(2)$ Å³, $Z=1$, $d=1.212$ g/cm³. $\lambda=0.71073$ Å, $\mu(\text{MoK}\alpha)=0.086$ mm⁻¹, $F(000)=226.0$, $T=298(2)$ K. A colorless crystal of dimensions $0.27 \times 0.19 \times 0.04$ mm was selected for X-ray analysis. A total of 3660 reflections, collected in the range $1.75^\circ \leq \theta \leq 28.30^\circ$, yielded 2480 unique reflections. The structure was solved using direct methods and was refined with the full-matrix least-squares on F^2 values for 2404 $I > 2\sigma(I)$. Hydrogen atoms were fixed at calculated positions. The final indices were $R_1=0.0550$ and $wR_2=0.1117$ with goodness-of-fit=1.029. Crystallographic data for structure **1** have been deposited at the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC 742975. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, U.K. (fax: +44 1223 336033; e-mail: deposit@ccdc.cam.ac.uk).

Vibrallactone E (2): Colorless oil; $[\alpha]_{\text{D}}^{26} +61.7$ ($c=0.41$, MeOH). IR (KBr) cm^{-1} : 3421, 2925, 1708, 1641, 1439, 1380, 1025. $^1\text{H-NMR}$ (CD_3OD , 500 MHz) and $^{13}\text{C-NMR}$ (125 MHz): see Table 1. Negative FAB-MS m/z : 225 $[\text{M}-\text{H}]^-$. HR-ESI-MS m/z : 225.1133 $[\text{M}-\text{H}]^-$ (Calcd for $\text{C}_{12}\text{H}_{17}\text{O}_4$, 225.1126).

Vibrallactone F (3): Colorless oil; $[\alpha]_{\text{D}}^{28} -53.2$ ($c=0.46$, MeOH). IR (KBr) cm^{-1} : 3424, 2919, 1703, 1450, 1383, 1028. $^1\text{H-NMR}$ (CD_3OD , 500 MHz) and $^{13}\text{C-NMR}$ (125 MHz): see Table 1. Negative FAB-MS m/z : 225 $[\text{M}-\text{H}]^-$. HR-ESI-MS m/z : 225.1124 $[\text{M}-\text{H}]^-$ (Calcd for $\text{C}_{12}\text{H}_{17}\text{O}_4$, 225.1126).

MTPA Esters of Compounds 1–3 A mixture of **1** (4.3 mg), (*S*)-MTPA (39.3 mg), 1,3-dicyclohexylcarbodiimide (DCC, 39.7 mg), and 4-(dimethylamino)pyridine (DMAP, 7.0 mg) was dissolved in 3 ml of dry CH_2Cl_2 and stirred at room temperature for 10 h. The reaction mixture was filtered, and the concentrated filtrate was chromatographed over silica gel (eluted with CHCl_3) to yield the purified Mosher ester of **1a** (5.0 mg). The methyl esters after reaction with trimethylsilyldiazomethane (TMSCHN_2) were prepared from **2** and **3** using a procedure published in the literature.⁵⁾ The other MTPA esters were prepared in the same manner as **1a** and characterized by measurement of their $^1\text{H-NMR}$ spectroscopic data in CDCl_3 .

(S)-MTPA Ester of 1 (1a): $^1\text{H-NMR}$ (500 MHz, CDCl_3) δ : 7.53–7.55 (2H, m, MTPA-ArH), 7.39–7.41 (3H, m, MTPA-ArH), 5.23 (1H, dd, $J=10.1, 4.1$ Hz, H-5), 5.15 (1H, dd, $J=8.2, 6.9$ Hz, H-9), 4.31 (1H, ddd, $J=10.5, 3.2, 1.4$ Hz, H-13a), 4.06 (1H, d, $J=10.5$ Hz, H-13b), 3.56 (3H, s, OCH_3), 2.62 (1H, m, H-4a), 2.55 (1H, dd, $J=14.4, 6.9$ Hz, H-8a), 2.44 (1H, dd, $J=14.4, 8.2$ Hz, H-8b), 2.43 (1H, m, H-3), 1.93 (1H, dd, $J=12.6, 2.5$ Hz, H-2a), 1.87 (1H, ddd, $J=12.6, 5.0, 1.8$ Hz, H-2b), 1.71 (3H, s, H-12), 1.59 (3H, s, H-11), 1.53 (1H, m, H-4b).

(R)-MTPA Ester of 1 (1b): $^1\text{H-NMR}$ (500 MHz, CDCl_3) δ : 7.54 (2H, m, MTPA-ArH), 7.40–7.42 (3H, m, MTPA-ArH), 5.13 (1H, dd, $J=10.1, 4.6$ Hz, H-5), 5.11 (1H, dd, $J=8.2, 6.9$ Hz, H-9), 4.36 (1H, dd, $J=10.5, 2.7$ Hz, H-13a), 4.18 (1H, d, $J=10.5$ Hz, H-13b), 3.50 (3H, s, OCH_3), 2.72 (1H, m, H-4a), 2.47 (1H, m, H-3), 2.46 (1H, dd, $J=14.6, 6.9$ Hz, H-8a), 2.39 (1H, dd, $J=14.6, 8.2$ Hz, H-8b), 1.93 (1H, d, $J=14.5$ Hz, H-2a), 1.87 (1H, m, H-2b), 1.71 (1H, m, H-4b), 1.70 (3H, s, H-12), 1.54 (3H, s, H-11).

