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ORIGINAL ARTICLE

Total synthesis of amidepsine B and revision of its absolute configuration

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The total synthesis of (−)-amidepsine B, a potent diacylglycerol acyltransferase inhibitor, has been achieved. This synthetic study resulted in the revision of the previously assigned stereostructure of the natural amidepsine B and determined the absolute configuration.
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INTRODUCTION

Amidepsines A–E were isolated from the culture broth of fungal strains FO-2942 and FO-5969 and found to be inhibitors of diacylglycerol acyltransferase (DGAT),[1–3] which is exclusively involved in triacylglycerol formation. Excessive accumulation of triacylglycerol can cause fatty liver, obesity and hypertriglyceridemia, which leads to serious diseases, such as atherosclerosis, diabetes and metabolic disorders. Therefore, DGAT inhibitors have the potential to become drugs. Spectroscopic analyses of the amidepsines elucidated depsipeptide structures consisting of three 4,6-dihydroxy-2-methylbenzoic acid derivatives and an amino acid (except for amidepsine D), as shown in Figure 1. The DGAT inhibitory activity of the amidepsines was tested by a cellular assay using Raji cells, and the results showed that amidepsine B (**1**) was the most potent inhibitor. Amidepsine B (**1**) was previously determined to be a mixture of stereoisomers at its single chiral center, the alanine alpha carbon. A 3:2 mixture of L- and D-alanines was revealed by acid hydrolysis followed by HPLC analyses using a chiral column (Amidepsines A and C were also reported as a 3:2 mixture of L- and D-amino acids[2]). Herein, the total synthesis of amidepsine B and a revision of its absolute configuration will be described.

RESULTS AND DISCUSSION

The synthesis used a known aldehyde **2**, which is a common starting material for the preparation of key substrates **5** and **10** (scheme 1).[4] Regioselective *p*-*O*-methylation (In this reaction, reactivity of the hydroxy group adjacent to the formyl group in **3** would be precluded by the formation of an intramolecular hydrogen bond. Similar reactivity was observed.[5]) followed by benzylation gave **4** quantitatively, which was treated with $NaClO_2$ to afford carboxylic acid **5** quantitatively. Furthermore, similar conversion of **2** to **8**, including the formation of a *p*-*O*-methoxymethyl ether instead of methylation, was also accomplished quantitatively. Esterification of **8** with allyl bromide followed by deprotection of the methoxymethyl ether afforded **10** quantitatively.

Next, the key substrates **5** and **10** were coupled by treatment with trifluoroacetic anhydride (TFAA) to give rise to **11** quantitatively. Deprotection of the allyl ester in the presence of a palladium catalyst gave carboxylic acid **12** in 98% yield.

Subsequent esterification of **12** with **10** was troublesome. Esterification conditions, such as TFAA, 1-[(3-dimethylamino)propyl]-3-ethylcarbodiimide/dimethylaminopyridine (DMAP) and benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate (BOP)/Et_3N/DMAP led to a low yield of the desired product **14** with side products, such as the anhydride of **12**. Therefore, we next focused on esterification through the acid fluoride.[6] Reaction of **12** with (diethylamino)sulfur trifluoride (DAST) proceeded in 89% yield to furnish the stable acid fluoride **13**. Treatment of **13** with sodium alkoxide, derived from **10** and sodium hydride, was effected to give the desired allyl ester **14** quantitatively. The allyl ester was then deprotected under palladium-catalyzed conditions to afford **15** quantitatively.

Having constructed the depside **15**, we next turned to condensation with L-alanine before preparing the 3:2 mixture of L- and D-alanines toward the total synthesis (scheme 2). Treatment of **15** with L-alanine benzyl ester in the presence of BOP and Et_3N produced benzyl ester (+)-**16** in 79% yield. Finally, global deprotection by hydrogenolysis completed the total synthesis of (−)-amidepsine B [(−)-**1**] in 83% yield.

The physical properties (^1H and ^{13}C NMR, m.p., IR and MS) of synthetic (−)-amidepsine B (**1**) were completely identical to those of a

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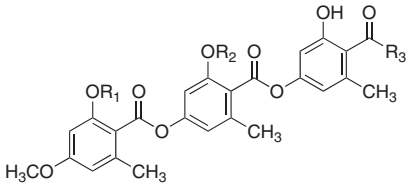
natural sample, and the optical rotation of synthetic (−)-**1** ($[\alpha]_D^{23}$ −17.3°; *c* 0.10, MeOH) also corresponded with that of the authentic sample of (**1**) ($[\alpha]_D^{25}$ −16°; *c* 0.1, MeOH). This result triggered the need for the re-assignment of the absolute configuration of natural amidepsine B (**1**). According to the procedure shown above, (+)-amidepsine B [(+)-**1**] by condensation of **15** with D-alanine was also synthesized.



