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Structural corrections of photinides A, B and their novel derivatives

Ryoko Yasumura [a], Kazuaki Tanaka [a], Tatsuo Nehira [b], Masaru Hashimoto [a,*]

[a] Department of Agriculture and Bioscience, Hirosaki University, 3-Bunkyo-cho, Hirosaki 036-8561, Japan
[b] Graduate School of Integrated Arts and Sciences, Hiroshima University, 1-7-1, Kagamiyama, Higashi-Hiroshima 739-8521, Japan

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ABSTRACT

Stereochemistries of the C2═C8 double bond in photinides A and B were corrected to be reversed forms. The present studies also revised their absolute configurations by comparing the CD spectra of the degradation product with the corresponding synthetic sample. *Discosia* sp. SH 125 produced novel derivatives photinides X and Y, of which stereochemistry was established by NMR and CD analyses as well as theoretical calculations of the CD spectra.

1. Introduction

In the course of our studies exploring metabolites from fungi with unique ecologies in northern Japan,[1–4] we found that *Discosia* sp. SH 125 produces photinides A and B, which had been reported by Che et al. in 2009. Our studies revealed structural errors in their C2═C8 double bonds as well as the absolute configurations to revise their structures as shown in Fig. 1. We also isolated their diastereomers photinides X (**3**) and Y (**4**) as their novel derivatives from the same source. Combination analyses of the CD spectral comparisons of **1** with **3** and their theoretical calculations led us to establish their absolute configurations. This paper reports the detail of these studies.

In our screening with *Cochlibolus miyabeanus*,[5] we found a potent growth inhibition in the EtOAc extracts from the culture broth of *Discosia* sp. SH 125. This fungus had been isolated by Tanaka, one of the present authors, from fallen leaves of *Castanea crenata* at Aomori prefecture, Japan in 2003. Chromatographic separation provided a mixture of photinides A, B, X, and Y (**1**–**4**, 650 mg) from 2 L of the culture broth. The ODS HPLC employing LiChrospher® RP-18e (4.0ϕ×250 mm) separated them (**1**: t_R=37 min, **3**: t_R=40 min, **4**, t_R=43 min, and **2**: t_R=56 min) by eluting with MeOH/H_2O (30:70, 1.0 mL/min flow). Other conditions (other column, eluents etc.) remarkably reduced the separation efficiency, so far we examined.[6] Due to such separation difficulty and their instability as described later, only part of the crude sample was subjected to the HPLC purification, and used for structural and biological studies.

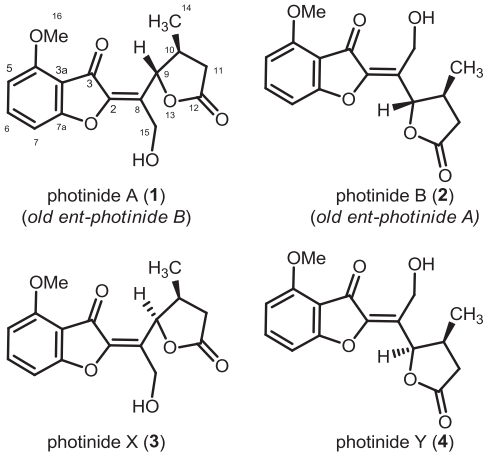

Fig. 1. Structures of photinides A, B, X and Y, as well as the old structures of photinides A and B.

2. Stereochemical corrections of photinide A and B

The ^{1}H and ^{13}C NMR data of **1** and **2** (Table 1) showed very good accordance with those of Che's photinides A and B,[7] respectively, in acetone-d_6. Our samples gave the protonated ions (**1**: *m*/*z* 305.1022, **2**: *m*/*z* 305.1029) in the ESI-MS, while Che reported sodium adduct

* Corresponding author. E-mail address: hmasaru@cc.hirosaki-u.ac.jp (M. Hashimoto).

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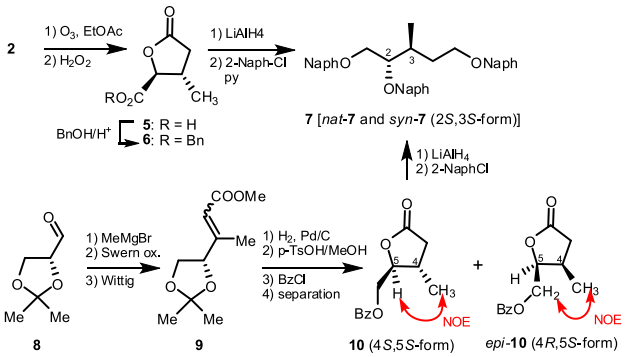
Table 1
^{1}H and ^{13}C NMR data for photinides A (**1**), B (**2**), X (**3**), and Y (**4**) in acetone-d_6

