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Total syntheses of neuroprotective mastigophorenes A and B

Yoshiyasu Fukuyama,[a,*] Keiji Matsumoto,[a] Yasutoshi Tonoi,[a] Ritsuko Yokoyama,[a] Hironobu Takahashi,[a] Hiroyuki Minami,[a] Hiroshi Okazaki[b] and Yasuhide Mitsumoto[b]

[a]*Faculty of Pharmaceutical Sciences, Institute of Pharmacognosy, Tokushima Bunri University, Yamashiro-cho, Tokushima 770-8514, Japan*

[b]*Tokushima New Drug Research Institute, Otsuka Pharmaceutical Co. Ltd., Tokushima 771-0192, Japan*

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Abstract—(−)-Herbertenediol (**3**) which is regarded as a biosynthetic precursor of mastigophorenes A and B has been effectively synthesized from (*R*)-1,2-dimethyl-2-cyclopentene carboxylic acid by applying an intramolecular Heck reaction to the construction of the quaternary carbon center, and then horseradish peroxidase-catalyzed oxidative coupling of **3** has given rise to (−)-mastigophorenes A and B. Mastigophorenes A and B have been found to exhibit significant neuroprotective activity in primary cultures of fetal rat cortical neurons.

1. Introduction

Mastigophorenes A (**1**) and B (**2**),[1,2] isolated from the Boruneo liverwort *Mastigophora diclados*, are unique herbertane-type sesquiterpene dimers which exhibit intriguing neurotrophic properties, i.e. promote neuronal outgrowth and enhance choline acethyltransferase activity in the primary cultures of fetal rat cerebral hemisphere.[2] These sesquiterpene dimers **1** and **2** (Fig. 1) are presumably formed by one-electron oxidative phenolic coupling of (−)-herbertenediol (**3**)[3] co-occurring in the above liverwort.[4] The first synthesis of **1** and **2** which involved the oxidative phenolic coupling of the mono-*o*-methyl derivative **19** was recently communicated by Bringmann et al.[5,6] and the two unique syntheses were followed by the atropo-diastereoselective constaction of the biaryl axis of **1** and **2**.[7,8] These prompted us to account in detail for our independent synthesis and biological evaluation of mastigophorenes A (**1**) and B (**2**).[9]

Herein, we report the synthesis of the optical active **1** and **2**, which features oxidative dimerization of (−)-**3** and mono-MOM derivative **18** by using HRP (horseradish peroxidase) and (*tert*-BuO)$_2$, respectively.

2. Results and discussion

2.1. Synthesis of (−)-herbertenediol (3)

In the preceding paper,[10] we reported the efficient synthesis of the racemic herbertenediol (**3**) by employing the intramolecular Heck reaction for the crucial contraction of the quaternary carbon directly bonded to the benzene ring. Thus the synthesis of optical active (−)-**3** started from the preparation of (*R*)-carboxylic acid **7**, to which the same procedure used for the synthesis of the racemic **3** was applied.

Koga et al.[11] reported a convenient procedure for the synthesis of both enantiomers of α,α-dialkyl-β-keto esters with a predictable chirality depending on the solvent system.

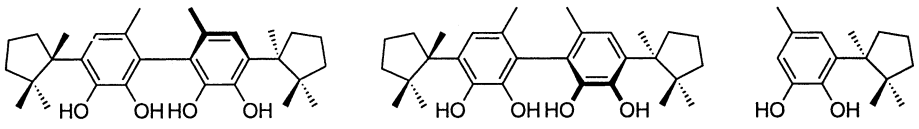

Figure 1. Structures of mastigophorenes A (**1**) and B (**2**), and (−)-herbertenediol (**3**).

Keywords: mastigophorene; herbertane-type sesquiterpene; Heck reaction; horseradish peroxidase; neuroprotective activity.
* Corresponding author. Tel.: +81-88-622-9611; fax: +81-88-655-3051; e-mail: fukuyama@ph.bunri-u.ac.jp