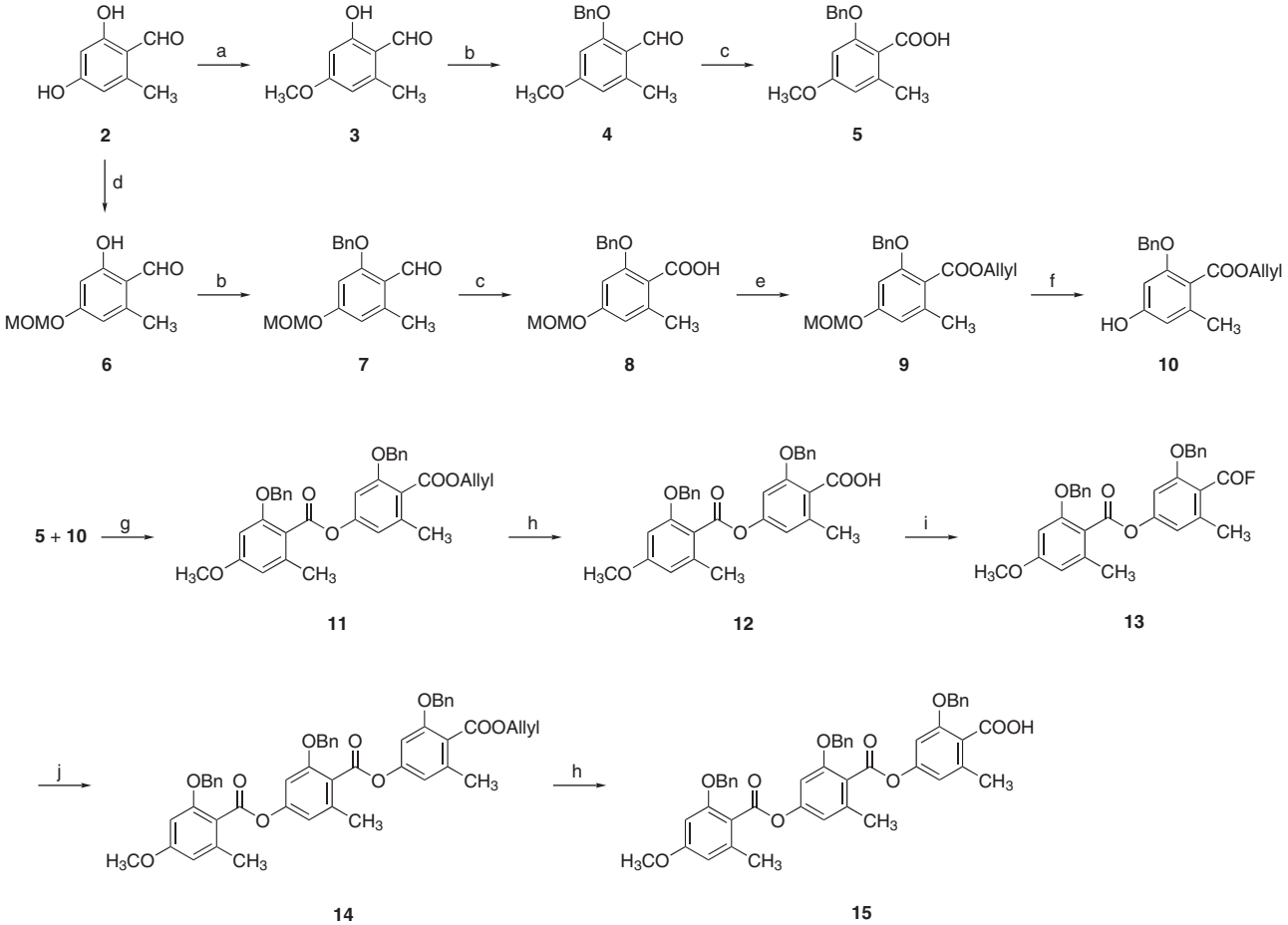
Amidepsine	A	B	C	D	E
R_1	Me	H	H	Me	Me
R_2	H	H	H	H	Me
R_3	alanine**	L-alanine*	valine**	OH	alanine

Figure 1 Structure of amidepsines A–E. *The original isomeric mixture reported for amidepsine B has been corrected by this work. **The actual configurations of amidepsines A and C are now in question.[2]

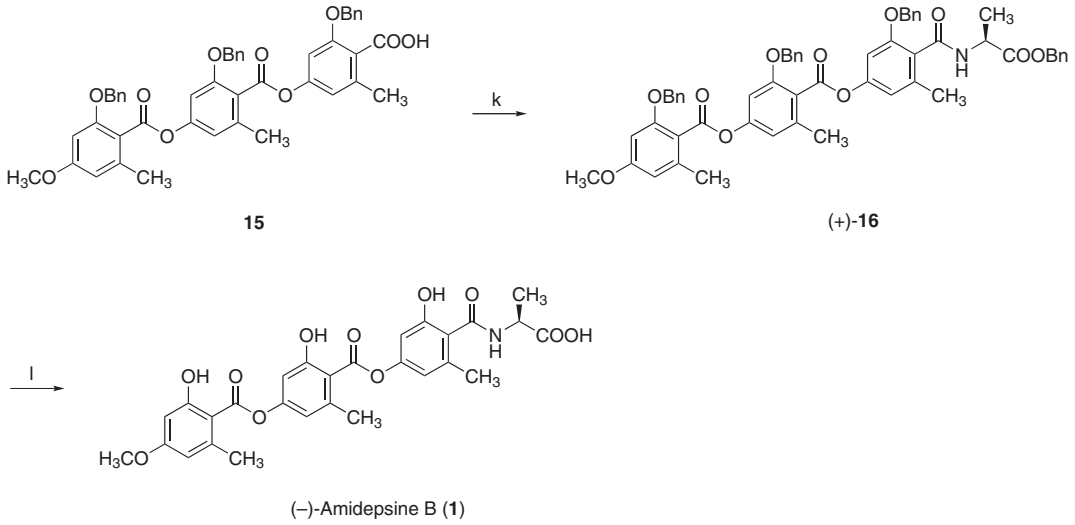
We next analyzed the synthetic amidepsines (+)-**1** and (−)-**1** and the natural amidepsine B (**1**) with a chiral HPLC column. When the 1:1 mixture of the synthetic amidepsines (+)-**1** and (−)-**1** was injected onto the chiral HPLC system, each enantiomer was separated completely as shown in Figure 2a. Figures 2b and c show the HPLC analytical data of (+)-**1** and (−)-**1**, respectively. Next, the natural amidepsine B (**1**) was also subjected to HPLC analysis and it was determined that the retention time was identical to that of (−)-amidepsine B [(−)-**1**] as shown in Figure 2d.

This result shows that the absolute configuration of amidepsine B is *S*, derived from L-alanine, and that the previous determination of the absolute configuration of amidepsine B was inaccurate. It is likely that the acid hydrolysis of amidepsine B must have led to the epimerization of the resulting alanine. It is highly likely that amidepsines A and C are also L-amino acids, which will be reported in due course.