Position	Photinide A (**1**)		Photinide B (**2**)		Photinide X (**3**)		Photinide Y (**4**)	
	δ_{1H} (J in Hz)	δ_{13C}	δ_{1H} (J in Hz)	δ_{13C}	δ_{1H} (J in Hz)	δ_{13C}	δ_{1H} (J in Hz)	δ_{13C}
2		146.5		146.8		145.7		145.9
3		182.4		182.4		182.4		182.2
3a		112.3		112.2		112.4		112.2
4		159.7		159.8		159.6		159.8
5	6.79 (d, 8.3)	106.5	6.79 (d, 8.3)	106.6	6.78 (d, 8.3)	106.4	6.80 (d, 8.2)	106.6
6	7.68 (t, 8.3)	140.3	7.69 (t, 8.3)	140.4	7.67 (t, 8.3)	140.2	7.69 (t, 8.2)	140.3
7	6.84 (d, 8.3)	105.1	6.83 (d, 8.3)	105.1	6.85 (d, 8.3)	105.2	6.83 (d, 8.2)	105.1
7a		167.2		167.2		167.5		167.0
8		128.7		129.6		129.3		130.2
9	6.19 (d, 7.3)	80.5	5.28 (d, 7.2)	83.1	6.42 (d, 8.7)	78.9	5.74 (d, 8.1)	80.6
10	2.86 (m)	37.5	2.82 (m)	37.0	3.13 (m)	36.0	3.12 (m)	36.5
11	2.30 (dd, 8.4, 17.0)	37.2	2.33 (dd, 9.1 17.0)	36.9	2.45 (dd, 8.9, 17.2)	34.3	2.44 (dd, 7.6, 17.0)	34.8
	2.77 (dd, 8.5, 17.0)		2.79 (dd, 8.6, 17.0)		2.65 (dd, 9.2, 17.2)		2.74 (dd, 8.8, 17.0)	
12		176.8		176.7		177.4		176.9
14	1.21 (d, 6.5)	18.0	1.23 (d, 6.6)	17.7	0.98 (d, 7.1)	15.6	1.05 (d, 7.1)	15.5
15	4.40 (d, 12.6)	56.6	4.39 (d, 13.0)	54.4	4.34 (d, 12.0)	56.5	4.36 (d, 13.5)	53.4
	4.69 (d, 12.6)		5.11 (d, 13.0)		4.69 (d, 12.6)		5.10 (d, 13.5)	
16	3.95 (s)	56.6	3.96 (s)	56.6	3.95 (s)	56.6	3.96 (s)	56.7

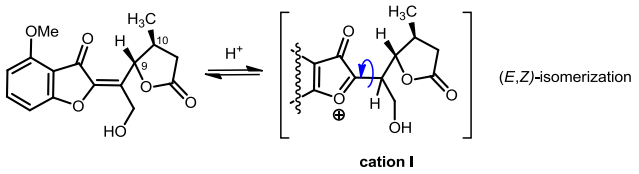
ions ([M+Na]$^{+}$) by the same ionization method. However, both indicated the same molecular formula $C_{16}H_{16}O_6$ for these molecules. Our own analyses involving 2D HMBC and HSQC spectra of these molecules consisted well with Che's photinides A and B except for the stereochemistry at C2=C8 double bonds. In spite of a (*Z*)-stereochemistry for **1** in Che's report, we concluded (*E*)-form based on the characteristically high frequency chemical shift of the C9H resonance (6.19 ppm), compare to that of **2** (5.28 ppm). We assumed that carbonyl oxygen at C3 carbonyl withdraws the electrons of the spatially close C9H, which magnetically deshields C9H of **1**.[8] However, Che considered a shielding effect by the C3 carbonyl to explain this high frequency shift. Since our assignment contradicted their conclusion, we further studied that with their theoretical chemical shifts.[9,10] Prior to these calculations, conformational searches for both **1** and **2** were performed using semi-empirical AM1 method on Spartan10[11] to find 12 and 14 stable conformers, respectively. Each conformer was precisely equilibrated using the EDF2/6-31G* density functional model[12] and then subjected to chemical shifts calculations with the same theory. Application of this scheme has been known to reproduce a wide range of experimental chemical shifts.[13] Theoretical chemical shifts were given after correction by Boltzmann distributions. As expected, the C9H of **1** localizes geometrically close position to the C3 carbonyl in all stable conformers within 10 kcal/mol steric energy from the global minimum conformation. These calculations suggested the C9H chemical shift of **1** to be 6.42 ppm, while that of **2** was estimated to be 5.12 ppm. These results accorded with our assignment as described. The experimental ^{13}C signal for the C9 of **1** (80.5 ppm) appeared 2.6 ppm lower frequency than that of **2** (83.1 ppm), while the C15 of **1** (56.6 ppm) resonated at 2.2 ppm higher frequency than that of **2** (54.4 ppm). These differences can be explained by steric compression effect[14,15] by the C3 carbonyl group. Calculations also reproduced those well [C9: 78.5 ppm (**1**), 80.3 ppm (**2**), $\Delta\delta$ −2.3 ppm, C15: 60.1 ppm (**1**), 57.2 (**2**) $\Delta\delta$ 2.9 ppm]. As described, the relative structure of photinides A and B should be revised as shown Fig. 1.