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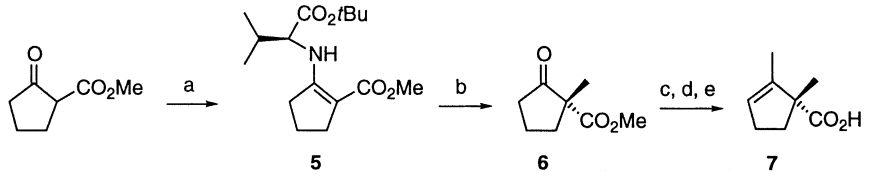
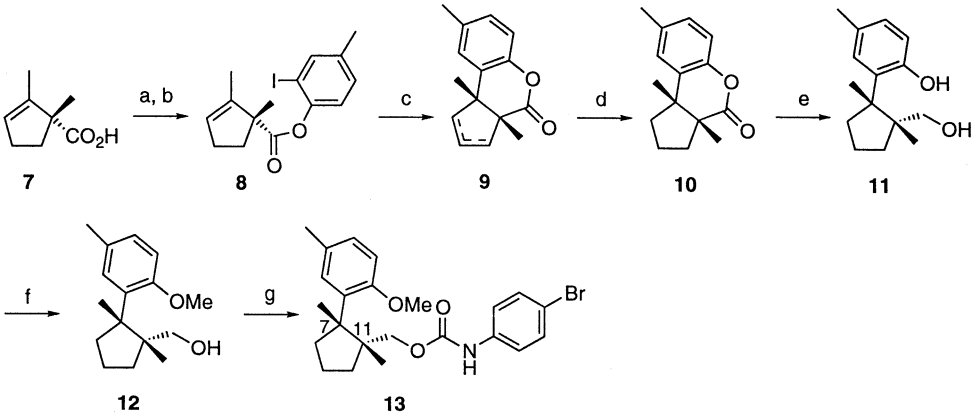


Scheme 1. Reagents, conditions and yields: (a) (*S*)-valine *tert*-butyl ester, $Et_2O{\cdot}BF_3$, benzene, 99%. (b) LDA, HMPA, MeI, −78°C and then −25°C, toluene, 40%. (c) MeMgI, Et_2O. (d) P_2O_{10}, benzene. (e) KOH, MeOH/H_2O, 33% over three steps.



Scheme 2. Reagents, conditions and yields: (a) 2,4,6-*tri*-ClBzCl, Et_3N, THF. (b) 2-iodo-*p*-cresol, DMAP, benzene, 65%. (c) 10 mol% $Pd(OAc)_2$, 20 mol% $(o\text{-tol})_3P$, $n\text{-}Bu_3N$, DMF, 120°C, 94%. (d) 10% Pd/C, H_2, EtOH, 95%. (e) $LiAlH_4$, THF. (f) MeI, K_2CO_3, acetone, 98% over two steps. (g) 4-bromophenyl isocyanate, DABCO, toluene.

According to the Koga protocol, lithiated chiral enamine **5** prepared from methyl 2-oxo-cyclopentanecarboxylate and (*S*)-valine *tert*-butyl ester can be alkylated with methyl iodide in a toluene solvent containing HMPA, after hydrolysis, to give (*R*)-**6**, whereas (*S*)-**6** can be prepared from the same enamine **5** if THF is added to a toluene solvent instead of HMPA (Scheme 1).

Thus, the chiral enamine **5** was lithiated with LDA in the presence of HMPA and then alkylated with methyl iodide at −25°C, after hydrolysis, to give **6** in 40% yield (98% ee). The absolute configuration of the newly formed quaternary carbon was determined as *R* based on the optical rotation which was identical with that of **6** prepared in the other way by Fujiwara.[12] On the other hand, the (*S*)-**6** was obtained in 67% ee when the reaction was run in the presence of THF instead of HMPA. This chirality was consistent with Koga's prediction. Next, the (*R*)-**6** was converted to an optical active carboxylic acid **7** by the same procedures as the racemic one.[10] Yamaguchi's coupling between **7** and 2-iodo-*p*-cresol gave an ester **8** in 65% yield. Intramolecular Heck reaction of **8** was employed under the established conditions such as 10 mol% $Pd(OAc)_2$, 20 mol% $(o\text{-tol})_3P$ and $n\text{-}Bu_3N$ in DMF giving rise to a δ-lactone **9** in 94% yield as a mixture of double bond isomers. Hydrogenation of **9**, followed by $LiAlH_4$ reduction, yielded the diol **11**, the phenolic hydroxyl group of which was selectively methylated under the basic conditions giving rise to **12** in 93% yield over three steps (Scheme 2).