In conclusion, we have achieved the total synthesis of amidepsine B. Moreover, we have revised the absolute configuration of natural amidepsine B. Extension of this chemistry to the synthesis of structural analogs of amidepsine B so that the structure–activity



Scheme 1 Synthesis of depside **15**. Reagents and conditions: (a) CH_3I, K_2CO_3, acetone, rt, quant.; (b) Benzyl chloride, K_2CO_3, DMF, rt, quant. for **4** and **7**; (c) $NaClO_2$, 2-methyl-2-butene, $NaH_2PO_4{\cdot}2H_2O$, *t*-BuOH/H_2O, 0°C to rt, quant. for **5** and **8**; (d) Methoxymethyl chloride, *i*-Pr_2NEt, CH_2Cl_2, 0°C to rt, quant.; (e) Allyl Br, NaH, DMF, 0°C to rt, quant.; (f) 6N HCl, MeOH, 60°C, quant.; (g) Trifluoroacetic anhydride, toluene, rt, quant.; (h) HCOOH, $Pd(PPh_3)_4$, Et_3N, THF, 0°C to rt, 98% for **12**, quant. for **15**; (i) (Diethylamino)sulfur trifluoride, Et_2O/CH_2Cl_2, rt, 89%; (j) **10**, NaH, THF, 0°C, then **13**, rt, quant.



Scheme 2 Completion of the total synthesis of (–)-amidepsine B [(–)-**1**]. Reagents and conditions: (k) L-alanine benzyl ester, BOP, Et_3N, DMF, rt, 79%. For (–)-**16**: D-alanine benzyl ester, BOP, Et_3N, DMF, rt, 89%; (l) H_2, $Pd(OH)_2$, THF/EtOH, rt, 83%. For (+)-**1**: 94%.

relationships can be explored is currently under way and will be reported in due course.

EXPERIMENTAL PROCEDURE

Commercially available solvents were dried and distilled before use. Reactions were monitored by TLC using Merck $F60_{254}$ silica gel plates (0.25 mm). Spots were visualized with ultraviolet (UV) light (254 nm) and stained with phosphomolybdic acid. Flash column chromatography was performed on silica gel 60N (spherical, neutral, particle size 40–50 μm). Preparative TLC was performed on Merck $F60_{254}$ silica gel plates (0.50 mm). The chiral HPLC system was comprised of a Daicel-CHIRALPAK IA column (0.46 cm ϕ×25 cm), and a mobile phase of hexane/THF (tetrahydrofuran)/TFAA (80:20:0.1) (Senshu HPLC system; 10 μl injection; UV, 254 nm; flow rate, 1.0 ml min^{-1}).

Melting points were determined on a Yanagimoto micro melting apparatus and uncorrected. Optical rotations were obtained with a JASCO DIP-1000 polarimeter. Mass spectra were recorded on a JEOL JMS-700 mass spectrometer. FT-IR spectra were recorded on a Horiba FT-710 spectrometer. ^{1}H-NMR spectra were recorded on a JEOL JNM-EX270 (270 MHz), MERCURY-300 (300 MHz) and UNITY-400 (400 MHz) spectrometers in $CDCl_3$ or DMSO-d_6. ^{1}H-NMR spectral data are reported according to the following conventions: chemical shifts relative to $CDCl_3$ (7.26 p.p.m.) or DMSO-d_6 (2.48 p.p.m.), multiplicity (s=singlet, d=doublet, t=triplet, q=quartet, m=multiplet, br s=broad singlet, br d=broad doublet and dd=double doublet), coupling constant and integration. ^{13}C-NMR spectral data are reported in p.p.m. relative to $CDCl_3$ (77.0 p.p.m.) or DMSO-d_6 (39.5 p.p.m.).

2-Hydroxy-4-methoxy-6-methylbenzaldehyde (3)

To a solution of 2,4-dihydroxy-6-methylbenzaldehyde (**2**) (0.56 g, 3.68 mmol) in acetone (36.8 ml), K_2CO_3 (0.51 g, 3.68 mmol) and CH_3I (3.44 ml, 55.2 mmol) were added. The reaction mixture was stirred for 2.5 h at room temperature, quenched with H_2O and extracted with EtOAc. The combined organic extracts were dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel with an eluent (20:1 hexanes/EtOAc) to give **3** (0.61 g, quant.) as a white powder; m.p. 65–67 °C; IR (KBr): 3084, 2966, 2906, 2846, 1635, 1576, 1473 and 1429 cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$) δ 12.45 (s, 1H), 10.05 (s, 1H), 6.23 (s, 2H), 3.80 (s, 3H), 2.49 (s, 3H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ 192.8, 166.6, 166.4, 143.7, 113.2, 110.4, 98.5, 55.4 and 18.2; HRMS (FAB, *m*–NBA): calcd. for $C_9H_{11}O_3$: 167.0708 $[M+H]^+$, found: *m/z* 167.0703.

2-Benzyloxy-4-methoxy-6-methylbenzaldehyde (4)

To a solution of **3** (0.60 g, 3.64 mmol) in *N*,*N*-dimethylformamide (DMF) (18.2 ml), K_2CO_3 (5.03 g, 36.4 mmol) and BnCl (6.29 ml, 56.4 mmol) were added at 0 °C. After stirring for 2.5 h at room temperature, the reaction was quenched with H_2O and extracted with CH_2Cl_2. The combined organic extracts were dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel with an eluent (20:1 to 10:1 hexanes/EtOAc) to give **4** (0.93 g, quant.) as a white powder; m.p. 78–80 °C; IR (KBr): 3321, 3091, 3049, 2966, 2925, 2871, 2789, 1666, 1603 and 1448 cm^{-1}; ^{1}H-NMR (270 MHz, $CDCl_3$) δ 10.59 (s, 1H), 7.38 (m, 5H), 6.40 (d, *J*=1.8 Hz, 1H), 6.34 (d, *J*=1.8 Hz, 1H), 5.14 (s, 2H), 3.84 (s, 3H), 2.60 (s, 3H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ 190.4, 164.2, 164.2, 144.6, 136.0, 128.6, 128.1, 127.2, 117.5, 109.1, 97.0, 70.5, 55.3 and 22.3; HRMS (FAB, *m*–NBA): calcd. for $C_{16}H_{17}O_3$: 257.1178 $[M+H]^+$, found: *m/z* 257.1176.