We next investigated their absolute sterochemistry. Che judged (9*R*)-configuration for both their photinides A and B by observing a negative Cotton effect at 210 and 219 nm, respectively, in the CD spectra. In fact, we also observed the effect. However their conclusion involves ambiguity because of only a few examples reported for their conclusion.[16,17] Thus, we determined it by chemical correlations. When **2** was oxidized with ozone, furanonecarboxylic acid **5** was obtained after oxidative work up. Since **5** was hardly detected by TLC, it was isolated in a form of benzyl ester **6** (2.5 mg) by heating with benzyl alcohol in the presence of *p*-toluenesulfonic acid. We sometimes experience low reliability in specific rotation values in cases of small quantity of sample and/or small $[\alpha]_D$ value. Since only small amount of the **6** was available, we investigated that by CD spectra. With expecting increment of the CD intensity,[18] **6** was converted into tris-*O*-(2-naphthoyl) derivative *nat*-**7** by $LiAlH_4$ reduction followed by treatment with 2-naphthoyl chloride in pyridine. Strong UV absorption of 2-naphthoyl group was also helpful in the preparative TLC. As expected, *nat*-**7** provided a typical splitting positive Cotton effect [$\Delta\varepsilon$ +118 (241 nm), −80 (227 nm), see supplemental data] with reliable quality. Then we synthesized *syn*-**7** (2*S*,3*S*-form) from acetone D-glyceraldehyde **8**[19] as shown in Scheme 1. Although **10** (4*S*,5*S*-form) was obtained along with its (4*R*,5*S*)-diastereomer *epi*-**10** in almost 1:1 ratio, these could be separated by silica gel chromatography.[20] Stereochemistry of **10** was established by observing a characteristic NOE between the C4-methyl and the C5-methine protons. The isomer *epi*-**10** showed no corresponding NOE between those protons but gave that between C4-methyl and C5-methylene protons, which consisted with above assignments. The ^{1}H NMR spectra of *nat*-**7** and *syn*-**7** were identical to correlate their relative stereochemistry. It was found that *syn*-**7** gave quite similar CD profile [$\Delta\varepsilon$ +88 (241 nm), −63 (228 nm)] to that of *nat*-**7**. The $|\Delta\varepsilon|$ values of *syn*-**7** were slightly smaller than those of *nat*-**7**. It could be explained by partial racemization in the Wittig reaction giving **9**, because the reaction required three days for completion to allow that. The (2*S*,3*R*)-isomer of **7**, prepared with *epi*-**10**, showed considerably different ^{1}H NMR spectra from that of *nat*-**7**, which led us to undoubtedly establish the (*Z*,9*S*,10*S*)-configuration for **2**.



Scheme 1. Preparations of *nat*-**7** and *syn*-**7** (2*S*,3*S*-form).

Since **1** could be isolated in smaller amount (3.5 mg) than **2** (14.3 mg) due to separation efficiency in the HPLC, **1** could not be applied to configurational determination in the similar manner. However, it was determined as (*E*,9*S*,10*S*)-form as described below. Readily isomerization was observed between **1** and **2**, for example, in acetone-d_6. This interconversion became faster in $CDCl_3$ and in aqueous CH_3CN solution containing 0.1% TFA after HPLC purification.[21] These suggested that protonation on the C2=C8 double bond generates cation **I**, which allows the bond rotation as shown in Scheme 2. The solvent $CDCl_3$ is gradually decomposed to result HCl, which would catalyze the isomerization even it was trace amount. However, this mechanism does not induce epimerization at C9 nor C10. These led us conclude the same configuration for the C9–C14 furanone moiety of **2**. As described, these experiments revised both relative and absolute stereochemistry of old photinides A and B by Che et al.



Scheme 2. Plausible isomerization mechanism.

3. Structure of photinide X (3) and Y (4)

Discosia sp. SH 125 also produces photinide X (**3**) and Y (**4**) as the minor components. Similarly to **1** and **2**, these were separable but gradually interconverted each other. Thus, we needed to complete the separation within a day. Also due to separation difficulty, only analytical samples could be prepared (2.1 and 1.6 mg, respectively). These molecules showed the same molecular formulas ($C_{16}H_{16}O_6$) as both **1** and **2**. The ^1H and ^{13}C NMR profiles of **3** and **4** (Table 1) showed considerable differences for the C9–C14 furanone moieties from those of **1** and **2**, respectively, but very resembled for other parts. These suggested that **3** and **4** are diastereomers about the furanone moiety but have the same 2-methylenebenzofuran-3(2*H*)-one substructure, *id est* (*E*,9*R**,10*S**)-form for **3** and (*Z*,9*R**,10*S**)-form for **4**. The relative stereochemistry of the C9–C14 furanone part was confirmed by employing **3**. Irradiation of C9H in **3** induced a characteristic NOE at C10H, while **1** gave the NOE between C9H and $C14H_3$.

Absolute chemistries of these minor components were investigated by combination analyses of molecular modeling and CD spectra. Conformational search was performed for **3** by the same scheme as those for **1** as described. When the most stable conformers of **1** [being established to be (9*S*,10*S*)] and (9*S*,10*R*)-enantiomer of **3** were overlaid, the chromophores (O1–C8 2-methylenebenzofuranone moiety, and C12 carbonyl) in **3** nearly coincided with those in **1**, as shown in Fig. 2. Conformational searches also provided other stable conformers for both **1** and **3**. In each case, these were conformational isomers due UV unabsorbent C4OMe and C15OH groups but showed no considerable conformational difference in their UV absorbent frameworks. Since geometrical relationship of chromophores affect in CD,[22] these isomers should provide the similar CD spectra and not disturb above discussions. Contrary to these assumption, **1** and **3** gave CD spectra in nearly inverse relationship, suggesting that **3** is the mirror image, (9*R*,10*S*)-enantiomer. This was verified by their theoretical CD spectra with DFT B3LYP with 6-31+G* basis set followed by spectral correction based on the Boltzmann distributions of the conformers. As shown in Fig. 3, these calculations reproduced the experimental CD spectra of both **1** and **3**. Compounds **3** and **4** were also readily isomerized each other. The similar discussion led the same configuration for the C9–C14 furanone moieties of **3** and **4**. Accordingly stereochemistry of **3** and **4** were concluded to be (*E*,9*R*,10*S*)- and (*Z*,9*R*,10*S*)-forms, respectively. Since C9H is expected be relatively acidic because of C3 C=O group, interconversion might be possible between **1** and **3**. However, we have not observed that so far.