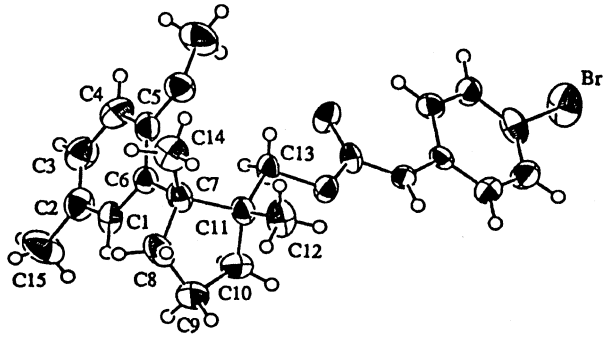
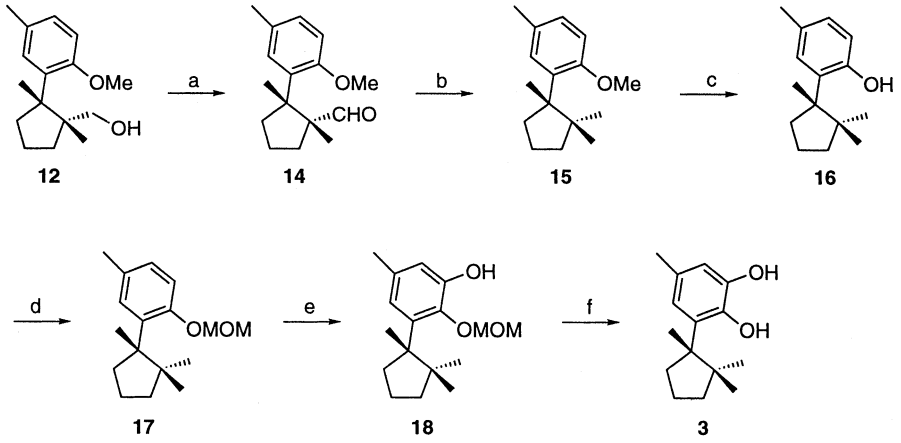


Figure 2. X-Ray ORTEP drawing of **13**.

To make sure of the configuration of the quaternary carbon newly formed by the intramolecular Heck reaction, **12** was converted to a *p*-bromophenyl carbamate **13**, which provided single crystals suitable for X-ray crystallographic analysis. The ORTEP drawing of **13** with the correct absolute configuration,[13] as shown in Fig. 2, indicates the favorable *S* configuration on the C-7 carbon.

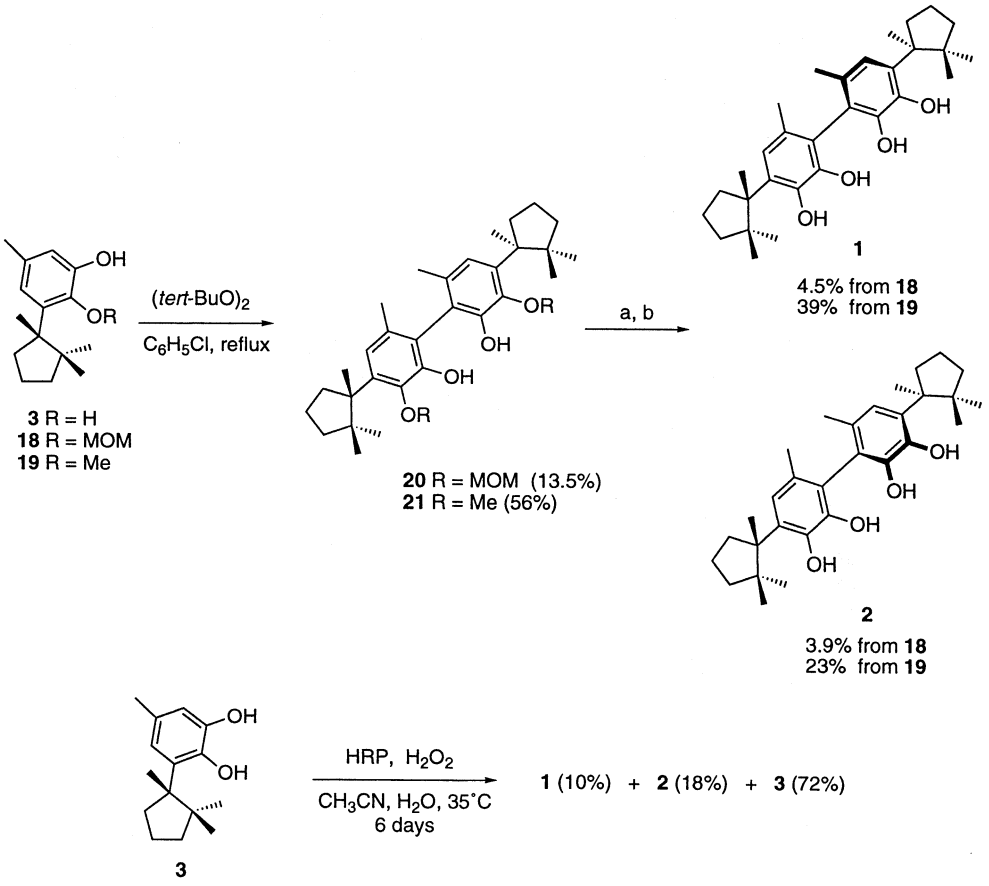
Swern oxidation of the primary alcohol in **12** afforded the aldehyde **14** in 96% yield. Subsequent Hunag–Minlon reduction of **14** yielded (−)-α-herbertenol (**16**)[3] after deprotection of the *o*-methyl group with BBr_3. Matsuo et al.[3] already converted **16** to a mixture of (−)-herbertenediol (**3**) and its positional isomer of the hydroxyl group by the direct oxidation of **16** with benzoyl peroxide in poor yield. Thus we exploited more efficient method of hydroxylation on the benzene ring. The hydroxyl group of **16** was first converted to MOM-ether **17**, which was lithiated with *sec*-BuLi regioselectively at the *ortho*-position to the MOM group and then treated with Davis reagent[14] giving rise to a hydoxylated product **18** in 51% yield. Treatment of **18** with 48% HBr gave (−)-herbertenediol (**3**), which was identical in all respects with natural one (Scheme 3).