2-Benzyloxy-4-methoxy-6-methylbenzoic acid (5)

To a solution of **4** (0.16 g, 0.61 mmol) in *t*-BuOH/H_2O (1:1) (6.2 ml) 2-methyl-2-butene (0.26 ml, 2.45 mmol), $NaH_2PO_4{\cdot}2H_2O$ (0.29 g, 1.84 mmol) and $NaClO_2$ (0.17 ml, 1.84 mmol) were added at 0 °C. The reaction mixture was stirred for 24 h at room temperature, diluted with H_2O and extracted with CH_2Cl_2. The combined organic extracts were dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel with an eluent (5:1 to 3:1 hexanes/EtOAc) to give **5** (0.18 g, quant.) as a white powder; m.p. 98–100 °C; IR (KBr): 3298, 3022, 2935, 2877, 2837, 1705, 1604 and 1448 cm^{-1}; ^{1}H-NMR (270 MHz, $CDCl_3$) δ 10.22 (br s, 1H), 7.40 (m, 5H), 6.48 (s, 2H), 5.20 (s, 2H), 3.83 (s, 3H), 2.61 (s, 3H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ 169.9, 162.0, 158.4, 142.7, 135.6, 128.7, 128.3, 127.3, 113.5, 108.9, 97.9, 71.3, 55.3 and 22.0; HRMS (FAB, *m*–NBA): calcd. for $C_{16}H_{16}O_4$: 272.1049 $[M]^+$, found: *m/z* 272.1049.

2-Hydroxy-4-methyoxymethoxy-6-methylbenzaldehyde (6)

To a solution of **5** (0.56 g, 3.70 mmol) in CH_2Cl_2 (37.0 ml) *i*-Pr_2NEt (0.71 ml, 4.07 mmol) and chloromethyl methyl ether (MOMCl) (0.56 ml, 7.39 mmol) were added at 0 °C. After stirring for 45 min at room temperature, the reaction was quenched with H_2O and extracted with CH_2Cl_2. The combined organic extracts were dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel with an eluent (30:1 hexanes/EtOAc) to give **6** (0.72 g, quant.) as a white powder; m.p. 46–48 °C; IR (KBr): 3089, 2964, 1631, 1493 and 1431 cm^{-1}; ^{1}H-NMR (270 MHz, $CDCl_3$) δ 12.31 (s, 1H), 10.11 (s, 1H), 6.42 (d, *J*=2.2 Hz, 1H), 6.36 (d, *J*=2.2 Hz, 1H), 5.19 (s, 2H), 3.46 (s, 3H), 2.53 (s, 3H); ^{13}C-NMR

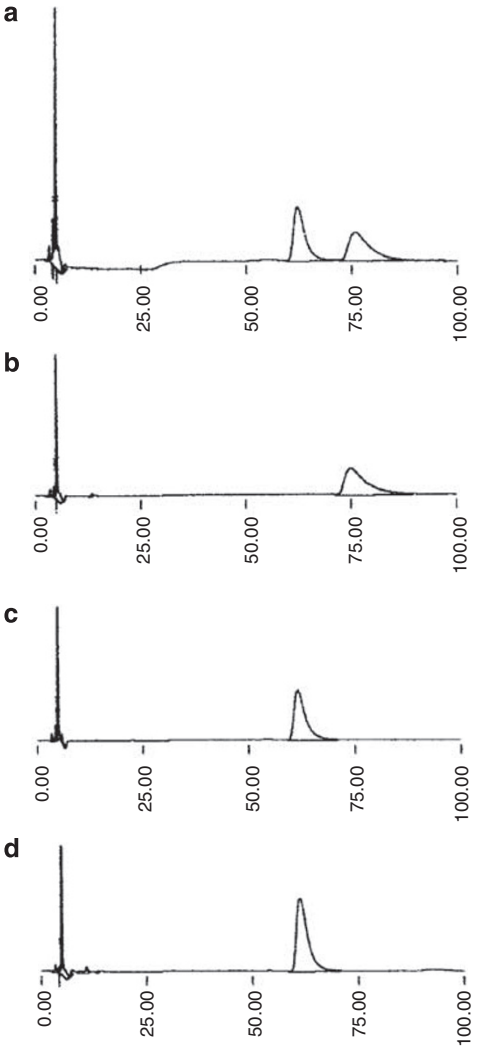


Figure 2 HPLC analysis of natural and synthetic amidepsines B. The abscissa axis indicates retention time. (**a**) a 1:1 mixture of synthetic amidepsines B (+)-**1** and (−)-**1**; (**b**) synthetic amidepsine B [(+)-**1**] (derived from D-alanine); (**c**) synthetic amidepsine B [(−)-**1**] (derived from L-alanine); (**d**) natural amidepsine B.

(75 MHz, $CDCl_3$) δ 193.1, 165.9, 164.1, 144.0, 113.9, 110.9, 101.3, 93.8, 56.3 and 183.3; HRMS (FAB, *m*–NBA): calcd. for $C_{10}H_{13}O_4$: 197.0814 $[M+H]^+$, found: *m/z* 197.0808.

2-Benzyloxy-4-methoxymethoxy-6-methylbenzaldehyde (7)

To a solution of **6** (0.76 g, 3.86 mmol) in DMF (19.3 ml) K_2CO_3 (5.33 g, 38.6 mmol) and BnCl (4.44 ml, 38.6 mmol) were added at 0 °C. After stirring for 45 min at room temperature, the mixture was warmed to 50 °C and stirred for 30 min. The mixture was quenched with H_2O and extracted with CH_2Cl_2. The combined organic extracts were dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel with an eluent (20:1 hexanes/EtOAc) to give **7** (1.09 g, 99%) as a white powder; m.p. 43–46°C; IR (KBr): 2927, 1674, 1601, 1450 and 1415 cm^{-1}; ^{1}H-NMR (270 MHz, $CDCl_3$) δ 10.59 (s, 1H), 7.39 (m, 5H), 6.57 (d, *J*=2.0 Hz, 1H), 6.48 (d, *J*=2.0 Hz, 1H), 5.19 (s, 2H), 5.13 (s, 2H), 3.48 (s, 3H), 2.58 (s, 3H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ 190.6, 164.1, 161.9, 144.5, 135.9, 128.6, 128.1, 127.3, 118.2, 111.3, 98.5, 93.9, 70.5, 56.2 and 22.1; HRMS (FAB, *m*–NBA): calcd. for $C_{17}H_{17}O_4$: 285.1132 $[M-H]^-$, found: *m/z* 285.1119.