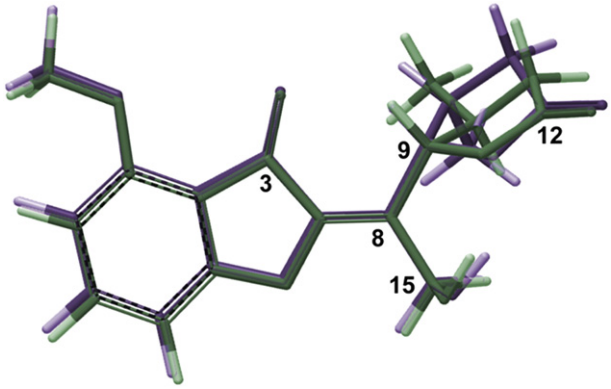


Fig. 2. Overlaid the most stable conformers of **1** [(*E*,9*S*,10*S*)-form, green] and (9*S*,10*R*)-enantiomer of **3** (purple) by DFT EDF2 with 6-31+G*.

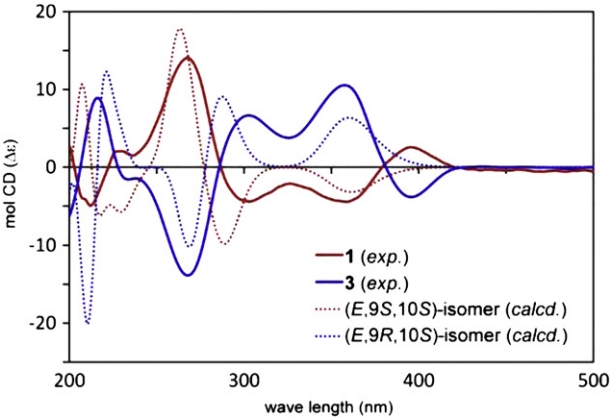


Fig. 3. Experimental and calculated CD spectra of **1** and **3**.

4. Biological assay

Finally their biological activity was studied to disclose that photinide A, B and X (**1**, **2**, and **3**) showed potent growth inhibition against *C. miyabeanus* with 10 μg/mL (IC_{50}), whereas photinide Y (**4**) showed considerably weaker activity (IC_{50} 100 μg/mol).

5. Conclusion

As described, we investigated structures of **1** and **2**. Comparisons of the experimental and theoretical NMR chemical shifts led us to revise their stereochemistry of C2=C8 double bonds. Absolute chemistry of old photinides A and B by Che was also corrected by CD spectral correlation between the degradation product and the synthetic sample. We also isolated **3** and **4** as the minor analogues. Spectral analyses disclosed that these are C9-epimers of **1** and **2**. Although their configurations were not determined directly, those could be disclosed by a combination of conformational and CD spectral comparisons between **1** and **3**. Theoretical CD calculations confirmed them.

6. Material, methods, and experiments

6.1. General

Ultraviolet (UV) spectra were obtained by a HITACHI U-2010 spectrometer. Circular dichroism (CD) spectra were measured on a JASCO J-725 spectropolarimeter. NMR spectra were recorded on a JEOL JNM-ECX500 spectrometer. In the case acetone-d_6 was used, CHD_2COCD_3 (^{1}H: 2.03 ppm) and $^{13}CD_3COCD_3$ (^{13}C: 29.9 ppm) were employed as the standards. In the cases $CDCl_3$ was used, tetramethylsilane (0.0 ppm for ^{1}H and ^{13}C NMR) was used as the internal standard. Mass spectra were measured by electrospray-ionization (ESI) mass spectrometry on a HITACHI NanoFrontier LD spectrometer.

6.2. Fungus

Discosia sp. SH 125 was collected from fallen leaves of *C. crenata* Aomori Prefecture, Japan in 2003, and the fungal isolate was deposited at the National Institute of Agrobiological Sciences, Japan as MAFF 242783.

6.3. Calculations

Conformational searches and chemical shift calculations were performed with Spartan 10 (Wavefunction, Irvine, CA) using a hand-made PC (operating System: Windows7 Professional, CPU: AMD Phenom(tm) X3 945 processor 3.00 GHz, RAM 8 GB). Theoretical CD spectra were calculated with Gaussian 09 (Revision A.02 by Gaussian, Wallingford, CT) with a PC (Operating System: CentOS a Linux, CPU: 2 Intel Xeon 3 5550 processors 2.67 GHz, RAM 24 GB).

6.4. Isolation

Discosia sp. was cultured in potato-sucrose medium (200 mL in 500 mL baffled Erlenmeyer flask×10) on a rotary shaker (110 rpm) at 25.8 °C for 10 days. After filtration in suction, the filtrate was concentrated bellow 25 °C under reduced pressure until the whole volume became 1.0 L. The resulting solution was extracted with EtOAc (500 mL×2) and the organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. Silica gel column chromatography of the residue eluting with 0, 1, and 3% acetone/chloroform and the fraction eluted with 1% acetone/chloroform was recovered and concentrated to give a mixture of photinides A–D (650 mg). Part of the mixture was subjected to HPLC [LiChrospher® RP-18e (4.0ϕ×250 mm) MeOH/H_2O (30:70), 1.0 mL/min flow] to give photinide A (1, 3.5 mg, t_R 37 min), photinide B (2, 14.3 mg, t_R 56 min), photinide X (3, 2.1 mg, t_R 40 min) and photinide Y (4, 1.6 mg, t_R 43 min) (photinide D).