Scheme 3. Reagents, conditions and yields: (a) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, 96%. (b) $N_2H_4{\cdot}H_2O$, NaOH, DEGL, 180°C, 83%. (c) BBr_3, CH_2Cl_2, −78°C and then rt, 66%. (d) MOMCl, *i*-Pr_2NEt, CH_2Cl_2, 71%. (e)*sec*-BuLi, TMEDA, Davis reagent, THF, 51%. (f) 48% HBr, MeOH, 98%.

2.2. Syntheses of mastigophorenes A (1) and B (2)

Mastigophorenes A and B are biogenetically derived from (−)-herbertenediol (**3**) by oxidative phenol coupling.[2] Bringmann et al.[6] already reported the oxidative dimerization of the mono-methly ether **19** using (*tert*-BuO)$_2$. We applied first this method to oxidative coupling of the mono-MOM ether **18**, thereby giving rise to dimers **20** as an inseparable diastereomeric mixture in 13.5% yield, which led, after deprotection, to **1** and **2** in 34 and 29% yield, respectively. On the other hand, we attempted the same oxidative dimerization of the monomethyl ether **19** to give **21** in 56% yield, much better than the reported yield (28%).[6] Chemical oxidative couplings of **3** involving (*tert*-BuO)$_2$, however, failed to directly yield dimers **1** and **2**. We were previously succeeded in the formation of the benzodioxane-type dimers from caffeic acid by using horseradish peroxidase (HRP) catalyzed oxidative phenolic coupling.[15] Thus we applied this enzyme method to the oxidative coupling of **3** itself. A solution of **3** in acetonitrile was incubated with HRP (200 units, type II) in phosphate buffer (pH 6.0) in the presence of hydrogen peroxide for 6 days,[16] resulting in the



Scheme 4. Oxidative coupling of herbertenediol (**3**) and its derivatives; reagents and conditions: (a) 48% HBr, MeOH for **20**. (b) BBr_3, CH_2Cl_2 for **21**.

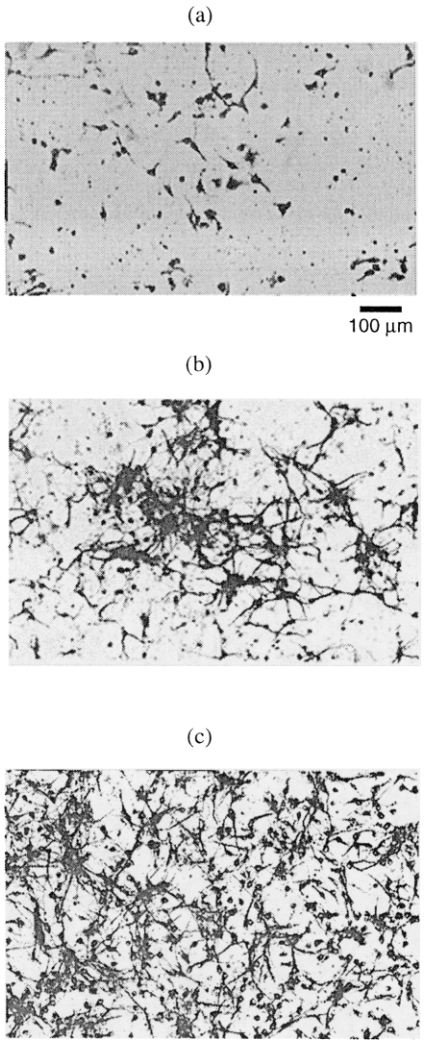


Figure 3. Enhancement of neurite outgrowth by mastigophorenes A and B in primary cultures of E18 SD rat cortical neurons. After the neuronal cells (5×10^5 cells cm^{-2}) cultured for 5 days in the presence of 0.5% EtOH and mastigophorenes A and B were fixed by 4% paraformaldehyde-PBS, the immunohistochemical staining for the microtuble associated protein-2 were performed. Pictures taken with 150 magnifications. (a) 0.5% EtOH, (b) 1 μM mastigophorene A, and (c) 1 μM mastigophorene B.

formation of **1** (10%) and **2** (18%) with the recovery **3** (72%).