2-Benzyloxy-4-methoxymethoxy-6-methylbenzoic acid (8)

To a solution of **7** (0.70 g, 2.45 mmol) in *t*-BuOH/H_2O (1:1) (24.4 ml), 2-methyl-2-butene (1.04 ml, 9.79 mmol), $NaH_2PO_4{\cdot}2H_2O$ (1.10 g, 7.34 mmol) and $NaClO_2$ (0.66 ml, 7.34 mmol) were added at 0 °C. The reaction mixture was stirred for 24 h at room temperature, diluted with H_2O and extracted with CH_2Cl_2. The combined organic extracts were dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel with an eluent (5:1 hexanes/EtOAc) to give **8** (0.74 g, quant.) as a yellow oil. IR (KBr): 3168, 2927, 1697, 1601 and 1448 cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$) δ 9.75 (br s, 1H), 7.41 (m, 5H), 6.59 (d, *J*=2.2 Hz, 1H), 6.58 (d, *J*=2.2 Hz, 1H), 5.17 (s, 2H), 5.16 (s, 2H), 3.47 (s, 3H), 2.51 (s, 3H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ 169.3, 159.6, 158.3, 142.7, 135.5, 128.7, 128.3, 127.4, 114.4, 111.5, 99.4, 94.1,71.4, 56.1 and 21.9; HRMS (FAB, *m*–NBA): calcd. for $C_{17}H_{19}O_5$: 303.1232 $[M+H]^+$, found: *m/z* 303.1244.

2-Benzyloxy-4-methoxymethoxy-6-methylbenzoic acid allyl ester (9)

To a solution of **8** (1.06 g, 3.51 mmol) in DMF (35.1 ml), NaH (0.21 g, 5.27 mmol) and allyl bromide (0.91 ml, 10.5 mmol) were added at 0 °C under N_2. After stirring for 30 min, the reaction mixture was warmed to room temperature and stirred for 1.5 h. The mixture was quenched with H_2O and extracted with EtOAc. The combined organic extracts were dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel with an eluent (15:1 hexanes/EtOAc) to give **9** (1.14 g, quant.) as a colorless oil. IR (KBr): 2945, 1726, 1599 and 1446 cm^{-1}; ^{1}H-NMR (270 MHz, $CDCl_3$) δ 7.33 (m, 5H), 6.50 (d, *J*=2.3 Hz, 2H), 5.95 (m, 1 H), 5.35 (dd, *J*=17.2, 1.5 Hz, 1H), 5.19 (dd, *J*=10.2, 1.5 Hz, 1H), 5.13 (s, 2H), 5.06 (s, 2H), 4.78 (br d, *J*=5.6 Hz, 2H), 3.44 (s, 3H), 2.30 (s, 3H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ 167.7, 158.8, 157.0, 138.2, 136.5, 132.1, 128.3, 127.7, 127.1, 118.4, 117.8, 109.6, 99.1, 94.2, 70.3, 65.6, 55.9 and 19.7; HRMS (FAB, *m*–NBA): calcd. for $C_{20}H_{23}O_5$: 343.1545 $[M+H]^+$, found: *m/z* 343.1556.

2-Benzyloxy-4-hydroxy-6-methylbenzoic acid allyl ester (10)

To a solution of **9** (2.10 g, 6.13 mmol) in CH_3OH (30.5 ml), 6 N HCl (30.5 ml) was added. After stirring for 30 min at 60 °C, the reaction mixture was neutralized with a saturated aqueous $NaHCO_3$ solution and extracted with CH_2Cl_2. The combined organic extracts were dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel with an eluent (10:1 hexanes/EtOAc) to give **10** (1.83 g, quant.) as a yellow oil. IR (KBr): 3381, 3080, 3033, 2937, 2875, 1697, 1603 and 1458 cm^{-1}; ^{1}H-NMR (270 MHz, $CDCl_3$) δ 7.31 (m, 5H), 6.26 (d, *J*=2.0 Hz, 1H), 6.20 (d, *J*=2.0 Hz, 1H), 5.93 (m, 1H), 5.35 (dd, *J*=17.2, 1.3 Hz, 1H), 5.19 (dd, *J*=10.6, 1.3 Hz, 1H), 4.92 (s, 2H), 4.77 (br d, *J*=5.6 Hz, 2H), 2.21 (s, 3H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ 169.0, 158.1, 157.5, 138.6, 136.4, 131.8, 128.3, 127.8, 127.1, 118.7, 115.7, 109.6, 98.2, 70.2, 66.0 and 19.6; HRMS (FAB, *m*–NBA): calcd. for $C_{18}H_{19}O_4$: 299.1283 $[M+H]^+$, found: *m/z* 299.1296.

2-Benzyloxy-4-(2-benzyloxy-4-methoxy-6-methyl) benzoyloxy-6-methylbenzoic acid allyl ester (11)

To a solution of **5** (0.19 g, 0.69 mmol) and **10** (0.17 g, 0.57 mmol) in toluene (2.3 ml), TFAA (0.57 ml) was added. The reaction mixture was stirred for 1 h at room temperature, quenched with H_2O and extracted with EtOAc. The combined organic extracts were dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel with an eluent (40:1 to 20:1 hexanes/EtOAc) to give **11** (0.32 g, quant.) as a white powder; m.p. 122–125 °C; IR (KBr): 2927, 2856, 1738, 1597 and 1437 cm^{-1}; ^{1}H-NMR (270 MHz, $CDCl_3$) δ 7.33 (m, 10H), 6.54 (d, *J*=1.7 Hz, 1H), 6.52 (d, *J*=1.7 Hz, 1H), 6.45 (d, *J*=1.7 Hz, 1H), 6.40 (d, *J*=2.0 Hz, 1H), 5.91 (m, 1H), 5.34 (dd, *J*=17.2, 1.2 Hz, 1H), 5.19 (dd, *J*=10.2, 1.2 Hz, 1H), 5.09 (s, 2H), 4.86 (s, 2H), 4.77 (br d, *J*=5.6 Hz, 2H), 3.82 (s, 3H), 2.43 (s, 3H), 2.26 (s, 3H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ 167.3, 166.4, 161.8, 157.8, 156.6, 152.2, 139.0, 137.9, 136.3, 136.2, 131.9, 128.5, 128.3, 128.1, 127.8, 127.7, 127.3, 121.6, 118.5, 115.8, 115.5, 107.2, 104.2, 97.4, 70.7, 70.5, 65.8, 55.4, 20.0 and 19.4; HRMS (FAB, *m*–NBA+NaI): calcd. for $C_{34}H_{32}O_7Na$: 575.2046 $[M+Na]^+$, found: *m/z* 575.2072.