*6.4.1. Photinide A (**1**)* UV (2.6×10^{-3} mg/mL in CH_3CN, nm) 218 (ε 12,500), 271 (ε 11,000), 309 (ε 3600), 377 (ε 4600). IR (NaCl) 3450, 2850, 1780, 1610, 1495, 1090 cm^{-1}. The CD spectrum (8.6×10^{-5} mol/L in CH_3CN) and NMR spectral data (acetone-d_6) are shown in Fig. 3 and Table 1. The ^{1}H and ^{13}C NMR data showed good accordance with those photinide A in Che's report.[7] ESIMS (rel int, %) *m*/*z* 305.1022 (85, calcd for $C_{16}H_{17}O_6$ $[M+H]^+$: 305.1025), 287.0910 (100, calcd for $C_{16}H_{15}O_5^+$, $[M+H-H_2O]^+$: 287.0914).

*6.4.2. Photinide B (**2**)* UV (5.9×10^{-3} mg/mL in CH_3CN, nm) 218 (ε 14,000), 271 (ε 11,000), 309 (ε 3600), 377 (ε 5700). IR (NaCl) 3450, 2970, 1780, 1490, 1260, 1090 cm^{-1}. CD (5.9×10^{-3} mol/L in CH_3CN, nm) 219 ($\Delta\varepsilon$ −2.4), 271 ($\Delta\varepsilon$ +1.3), 327($\Delta\varepsilon$ 0), 385 ($\Delta\varepsilon$ +1.0). The NMR spectral data (acetone-d_6) are shown in Table 1. The ^{1}H and ^{13}C NMR data showed good accordance with those of photinide B in Che's report.[7] ESIMS (rel int, %) *m*/*z* 305.1029 (85, calcd for $C_{16}H_{17}O_6$ $[M+H]^+$: 305.1025), 287.0910 (100, calcd for $C_{16}H_{15}O_5^+$, $[M+H-H_2O]^+$: 287.0914).

*6.4.3. Photinide X (**3**)* UV (1.5×10^{-3} mg/mL, CH_3CN, nm) 218 (ε 13,000), 272 (ε 11,000), 304 (ε 3900), 367 (ε 4900). IR (NaCl) 3440 (br), 2925, 1775, 1610, 1260 cm^{-1}. The CD (5.1×10^{-5} mol/L, CH_3CN) and NMR spectral data (acetone-d_6) are shown in Fig. 3 and Table 1, respectively. ESIMS (rel int, %) *m*/*z* 305.1033 (60, calcd for $C_{16}H_{17}O_6$ $[M+H]^+$: 305.1019), 287.0927 (100, calcd for $C_{16}H_{15}O_5^+$, $[M+H-H_2O]^+$: 287.0914).

*6.4.4. Photinide Y (**4**)* UV (1.4×10^{-3} mg/mL, CH_3CN, nm) 218 nm (ε 15,000), 271 (ε 11,000), 307 (ε 3500), 366 (ε 4300). IR (NaCl) 3400, 2925, 1780, 1610, 1260 cm^{-1}. CD (4.6×10^{-5} mol/L, CH_3CN) 213 nm ($\Delta\varepsilon$ +1.5), 273 nm ($\Delta\varepsilon$ −2.0), 332 nm ($\Delta\varepsilon$ +0.7), 362 nm ($\Delta\varepsilon$ +1.7). The CD spectrum of this sample may not be not reliable due to the impurity. The NMR spectral data (acetone-d_6) are shown in Table 1. ESIMS (rel int, %) *m*/*z* 305.1025 (15, calcd for $C_{16}H_{17}O_6$ $[M+H]^+$: 305.1019), 287.0923 (100, calcd for $C_{16}H_{15}O_5^+$, $[M+H-H_2O]^+$: 287.0914).

6.5. Preparation of tris(2-naphthoyl)ester of (2*S*,3*S*)-3-methylpentane-1,2,5-triol (*nat*-7) from 2

*6.5.1. (2S,3S)-Benzyl 3-methyl-5-oxotetrahydrofuran-2-carboxylate (**6**)* Ozone was bubbled to a solution of **2** (10 mg, 33 μmol) in MeOH (8.0 mL) at −78 °C and the mixture was stirred at the same temperature for 30 min. Aqueous H_2O_2 solution (30%, 50 μL) was added in order to decompose the ozonide, the mixture was further stirred at room temperature for additional 30 min. After the mixture was diluted with H_2O (20 mL), methanol was removed by rotary evaporator to obtain the aqueous solution. It was extracted with EtOAc (50 mL) and the organic layer was washed with 1.0 M aqueous HCl solution (30 mL). The organic solution was dried over $MgSO_4$, and then concentrated under reduced pressure. The residue was dissolved in benzyl alcohol (100 μL) and benzene (1.0 mL). The solution was stirred with *p*-TsOH (3.0 mg) at 90 °C for 10 h. After Et_3N (50 μL) was added, the mixture was concentrated and the resulting oily material was further concentrated by bulb to bulb distillation. Silica gel column chromatography of the residue with EtOAc/benzene (3:97) gave **6** containing benzyl alcohol as the impurity (total 2.5 mg) ^{1}H NMR ($CDCl_3$) δ 1.24 (3H, d, *J*=6.9 Hz), 2.14 (1H, dd, *J*=5.6, 17.4 Hz), 2.63 (1H, m), 2.74 (1H, dd, *J*=8.4, 17.4 Hz), 4.50 (1H, d, *J*=4.8 Hz), 5.22 and 5.23 (each 1H, *d* (AB), *J*=12.3 Hz), 7.35 (aromatic protons), Other than above signals, benzyl proton (4.67 ppm, s, 2H proportion) was observed. Irradiation of the doublet methyl at 1.25 ppm induced NOEs at the signals of 2.15, 2.63, 4.52 ppm. LC-ESIMS *m*/*z* 273.0533 (25, calcd for $C_{13}H_{14}O_4K$, $[M+K]^+$: 273.0529), 257.0796 (35, calcd for $C_{13}H_{14}O_4Na$, $[M+Na]^+$: 257.0790), 252.1239 (100, calcd for $C_{13}H_{18}O_4N$, $[M+NH_4]^+$: 252.1236), 235.0972 (20, calcd for $C_{13}H_{15}O_4$, $[M+H]^+$: 235.0970).