It should be noted that HRP-catalyzed oxidative coupling of **3** is superior to chemical oxidative ones in terms of simple operation as well as of recycling **3** (Scheme 4).

3. Neuroprotective activity of 1 and 2

We reported that mastigophorenes A (**1**) and B (**2**) showed an interesting neurotrophic activity in the primary neuronal cultures in the serum-containing medium.[1,2] With synthetic **1** and **2** in hand, we are now in position to be able to evaluate more neurotrophic property of them. The present primary cultures were performed using 18-day fetal rat cortical neurons in the serum-free DMEM medium supplemented with N2 as described by Abe et al.[17] Morphorogical evaluations of neurons, as shown in Fig. 3, indicated that mastigophorenes A and B not only promoted significantly neurite outgrowth but also maintained the neuronal survival at concentrations of 0.1 and 1 μM. However, they were found to be toxic against neurons at a concentration of 10 μM. Neuronal survival was measured by the WST-8 reduction assay.[18] As shown in Fig. 4, **1** and **2** exhibited potent effects on the neuronal survival in primary cultures. Particularly, they enhanced significantly the survival of neurons at a concentration of 1 μM in comparison of the control cultures, but they lost their survival effect at 10 μM. These results suggest that mastigophorenes A and B can protect the neurons from being damaged by toxic substances such as oxygen free radicals.

In conclusion, we have synthesized (−)-herbertenediol (**3**) by following our previously developed procedure based on the intramolecular Heck reaction, and then have found that HRP catalyzed oxidative coupling serves as an effective method for **3** being dimerized into mastigophorenes A (**1**) and B (**2**). Moreover, our biological assay results indicate that mastigophorenes A and B have potent neuroprotective effect on the neuronal survival in the primary cultures in addition to their outgrowth promoting activity. Further

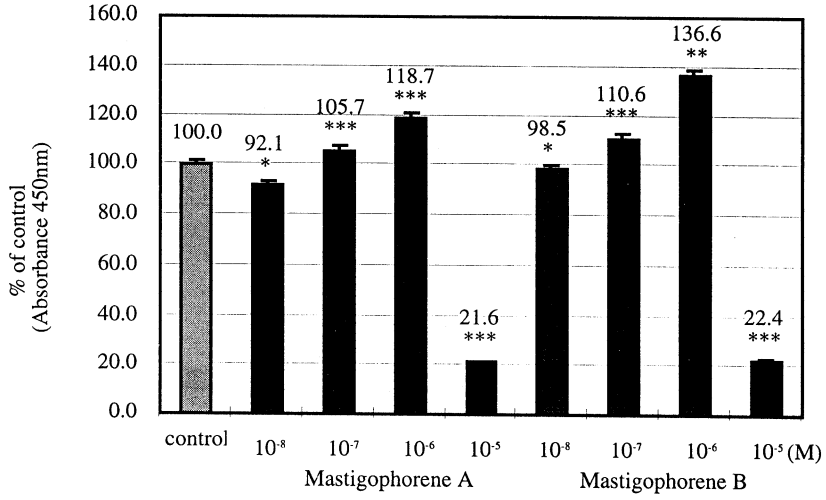


Figure 4. Enhancement of survival of rat cortical neurons by mastigophorenes A and B in primary cultures. Effects of survival were assessed by the WST-8 reduction assay. The data are expressed as means SE (n=4); $^{*}P<0.05$, $^{**}P<0.015$, $^{***}P<0.005$ versus control.

biological studies on significant neuroprotective effects are under way.

4. Experimental

4.1. General

Melting points were determined on a Yanagimoto micro melting point apparatus without correction. IR, UV and CD spectra were recorded on JASCO 5300 FT-IR, Shimazu UV-300 and JASCO J-500 spectrometers, respectively. ^{1}H- and ^{13}C-NMR spectra were taken on a Varian unity-200 or a JEOL GX-400 spectrometer. Chemical shifts are expressed in δ units (part per million downfield from Me_4Si). Mass spectra (MS) were recorded on a JEOL AX-500. Air- and moisture-sensitive reagents were transferred via syringe or cannula, and reactions involving these materials were carried out in oven-dried flasks under a positive pressure of argon. Silica gel (Wako, C-300) was used for column chromatography. Analytical thin-layer chromatographies (TLC) were performed with Merck precoated TLC plates (Kiselgel 60 F_{254}, 0.25 mm), and spots were visualized with ultraviolet light, iodide, and 40% $CeSO_4/H_2SO_4$.