2-Benzyloxy-4-(2-benzyloxy-4-methoxy-6-methyl) benzoyloxy-6-methylbenzoic acid (12)

To a solution of **11** (0.32 g, 0.58 mmol) in THF (5.8 ml), HCOOH (0.10 ml, 2.90 mmol), Et_3N (0.40 ml, 2.90 mmol) and $Pd(PPh_3)_4$ (34.0 mg, 29.0 μmol) were added at 0 °C. The reaction mixture was stirred for 24 h at room temperature, quenched with H_2O and extracted with EtOAc. The combined organic extracts were dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel with an eluent (1:1 hexanes/EtOAc) to give **12** (0.29 g, 98%) as a white powder; m.p. 155–158 °C; IR (KBr): 3475, 2991, 2958, 2931, 2873, 2659, 2561, 1741, 1691, 1597 and 1442 cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.36 (m, 10H), 6.62 (d, *J*=1.8 Hz, 1H), 6.61 (d, 1H, *J*=1.8 Hz), 6.47 (d, 1H, *J*=1.9 Hz), 6.43 (d, *J*=2.1 Hz, 1H), 5.10 (s, 2H), 4.93 (s, 2H), 3.83 (s, 3H), 2.45 (s, 3H), 2.43 (s, 3H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ 170.1, 166.3, 161.9, 157.9, 157.3, 152.9, 140.7, 139.2, 136.3, 135.5, 128.6, 128.5, 128.2, 128.2, 127.7, 127.4, 119.2, 117.0, 115.3, 107.3, 104.7, 97.4, 71.2, 70.7, 55.4, 20.8 and 20.1; HRMS (FAB, *m*–NBA+NaI): calcd. for $C_{31}H_{28}O_7Na$: 535.1733 $[M+Na]^+$, found: *m/z* 535.1758.

2-Benzyloxy-4-(2-benzyloxy-4-methoxy-6-methyl) benzoyloxy-6-methylbenzoyl fluoride (13)

To a solution of **12** (0.03 g, 0.06 mmol) in Et_2O/CH_2Cl_2 (2:1) (0.9 ml) DAST (18.6 μl, 0.14 mmol) was added. The reaction mixture was stirred for 1.5 h at room temperature, quenched with H_2O and extracted with CH_2Cl_2. The combined organic extracts were dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel with an eluent (25:1 hexanes/EtOAc) to give **13** (27.0 mg, 89%) as a white powder; m.p. 111–114 °C; IR (KBr): 3624, 3458, 3035, 2924, 2852, 1817, 1732, 1593 and 1442 cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.38 (m, 10H), 6.62 (s, 1H), 6.48 (d, *J*=2.0 Hz, 1H), 6.44 (d, *J*=1.9 Hz, 1H), 5.10 (s, 2H), 4.92 (s, 2H), 3.84 (s, 3H), 2.46 (s, 3H), 2.41 (s, 3H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ 166.0, 162.0, 158.9 ($^3J_{CF}$=2.2 Hz), 158.0, 156.6 ($^1J_{CF}$=351.7 Hz), 154.5, 141.8 ($^3J_{CF}$=2.4 Hz), 139.2, 136.2, 135.6, 128.5, 128.2, 128.0, 127.7, 127.1, 116.6 ($^4J_{CF}$=2.4 Hz), 115.0, 114.5 ($^2J_{CF}$=56.2 Hz), 107.3, 104.7, 97.4, 70.7, 70.6, 55.3, 20.5 ($^4J_{CF}$=1.2 Hz), 20.0; HR-MS (FAB, *m*–NBA+NaI): calcd. for $C_{31}H_{27}FO_6Na$: 537.1684 $[M+Na]^+$, found: *m/z* 537.1687.

2-Benzyloxy-4-[2-benzyloxy-4-(2-benzyloxy-4-methoxy-6-methyl) benzoyloxy-6-methyl]benzoyloxy-6-methylbenzoic acid allyl ester (14)

To a solution of **10** (22.0 mg, 0.07 mmol) in THF (0.5 ml), NaH (6.0 mg, 0.15 mmol) was added at 0 °C under N_2. After stirring for 5 min at room temperature, a solution of **13** (26.0 mg, 0.05 mmol) in THF (0.5 ml) was added dropwise. After 30 min, the mixture was treated with MOMCl (3.8 μl, 0.05 mmol) for the conversion of an excess amount of **10** into **9** and then stirred for 30 min. The resulting mixture was quenched with H_2O and extracted with EtOAc. The combined organic extracts were dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel with an eluent (10:1 to 1:1 hexanes/EtOAc) to give **14** (40.0 mg, quant.) as a white powder; m.p. 183–187 °C; IR (KBr): 2925, 2858, 1741, 1597 and 1439 cm^{-1}; ^{1}H-NMR (270 MHz, $CDCl_3$) δ 7.33 (m, 15H), 6.59 (d, *J*=2.0 Hz, 1H), 6.58 (d, *J*=2.0 Hz, 1H), 6.49 (d, *J*=2.1 Hz, 1H), 6.47 (d, *J*=2.0 Hz, 1H), 6.46 (d, *J*=2.1 Hz, 1H), 6.41 (d, *J*=2.0 Hz, 1H), 5.91 (m, 1H), 5.33 (dd, *J*=17.2, 1.5 Hz, 1H), 5.18 (dd, *J*=10.5, 1.5 Hz, 1H), 5.10 (s, 2H), 4.85 (s, 2H), 4.82 (s, 2H), 4.76 (br d, *J*=5.9 Hz, 2H), 3.83 (s, 3H), 2.45 (s, 3H), 2.37 (s, 3H), 2.24 (s, 3H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ 167.3, 166.4, 166.0, 161.9, 157.9, 157.0, 156.6, 152.8, 152.0, 139.1, 138.2, 137.9, 136.3, 136.2, 136.0, 132.0, 128.5, 128.5, 128.4, 128.2, 128.2, 127.9, 127.9, 127.8, 127.3, 121.8, 120.5, 118.6, 116.0, 115.7, 115.4, 107.2, 104.1, 97.4, 70.8, 70.7, 70.5, 65.8, 55.4, 20.1, 19.4 and 19.3; HRMS (FAB, *m*–NBA+NaI): calcd. for $C_{49}H_{44}O_{10}Na$: 815.2832 $[M+Na]^+$, found: *m/z* 815.2806.