*6.5.2. Tris-O-(2-naphthoyl) ester of (2S,3S)-3-methylpentane-1,2,5-triol (nat-**7**)* The obtained benzyl ester **6** containing benzyl alcohol (2.5 mg) was stirred with $LiAlH_4$ (3.0 mg, 79 μmol) in THF (2.0 mL) at 0 °C for 1 h. Saturated aqueous NH_4Cl solution (two drops) and Celite (ca. 50 mg) were added and the mixture was further stirred at room temperature for 30 min. After filtration through Celite pad, the filtrate was concentrated in vacuo. Silica gel column chromatography of the residue with MeOH/EtOAc (3:97) gave the triol (ca. 2.0 mg). ^{1}H NMR ($CDCl_3$) δ 0.88 (3H, d, *J*=6.8 Hz), 1.5–1.7 (3H, m), 3.46 (2H, m), 3.61 (1H, ddd, *J*=4.5, 7.7, 10.9 Hz), 3.67 (1H, dd, *J*=2.1, 9.9 Hz), 3.75 (1H, ddd. *J*=4.5, 6.6, 10.9 Hz). The obtained triol (2.0 mg, 15 μmol) was stirred with 2-naphthoyl chloride (14 mg, 75 μmol) in pyridine (1.0 mL) at room temperature for 12 h in the dark. MeOH (100 μL) was added to decompose

the excess reagent. The mixture was poured into saturated aqueous $NaHCO_3$ solution (20 mL) and extracted ether (20 mL×3). Combined ethereal solution was washed with brine, dried over $MgSO_4$, and then concentrated in vacuo. Preparative TLC of the residue with EtOAc/hexane (20:80) gave *nat*-**7** (4.0 mg, 6.7×10^{-3} mmol). CD (6.0 μmol/μL, MeOH) $\Delta\varepsilon$ +118 (241 nm), −82 (227 nm)]. IR (NaCl) 2920, 2850, 1715, 1280, 1195 cm^{-1}. ^1H NMR 1.26 (3H, d, *J*=6.9 Hz), 1.86, 2.28, and 2.43 (each 1H, m), 4.51 (1H, ddd, *J*=6.1, 8.2, 11.0 Hz), 4.58 (1H, ddd, *J*=5.5, 6.7, 11.0 Hz), 4.64 (1H, dd, *J*=7.2, 11.9 Hz), 4.78 (1H, dd, *J*=3.6, 11.9 Hz), 5.61 (1H, ddd, *J*=3.6, 6.2, 7.2 Hz), 7.49 (3H, m), 7.52 (3H, m), 7.73–7.98 (9H), 7.95, 8.02, and 8.07 (each 1H, dd, *J*=1.7, 8.6 Hz), 8.50, 8.57, 8.62 (each 1H, br s). ESIMS (rel int, %) *m*/*z* 614.2526 (100, calcd for $C_{39}H_{36}O_6N$, $[M+NH_4]^+$: 614.2537), 619.2084 (35, calcd for $C_{39}H_{32}O_6Na$, $[M+Na]^+$: 619.2091), 597.2265 (10, calcd for $C_{39}H_{33}O_6$, $[M+H]^+$: 597.2272).

6.6. Preparation of *syn*-7 (2*S*,3*S*-form)

*6.6.1. (S)-Methyl 3-(2,2-dimethyl-1,3-dioxolan-4-yl)but-2-enoate (**9**)* Crude acetone D-glyceraldehyde, prepared from 1,2,5,6-di-*O*-isopropylidene-D-mannitol (500 mg, 1.90 mmol) by $NaIO_4$ oxidation under standard conditions,[19] was stirred with methylmagesium bromide (0.99 M in THF, 6.5 mL) in THF (10 mL) at 0 °C for 2 h. The mixture was poured into water and extracted with ether (20 mL×3). Combined ethereal solution was washed with brine (30 mL), dried over $MgSO_4$ and the concentrated under reduced pressure. Silica gel column chromatography of the residue with EtOAc/hexane (12:88) gave the alcohol (458 mg, 3.13 mmol, 82% in two steps) as 2:1 diastereomeric mixture about the methylcarbinol moiety. IR (NaCl) 3450, 2990, 2890, 1370, 1066 cm^{-1}. ^1H NMR (the sample was composed of diastereomers. $CDCl_3$) δ 1.08 (3H×0.33 (for minor) d, *J*=6.2 Hz), 1.10 (3H×0.67 (for major), d, *J*=6.4 Hz), 1.30 and 1.37 (each 3H, br s) 3.62 (1H×0.33 (for minor), dd, *J*=6.3, 8.2 Hz), 3.85–3.97 (4H×0.67 (for major) and 3H×0.33 (for minor)). The obtained alcohol (200 mg, 1.37 mmol) was oxidized under standard Swern's condition[23] in CH_2Cl_2 (15 mL) employing dimethylsulfoxide (500 μL), oxalyl chloride (240 μL), and Et_3N (2.0 mL) to give the methyl ketone (190 mg, 96%) after silica gel column chromatography. IR (NaCl) 2985, 1720, 1460, 1380, 1065 cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.36, 1.46, and 2.22 (each 3H, s), 3.97 (1H, dd, *J*=5.6, 8.7 Hz), 4.17 (1H, dd, *J*=7.8, 8.7 Hz), 4.38 (1H, dd, *J*=5.6, 7.8 Hz). Whole amount of the ketone thus obtained was dissolved in MeOH (6.0 mL) and stirred with methyl (triphenylphosphoranylidene) acetate (670 mg, 2.8 mmol) at room temperature for 3 days. After concentration, the residue was purified with silica gel column chromatography (EtOAc/hexane 4:96) to give the (*Z*)-isomer of 9 (112 mg, 42%) and the (*E*)-isomer of 9 (94.0 mg, 36%).