4.2. Synthesis of (−)-herbertenediol

4.2.1. *N*-(2-Carbomethoxy-1-cyclopenten-1-yl)-(*S*)-valine *tert*-butyl ester (5). To a solution of methyl 2-cyclopentanonecarboxylate (1.56 g, 10.9 mmol) and (*S*)-valine *tert*-butyl ester (3 g, 14.31 mmol) in benzene (60 mL) was added boron trifluroride diethyl etherate (0.14 mL, 0.55 mmol), and resulting solution was heated to reflux using Dean–Stark apparatus for 12 h. This reaction mixture was extracted with ether, washed with aqueous $NaHCO_3$, water and saturated NaCl solution and dried over $MgSO_4$. Evaporation of the solvent gave a crude mixture, which was chromatographed on silica gel (hexane/EtOAc=10:1) to give **5** (3.24 g, 99%) as an oil: $[\alpha]^{22}_D$=+96.8° (*c* 1.0, $CHCl_3$); IR (film) 3315, 1736, 1666, 1606, 1462, 1269, and 1134 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.97 (3H, d, *J*=2.3 Hz), 1.00 (3H, d, *J*=2.3 Hz), 1.46 (9H, s), 1.82 (2H, q, *J*=7.4 Hz), 2.10 (1H, m), 2.47 (4H, m), 3.64 (1H, dd, *J*=10.9, 5.9 Hz), 3.70 (3H, s), and 7.52 (1H, m); EIMS *m/z* (rel. int.) 297 [M^+] (24), 266 (3), 241 (16), 210 (6), 196 (100), and 164 (92); HREIMS *m/z* 297.1958 [M^+] (Calcd 297.1940 for $C_{16}H_{27}O_4N$).

4.2.2. Methyl (*R*)-1-methyl-2-cyclopentanonecarboxylate (6). A *n*-BuLi (1.6 M solution in hexane, 6.57 mL, 10.5 mmol) was added to a solution of diisopropylamine (1.47 mL, 10.5 mmol) in toluene (14 mL) at −78°C and the mixture was stirred for 30 min at 0°C. To this in situ prepared LDA solution was added a solution of **5** (2.60 g, 8.75 mmol) in toluene (5 mL) at −78°C and the resulting solution was stirred for 30 min. HMPA (1.83 mL, 10.51 mmol) was added and the reaction mixture was stirred for 30 min. Methyl iodide (0.67 mL, 10.5 mmol) was added and the reaction mixture was stirred at −25°C for 5 h. The reaction was quenched with 4% HCl (20 mL) and the whole mixture was stirred at 0°C for 30 min and was then extracted with ether. The organic layer was washed with aqueous $NaHCO_3$, 5% Na_2SO_3, water and saturated NaCl solution, dried over $MgSO_4$, concentrated in vacuo to leave the residue, which was purified by column chromatography (hexane/ether=10:1) to give **6** [(297 mg, 40% based on 54% recovery of **5** (1.19 g)] as an oil. The ee for **6** was determined to be 98% by GC analysis with chiral column (column: CYCLODEX-B; film thickness: 0.25 mm; ∅ 0.254 mm×30 m; 50 mL He/min; temperature: 70°C; retention time *R*: 60.03 min, *S*: 63.17 min); $[\alpha]^{19}_D$=−10.8° (*c* 1.28, $CHCl_3$); ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.31 (3H, s), 1.83–2.06 (3H, m), 2.31–2.55 (3H, m), 3.70 (3H, s).

4.2.3. (*R*)-1,2-Dimethyl-2-cyclopentenecarboxylate (7). A solution of MeMgI was prepared from Mg (110 mg, 4.54 mmol) and MeI (0.29 mL, 4.54 mmol) in ether (5 mL). To the stirred Grignard solution, **6** (590 mg, 3.78 mmol) in ether (5 mL) was added at room temperature for 30 min. The mixture quenched by the addition of saturated NH_4Cl solution, extracted with ether, washed with saturated $NaHCO_3$ solution, water and saturated NaCl solution, dried over $MgSO_4$. Evaporation of ether afforded a residue. To a solution of the residue in benzene (5 mL) was added phosphorus pentaoxide (4 g). The mixture was stirred at room temperature for 2 h. Evaporation of solvent gave the residue, which was dissolved in a mixture of methanol/water (1:1, 10 mL), added KOH (636 mg, 11.34 mmol), and the mixture was stirred for 3 days. This solution was acidified with 2N HCl, extracted with ether. The organic layer was extracted with sat. $NaHCO_3$ solution. The resulting aqueous layer was acidified with 2N HCl, and then extracted with ether. The extract was washed with water and saturated NaCl solution, dried over $MgSO_4$, concentrated, and purified by column chromatography (hexane/EtOAc=4:1) to give **7** (175 mg, 33%) as an oil: $[\alpha]^{21}_D$=+128.8° (*c* 0.86, $CHCl_3$); IR (film) 3400, 1698, 1448, 1408, 1282, and 1184 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.30 (3H, s), 1.72 (1H, t, *J*=1.5 Hz), 1.80 (1H, m), 2.44 (3H, m), and 5.51 (1H, m); EIMS *m/z* (rel. int.) 140 [M^+] (14), 95 (100), and 79 (10); HREIMS *m/z* 140.0833 [M^+] (Calcd 140.0837 for $C_8H_{12}O_2$).