Bis[(S)-MTPA] Ester of 2 (2a): $^1\text{H-NMR}$ (500 MHz, CDCl_3) δ : 7.49 (2H, m, MTPA-ArH), 7.46 (2H, m, MTPA-ArH), 7.39–7.43 (6H, m, MTPA-ArH), 5.81 (1H, s, H-2), 5.40 (1H, dd, $J=6.0, 2.5$ Hz, H-5), 4.95 (1H, dd, $J=8.1, 6.9$ Hz, H-9), 4.83 (2H, s, H-13), 3.51 (3H, s, OCH_3), 3.49 (3H, s,

OCH₃), 3.43 (3H, s, OCH₃), 2.88 (1H, dd, $J=17.5, 6.0$ Hz, H-4a), 2.58 (1H, dd, $J=14.0, 6.9$ Hz, H-8a), 2.35 (1H, dd, $J=17.5, 2.5$ Hz, H-4b), 2.30 (1H, dd, $J=14.0, 8.1$ Hz, H-8b), 1.66 (3H, s, H-12), 1.55 (3H, s, H-11).

Bis[(*R*)-MTPA] Ester of **2** (**2b**): ¹H-NMR (500 MHz, CDCl₃) δ : 7.49 (2H, m, MTPA-ArH), 7.47 (2H, m, MTPA-ArH), 7.37—7.43 (6H, m, MTPA-ArH), 5.87 (1H, s, H-2), 5.35 (1H, dd, $J=5.7, 1.9$ Hz, H-5), 4.94 (1H, d, $J=13.3$ Hz, H-13a), 4.93 (1H, dd, $J=8.0, 7.1$ Hz, H-9), 4.81 (1H, d, $J=13.3$ Hz, H-13b), 3.54 (3H, s, OCH₃), 3.43 (3H, s, OCH₃), 3.28 (3H, s, OCH₃), 2.83 (1H, ddd, $J=17.0, 5.7, 6.0$ Hz, H-4a), 2.54 (1H, dd, $J=14.0, 7.1$ Hz, H-8a), 2.42 (1H, dd, $J=17.0, 1.9$ Hz, H-4b), 2.25 (1H, dd, $J=14.0, 8.0$ Hz, H-8b), 1.61 (3H, s, H-12), 1.54 (3H, s, H-11).

Bis[(*S*)-MTPA] Ester of **3** (**3a**): ¹H-NMR (500 MHz, CDCl₃) δ : 7.49—7.54 (4H, m, MTPA-ArH), 7.39—7.41 (6H, m, MTPA-ArH), 5.77 (1H, dd, $J=7.0, 5.2$ Hz, H-5), 5.74 (1H, s, H-2), 4.89 (1H, d, $J=13.5$ Hz, H-13a), 4.82 (1H, dd, $J=8.0, 6.8$ Hz, H-9), 4.77 (1H, d, $J=13.5$ Hz, H-13b), 3.65 (3H, s, OCH₃), 3.54 (3H, s, OCH₃), 3.52 (3H, s, OCH₃), 2.84 (1H, dd, $J=16.1, 7.0$ Hz, H-4a), 2.36 (1H, dd, $J=16.1, 5.2$ Hz, H-4b), 2.32 (1H, dd, $J=14.4, 6.8$ Hz, H-8a), 2.22 (1H, dd, $J=14.4, 8.0$ Hz, H-8b), 1.56 (3H, s, H-12), 1.40 (3H, s, H-11).

Bis[(*R*)-MTPA] Ester of **3** (**3b**): ¹H-NMR (500 MHz, CDCl₃) δ : 7.55 (2H, m, MTPA-ArH), 7.49 (2H, m, MTPA-ArH), 7.41 (4H, m, MTPA-ArH), 7.39 (2H, m, MTPA-ArH), 5.76 (1H, s, H-2), 5.72 (1H, dd, $J=7.7, 6.1$ Hz, H-5),

4.86 (1H, dd, $J=7.7, 7.2$ Hz, H-9), 4.84 (1H, d, $J=13.8$ Hz, H-13a), 4.76 (3H, d, $J=13.8$ Hz, H-13b), 3.69 (3H, s, OCH₃), 3.55 (3H, s, OCH₃), 3.53 (3H, s, OCH₃), 2.88 (1H, dd, $J=16.2, 7.7$ Hz, H-4a), 2.45 (1H, dd, $J=14.4, 7.2$ Hz, H-8a), 2.28 (1H, dd, $J=14.4, 7.7$ Hz, H-8b), 2.22 (1H, dd, $J=16.2, 6.1$ Hz, H-4b), 1.57 (3H, s, H-12), 1.43 (3H, s, H-11).

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