6.6.1.1. Physical data for the (E)-isomer. $[\alpha]_D$ +27 (*c* 1.0, $CHCl_3$). IR (NaCl) 2990, 2930, 1720, 1660, 1465, 1230 cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.39, 1.44 (each 3H, s), 2.08 (3H, d, *J*=1.0 Hz), 3.62 (1H, dd, *J*=7.9, 8.1 Hz), 3.69 (3H, s), 4.19 (1H, dd, *J*=6.9, 8.1 Hz), 4.50 (1H, dd, *J*=8.0, 7.9 Hz), 6.02 (quint, *J*=1.0 Hz). (*E*)-Stereochemistry for the double bond was established by a comparison of the experimental and the theoretical chemical shift for the oxymethine proton (exp.: 4.50 ppm, calcd 4.46 ppm). Calculations were performed in the similar manner as described in the text. The ESI method did not ionize this sample.

6.6.1.2. Physical data for the (Z)-isomer. $[\alpha]_D$ +49 (*c* 1.0, $CHCl_3$). IR (NaCl) 2990, 2850, 1720, 1450, 1160 cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.37, 1.46 (each 3H, s), 1.97 (3H, d, *J*=1.0 Hz), 3.54 (1H, dd, *J*=6.7, 8.1 Hz), 3.66 (3H, s), 4.37 (1H, dd, *J*=7.5, 8.1 Hz), 5.70 (1H, br t, *J*=8 Hz), 5.74 (1H, quint, *J*=1.0 Hz). (*Z*)-Stereochemistry for the double bond was established by a comparison of experimental and theoretical chemical shift for the oxymethine proton (exp.: 5.70 ppm, calcd 5.70 ppm). Calculations were performed in the similar manner as described in the text. The ESI method did not ionize this sample.

*6.6.2. (4S,5S)-5-Benzoyloxymethyl-3,4-dihydro-3-methyl-furanone (**10**) and its (4R)-isomer(epi-**10**)* (*Z*)-Isomer of **9** (30 mg, 150 μmol) was stirred vigorously with 10% Pd/C (7.0 mg) in MeOH (2.0 mL) under H_2 atmosphere (5.0 atm) at room temperature for 12 h. The mixture was filtered and the filtrate was concentrated in vacuo. The residue was diluted with MeOH (2.0 mL) again and stirred with *p*-TsOH (2.0 mg) at room temperature for 12 h. After Et_3N (two drop) was added to neutralize the mixture, then it was concentrated under reduced pressure. The residue was stirred with benzoyl chloride (100 mL) in pyridine (1.0 mL) at room temperature for 12 h. MeOH was added to destroy the excess reagent. The mixture was poured into saturated aqueous $NaHCO_3$ solution (20 mL) and then extracted with ether (20 mL×2). The combined organic solution was washed with brine (20 mL), dried over $MgSO_4$, and then concentrated in vacuo. Silica gel column chromatography of the residue (EtOAc/hexane=80:20) gave a mixture of 10 [(4*S*,5*S*)-form] and *epi*-**10** [(4*R*,5*S*)-form] (28 mg, 79%). Employing part of the mixture (14 mg), preparative silica gel TLC (EtOAc/hexane=40:60) was performed to provide 10 (6.0 mg, R_f 0.5) and *epi*-**10** (7.0 mg, R_f 0.45). The reaction employing the (*E*)-**9** gave the same products in the almost same diastereomeric ratio.

*6.6.2.1. Physical data for **10** [(4S,5S)-form].* $[\alpha]_D$ +50 (*c* 1.0, $CHCl_3$). IR (NaCl) 2970, 1720, 1470, 1270, 1120 cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.89 (3H, d, *J*=7.0 Hz), 2.20 (1H, dd, *J*=8.7, 17.5 Hz), 2.43 (1H, m), 2.72 (1H, dd, *J*=8.4, 17.5 Hz), 4.32 (1H, ddd, *J*=3.1, 5.4, 8.3 Hz), 4.39 (1H, dd, *J*=5.4, 12.2 Hz), 4.51 (1H, dd, *J*=3.1, 12.2 Hz), 7.39 (2H, br t, *J*=8 Hz), 7.52 (1H, br t, *J*=8 Hz), 7.97 (2H, br d, *J*=8 Hz). Irradiation of the methyl doublet (1.89 ppm) induced an NOE at the oxymethine proton (4.32 ppm). ESIMS (rel int, %) *m*/*z* 257.0809 (40, calcd for $C_{13}H_{14}O_4Na$, $[M+Na]^+$: 257.0784), 252.1254 (30, calcd for $C_{13}H_{18}O_4N$, $[M+NH_4]^+$: 252.1230), 235.0989 (100, calcd for $C_{13}H_{15}O_4$, $[M+H]^+$: 235.0965).