4.2.4. 2-Iodo-4-methylphenyl 1,2-dimethyl-2-cyclopentenecarboxylate (8). 2,4,6-Trichlorobenzoyl chloride (0.28 mL, 1.8 mmol) was added to a mixture of **7** (250 mg, 1.79 mmol) and triethylamine (0.25 mL, 1.8 mmol) in tetrahydrofuran (5 mL). The mixture was stirred for 40 min at room temperature. After the removal of triethylamine hydrochloride by filtration, the filtrate was evaporated and the residue was dissolved in benzene (10 mL). To this solution was added a mixture of 2-iodo-*p*-cresol (632 mg, 2.7 mmol) and 4-dimethylaminopyridine (440 mg, 3.6 mmol) and the resulting mixture was stirred at room temperature for 41 h. The reaction mixture was extracted with ether and the ether extract was washed with water, $Cu(NO_3)_2$ solution, $NaHCO_3$ solution and saturated NaCl solution and dried over $MgSO_4$. Evaporation of the solvent gave a crude product, which was chromatographed on silica gel (hexane/CH_2Cl_2=9:1) to afford **8** (416 mg, 65%) as an oil: $[\alpha]^{23}_D$=+46.3° (*c* 16.0, $CHCl_3$); IR (film) 1751, 1599, 1481, 1217, 1130, and 1074 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.46 (3H, s, 1.82 (3H, d, *J*=1.9 Hz), 1.91 (1H, m), 2.30 (3H, s), 2.49 (2H, m), 2.77 (1H, m), 5.57 (1H, m), 6.89 (1H, d, *J*=8.1 Hz), 7.13 (1H, dd, *J*=8.1, 1.8 Hz), and

7.63 (1H, d, J=1.8 Hz); EIMS m/z (rel. int.) 356 [M^+] (3), 328 (2), 234 (7), and 95 (100); HREIMS m/z 356.0258 [M^+] (Calcd for 356.0273 for $C_{15}H_{17}O_2I$).

4.2.5. Intramolecular Heck reaction of 8. A mixture of **8** (110 mg, 0.31 mmol), palladium(II) acetate (7 mg, 0.03 mmol), tri(*o*-tolyl)phosphine (19 mg, 0.06 mmol) and tributhyl amine (0.15 mL, 0.62 mmol) in DMF (31 mL) was heated at 120°C for 20 h. The solvent was removed in vacuo to leave the residue, which was dissolved in ether. The resulting organic solution was washed with 2N HCl, water and saturated NaCl, dried over $MgSO_4$. The solvent was removed and the residue was chromatographed on silica gel (hexane/EtOAc=20:1) to afford **9** (66 mg, 94%) as an oil: IR (film) 1751, 1614, 1496, 1248, 1221, and 1084 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 1.34 and 1.35 (total 3H, s), 1.37 and 1.38 (total 3H, s), 2.33 (3H, s), 2.57 (1H, dt, J=16.2, 1.8 Hz), 2.83 (1H, dt, J=16.2, 1.8 Hz), 5.74–5.86 (2H, m), 6.88 (1H, d, J=8.2 Hz), 7.02 (1H, dd, J=8.2, 1.8 Hz), and 7.14 (1H, d, J=1.8 Hz); EIMS m/z (rel. int.) 228 [M^+] (100), 213 (27), 200 (85), 185 (76), and 159 (26); HREIMS m/z 228.1145 [M^+] (Calcd for 228.1150 for $C_{15}H_{16}O_2$).