2-Benzyloxy-4-[2-benzyloxy-4-(2-benzyloxy-4-methoxy-6-methyl)benzoyloxy-6-methyl]benzoyloxy-6-methylbenzoic acid (15)

To a solution of **14** (40.0 mg, 0.05 mmol) in THF (0.5 ml), HCOOH (9.4 μl, 0.25 mmol), Et_3N (34.8 μl, 0.25 mmol) and $Pd(PPh_3)_4$ (29.0 mg, 3.00 μmol) were added at 0 °C. The reaction mixture was stirred for 24 h at room temperature, quenched with H_2O and extracted with EtOAc. The combined organic extracts were dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel with an eluent (2:1 hexanes/EtOAc) to give **15** (38.0 mg, quant.) as a white powder; m.p. 215–216 °C; IR (KBr): 3446, 2924, 2858, 1745, 1691, 1597 and 1441 cm^{-1}; ^{1}H-NMR (270 MHz, $CDCl_3$) δ 7.40 (m, 15H), 6.60 (br s, 2H), 6.58 (d, *J*=2.0 Hz, 1H), 6.56 (d, *J*=2.1 Hz, 1H), 6.47 (d, *J*=2.2 Hz, 1H), 6.42 (d, *J*=2.3 Hz, 1H), 5.10 (s, 2H), 4.88 (s, 2H), 4.85 (s, 2H), 3.83 (s, 3H), 2.47 (s, 3H), 2.44 (s, 3H), 2.38 (s, 3H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ 167.2, 166.4, 165.9, 161.9, 158.0, 157.5, 157.2, 153.0, 152.9, 142.4, 139.2, 138.4, 136.3, 136.0, 135.1, 128.8, 128.6, 128.6, 128.5, 128.3, 128.2, 128.0, 127.9, 127.7, 120.2, 118.4, 117.6, 116.1, 115.4, 107.3, 104.6, 104.2, 97.5, 71.6, 70.9, 70.8, 55.5, 21.5, 20.1 and 19.5; HRMS (FAB, *m*–NBA+NaI): calcd. for $C_{46}H_{40}O_{10}Na$: 775.2519 $[M+Na]^+$, found: *m/z* 775.2510.

(2*S*)-2{2-Benzyloxy-4-[2-benzyloxy-4-(2-benzyloxy-4-methoxy-6-methyl)benzoyloxy-6-methyl]benzoyloxy-6-methylbenzoy}aminopropionic acid benzyl ester [(+)-(16)]

To a solution of **15** (92.0 mg, 0.12 mmol) in DMF (1.2 ml), L-Ala-OBn · HCl (58.0 mg, 0.27 mmol), Et_3N (68.0 μl, 0.49 mmol) and BOP (135.0 mg, 0.30 mmol) were added. The reaction mixture was stirred for 3.5 h at room temperature, quenched with H_2O and extracted with EtOAc. The combined organic extracts were dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel with an eluent (3:1 to 2:1 hexanes/EtOAc) to give (+)-(**16**) (88.0 mg, 79%) as a white powder; m.p. 179–181 °C; $[\alpha]^{27}_D$ +21.5 (*c* 0.1, $CHCl_3$); IR (KBr): 2925, 1741, 1595 and 1446 cm^{-1}; ^{1}H-NMR (270 MHz, $CDCl_3$) δ 7.38 (m, 20H), 6.61 (br s, 2H), 6.51 (d, *J*=1.3 Hz, 1H), 6.48 (br s, 2H), 6.43 (d, *J*=1.6 Hz, 1H), 6.41 (d, *J*=7.6 Hz, 1H), 5.18 (d, *J*=12.3 Hz, 1H), 5.13 (d, *J*=12.3 Hz, 1H), 5.11 (s, 2H), 4.87 (s, 2H), 4.79 (m, 1H), 4.78 (s, 2H), 3.84 (s, 3H), 2.47 (s, 3H), 2.39 (s, 3H), 2.28 (s, 3H), 1.35 (d, *J*=7.0 Hz, 3H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ 172.5, 166.4, 166.4, 166.2, 161.9, 157.9, 157.1, 156.2, 152.8, 151.8, 139.1, 139.1, 138.3, 136.3, 136.0, 136.0, 135.3, 128.6, 128.6, 128.6, 128.4, 128.4, 128.3, 128.2, 128.2, 128.0, 128.0, 127.8, 127.6, 123.8, 120.5, 116.2, 116.0, 115.4, 107.3, 104.2, 104.0, 97.4, 70.8, 70.8, 70.5, 67.0, 55.4, 48.1, 20.1, 19.5 and 18.3; HRMS (FAB, *m*–NBA): calcd. for $C_{56}H_{52}NO_{11}$: 914.3540 $[M+H]^+$, found: *m/z* 914.3497.