*6.6.2.2. Physical data for epi-**10** [(4R,5S)-form].* $[\alpha]_D$ +71 (*c* 1.0, $CHCl_3$). IR (NaCl) 2970, 2850, 1790, 1450, 1270, 1110 cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.15 (3H, d, *J*=6.7 Hz), 2.34 (1H, dd, *J*=7.0, 17.5 Hz), 2.70 (1H, dd, *J*=8.3, 17.5 Hz), 2.83 (1H, m), 4.45 (1H, dd, *J*=5.5, 12.6 Hz), 4.55 (1H, dd, *J*=3.5, 12.6 Hz), 4.78 (1H, ddd, *J*=3.5, 5.5, 7.0 Hz), 7.43 (2H, br t, *J*=8 Hz), 7.56 (1H, br t, *J*=8 Hz), 7.99 (2H, br d, *J*=8 Hz). Irradiation of the methyl doublet signal (1.19 ppm) induced an NOE at the oxymethylene protons (4.41 and 4.51 ppm). ESIMS (rel int, %) *m*/*z* 257.0799 (45, calcd for $C_{13}H_{14}O_4Na$, $[M+Na]^+$: 257.0784), 252.1245 (25, calcd for $C_{13}H_{18}O_4N$, $[M+NH_4]^+$: 252.1230), 235.0977 (100, calcd for $C_{13}H_{15}O_4$, $[M+H]^+$: 235.0965).

*6.6.3. Tris(2-naphthoyl) ester of (2S,3S)-3-methylpentane-1,2,5-triol (syn-**7**)* Benzoyl ester **10** (40 mg, 171 μmol) in THF (1.0 mL) was stirred with $LiAlH_4$ (5.0 mg) at 0 °C for 30 min. The work up and purification were performed in the similar manner as the described in Section 6.5.2 to give the triol (20 mg, 147 μmol). The ^1H NMR spectrum of the crude sample was identical with that from natural sample. Triol thus obtained (20 mg, 147 μmol) was stirred with 2-naphthoyl chloride (140 mg, 750 μmol) and pyridine (1.5 mL) in the similar manner as described in the Section 6.5.2 to give *syn*-**7** (8.0 mg, 20%) after silica gel column chromatography. Chromatographic behavior and the ^1H NMR spectrum of *syn*-**7** were identical with that of *nat*-**7**. CD (9.6×10^{-3} mmol/mL, MeOH) $\Delta\varepsilon$ +88 (241 nm), −63 (228 nm). IR (NaCl) 3060, 2925, 2850, 1715, 1280, 1195 cm^{-1}. The ^1H NMR spectrum of this sample was identical to that from natural *nat*-**7**. ESIMS (rel int, %) *m*/*z* 597.2275 (15, calcd for $C_{39}H_{33}O_6$, $[M+H]^+$: 597.2271), 614.2545 (100, calcd for $C_{39}H_{36}O_6N$,

$[M+NH_4]^+$: 614.2537), 619.2095 (50, calcd for $C_{39}H_{33}O_6Na$, $[M+Na]^+$: 619.2097).

*6.6.3.1. (2S,3R)-isomer of syn-**7**.* The (2*S*,3*R*)-isomer *epi*-**10** (40 mg, 170 μmol) obtained in Section 6.6.2.2 was also reduced with $LiAlH_4$ (5.0 mg) in THF (1.0 mL) and then 2-naphthylated with 2-naphthoyl chloride (140 mg, 750 μmol) and pyridine (1.5 mL) in the same manner as described in Section 6.6.3 to afford (2*S*,3*R*)-isomer of 7 (10 mg). CD (7.2×10^{-3} mmol/mL, MeOH) $\Delta\varepsilon$ +56 (242 nm), −32 (228 nm). IR (NaCl) 3060, 2920, 1715, 1280, 1190 cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.29 (3H, d, *J*=6.9 Hz), 1.84 (1H, m), 2.21 (1H, m), 2.41 (1H, m), 4.53 (1H, ddd, *J*=5.9, 6.4, 11.2 Hz), 4.57 (1H, dd, *J*=6.1, 11.2 Hz), 4.68 (1H, dd, *J*=7.3, 11.8 Hz), 4.73 (1H, dd, *J*=4.7, 11.8 Hz), 5.71 (1H, ddd, *J*=4.3, 4.6, 7.3 Hz), 7.41–7.58 (6H), 7.71–7.97 (9H), 7.94, 7.96, 8.06 (each 1H, dd, *J*=1.7, 8.6 Hz), 8.51, 8.52, 8.61 (each 1H, s). ESIMS (rel int, %) *m*/*z* 597.2259 (5, calcd for $C_{39}H_{33}O_6$, $[M+H]^+$: 597.2271), 614.2526 (100, calcd for $C_{39}H_{36}O_6N$, $[M+NH_4]^+$: 614.2537), 619.2078 (55, calcd for $C_{39}H_{33}O_6Na$, $[M+Na]^+$: 619.2097).

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Supplementary data

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