(2*S*)-2{2-Benzyloxy-4-[2-benzyloxy-4-(2-benzyloxy-4-methoxy-6-methyl)benzoyloxy-6-methyl]benzoyloxy-6-methylbenzoy}aminopropionic acid{(−)-Amidepsine B [(−)-1]}

A mixture of (+)-(**16**) (25.1 mg, 0.03 mmol) and $Pd(OH)_2$ (12.2 mg) in THF/EtOH (1:1) (2.8 ml) was stirred under H_2 atmosphere for 16 h. The mixture was filtered through a pad of Celite and the filtrate was concentrated. The residue was purified by preparative TLC on silica gel (5:1 $CHCl_3/CH_3OH$) with an eluent (3:1 $CHCl_3/CH_3OH$) to give (−)-(**1**) as a white powder (13.0 mg, 87%). Moreover, this sample was dissolved in $CHCl_3/CH_3OH$ (25:1) (5 ml) and washed with 1% aqueous H_3PO_4 (1 ml). The organic phase was dried over anhydrous Na_2SO_4 and concentrated *in vacuo* to give (−)-(**1**) (12.4 mg, 83%) as a white powder; m.p. 153–156 °C; $[\alpha]^{29}_D$ –17.3 (*c* 0.1, CH_3OH); IR (KBr): 3087, 2937, 2858, 1668, 1610 and 1454 cm^{-1}; ^{1}H-NMR (270 MHz, $CDCl_3$) δ 10.4 (br s, 1H), 8.44 (d, *J*=6.8 Hz, 1 H), 6.68 (br s, 1H), 6.66 (br s, 1H), 6.56 (br s, 1H), 6.53 (br s, 1H), 6.39 (br s, 1H), 6.36 (br s, 1H), 4.35 (m, 1H), 3.74 (s, 3H), 2.45 (s, 3H), 2.38 (s, 3H), 2.35 (s, 3H), 1.31 (d, *J*=7.1 Hz, 3H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ 174.1, 166.7, 166.4, 165.8, 162.1, 159.1, 156.1, 155.0, 152.0, 150.5, 139.6, 137.8, 137.6, 123.5, 118.4, 114.1, 113.3, 110.7, 108.1, 107.1, 106.4, 99.0, 55.2, 47.6, 20.8, 19.2, 18.8 and 16.9; HRMS (FAB, *m*–NBA): calcd. for $C_{28}H_{26}NO_{11}$: 552.1506 $[M-H]^-$, found: *m/z* 552.1510.

(2*R*)-2{2-Benzyloxy-4-[2-benzyloxy-4-(2-benzyloxy-4-methoxy-6-methyl)benzoyloxy-6-methyl]benzoyloxy-6-methylbenzoy}aminopropionic acid benzyl ester [(−)-(16)]

According to the conversion of **15** into (+)-**16**, **15** (30.0 mg, 0.04 mmol) was subjected to a condensation reaction with D-Ala-OBn·*p*-TsOH (21.8 mg, 0.060 mmol) to afford (−)-(**16**) (32.6 mg, 89 %) as a white powder; m.p. 181–183 °C; $[\alpha]^{23}_D$ –8.7 (*c* 0.1, $CHCl_3$); IR (KBr): 2925, 1741, 1595 and

1446 cm^{-1}; ^{1}H-NMR (270 MHz, $CDCl_3$) δ 7.38 (m, 20H), 6.61 (br s, 2H), 6.51 (d, *J*=1.3 Hz, 1H), 6.48 (br s, 2H), 6.43 (d, *J*=1.6 Hz, 1H), 6.41 (d, *J*=7.6 Hz, 1H), 5.18 (d, *J*=12.3 Hz, 1H), 5.13 (d, *J*=12.3 Hz, 1H), 5.11 (s, 2H), 4.87 (s, 2H), 4.79 (m, 1H), 4.78 (s, 2H), 3.84 (s, 3H), 2.47 (s, 3H), 2.39 (s, 3H), 2.28 (s, 3H), 1.35 (d, *J*=7.0 Hz, 3H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ 172.5, 166.4, 166.4, 166.2, 161.9, 157.9, 157.1, 156.2, 152.8, 151.8, 139.1, 139.1, 138.3, 136.3, 136.0, 136.0, 135.3, 128.6, 128.6, 128.6, 128.4, 128.4, 128.3, 128.2, 128.2, 128.0, 128.0, 127.8, 127.6, 123.8, 120.5, 116.2, 116.0, 115.4, 107.3, 104.2, 104.0, 97.4, 70.8, 70.8, 70.5, 67.0, 55.4, 48.1, 20.1, 19.5 and 18.3; HRMS (FAB, *m*–NBA+NaI): calcd. for $C_{56}H_{51}NO_{11}Na$: 936.3360 $[M+Na]^+$, found: *m*/*z* 936.3383.

(2*R*)-2{2-Benzyloxy-4-[2-benzyloxy-4-(2-benzyloxy-4-methoxy-6-methyl)benzoyloxy-6-methyl]benzoyloxy-6-methylbenzoy}aminopropionic acid{(+)-Amidepsine B [(+)-1]}

According to the conversion of (+)-**16** into (−)-**1**, (−)-(**16**) (30.8 mg, 0.03 mmol) gave (+)-(**1**) (17.7 mg, 94%) as a white powder; m.p. 156–159°C; $[\alpha]^{25}_D$ +10.4 (*c* 0.1, CH_3OH); IR (KBr): 3087, 2937, 2858, 1668, 1610 and 1454 cm^{-1}; ^{1}H-NMR (270 MHz, $CDCl_3$) δ 10.4 (br s, 1H), 8.44 (d, *J*=6.8 Hz, 1H), 6.68 (br s, 1H), 6.66 (br s, 1H), 6.56 (br s, 1H), 6.53 (br s, 1H), 6.39 (br s, 1H), 6.36 (br s, 1H), 4.35 (m, 1H), 3.74 (s, 3H), 2.45 (s, 3H), 2.38 (s, 3H), 2.35 (s, 3H), 1.31 (d, *J*=7.1 Hz, 3H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ 174.1, 166.7, 166.4, 165.8, 162.1, 159.1, 156.1, 155.0, 152.0, 150.5, 139.6, 137.8, 137.6, 123.5, 118.4, 114.1, 113.3, 110.7, 108.1, 107.1, 106.4, 99.0, 55.2, 47.6, 20.8, 19.2, 18.8 and 16.9; HRMS (FAB, *m*–NBA): calcd. for $C_{28}H_{28}NO_{11}$: 554.1662 $[M+H]^+$, found: *m*/*z* 554.1663.

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