4.2.6. Hydorgenation of 9. A solution of **9** (60 mg, 0.26 mmol) in ethanol (5 mL) containing palladium carbon (Pd/C, Pd: 10%, 5 mg) was stirred under an atmospheric pressure of hydrogen for 18 h. The reaction mixture was filtered, concentrated, and the residue was purified by column chromatography (hexane/EtOAc=4:1) to afford **10** (58 mg, 95%) as an oil: $[\alpha]^{21}_D$=+32.7° (c 1.38, $CHCl_3$); IR (film) 1755, 1614, 1495, 1238, and 1118 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 1.24 (3H, s), 1.27 (3H, s), 1.38 (1H, m), 2.34 (3H, s), 6.90 (1H, d, J=8.2 Hz), 7.03 (1H, dd, J=8.2, 2.0 Hz), and 7.12 (1H, d, J=2.0 Hz); EIMS m/z (rel. int.) 230 [M^+] (100), 215 (15), 202 (30), 187 (65), 173 (13), and 159 (41); HREIMS m/z 230.1312 [M^+] (Calcd for 230.1307 for $C_{15}H_{18}O_2$).

4.2.7. 1,2-Dimethyl-1-hydroxylmethyl-2-(2-hydroxy-5-methylphenyl)cyclopentane (11). To a solution of **10** (55 mg, 0.24 mmol) in tetrahydrofuran (3 mL) was added $LiAlH_4$ (100 mg, 2.63 mmol) at 0°C and the mixture was stirred for 3 h at room temperature. The reaction mixture was treated with water, and then was extracted with ether. The ether layer was washed with saturated NaCl, dried over $MgSO_4$. Removal of the solvent gave **11** (56 mg) as a colorless needle: mp 119.5–120.5°C (colorless plate from CH_2Cl_2 and hexane); $[\alpha]^{27}_D$=+15.5° (c 1.14, $CHCl_3$); IR (film) 3163, 1608, 1464, 1230, and 1024 cm^{-1}; EIMS m/z (rel. int.) 234 [M^+] (38), 216 (11), 201 (25), 173 (12), 161 (35), 148 (35), 135 (100); HREIMS m/z 234.1622 [M^+] (Calcd for 234.1620 for $C_{15}H_{22}O_2$); ^1H NMR (200 MHz, $CDCl_3$) δ 1.23 (3H, s), 1.56 (3H, s), 1.93 (1H, m), 2.27 (3H, s), 2.45 (1H, m), 3.31 (1H, d, J=11.1 Hz), 3.36 (1H, d, J=11.1 Hz), 6.74 (1H, d, J=7.8 Hz), 6.92 (1H, dd, J=7.8, 1.4 Hz), and 6.95 (1H, d, J=1.4 Hz); ^{13}C NMR (50 MHz, $CDCl_3$) δ 20.6, 21.0, 21.2, 24.1, 36.0, 42.4, 49.0, 51.0, 70.5, 117.2, 127.9, 129.1, 129.9, 132.9, and 152.9; Anal. Calcd for $C_{15}H_{22}O_2$: C, 76.88; H, 9.46. Found C, 76.91, H 9.66.

4.2.8. 1,2-Dimethyl-1-hydroxylmethyl-2-(2-methoxy-5-methylphenyl)cyclopentane (12). To a mixture of **11** (92 mg, 0.39 mmol) and K_2CO_3 (85 mg, 0.61 mmol) in acetone (10 mL) were added iodomethane (0.20 mL, 3.22 mmol) and reaction mixture was refluxed for 11 h. The reaction mixture was diluted with water and extracted with ether. The resulting organic layer was washed with saturated NaCl, dried over $MgSO_4$. Evaporation of the organic solvent gave the residue, which was chromatographed on silica gel (hexane/EtOAc=6:1) to afford **12** (95 mg, 98%) as an oil: $[\alpha]^{22}_D$=−19.4° (c 0.68, $CHCl_3$); IR (film) 3435, 1604, 1496, 1242, and 1028 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 1.20 (3H, s), 1.42 (3H, s), 2.28 (3H, s), 2.44 (1H, m), 3.10 (1H, bs), 3.13 (1H, bs), 3.80 (3H, s), 6.81 (1H, d, J=8.1 Hz), 7.01 (1H, dd, J=8.1, 2.0 Hz), and 7.09 (1H, d, J=2.0 Hz); EIMS m/z (rel. int.) 248 [M^+] (45), 215 (9), 175 (29), 162 (26), and 149 (100); HREIMS m/z 248.1774 [M^+] (Calcd for 248.1776 for $C_{16}H_{24}O_2$).

4.2.9. *p*-Bromophenylcarbamate of 12. To a mixture of **12** (12 mg, 0.05 mmol) and DABCO (8 mg, 0.07 mmol) in toluene (1 mL) were added a solution of 4-bromophenyl isocyanate (14 mg, 0.07 mmol) in toluene (0.5 mL) and reaction mixture was stirred for 1 h at room temperature. The reaction mixture was diluted with water and extracted with ether. The organic layer was washed with water and saturated NaCl solution, dried over $MgSO_4$ and concentrated. Purification of the residue by column chromatography on silica gel (hexane/EtOAc=1:1) gave **13** (10 mg): mp 143–145°C (colorless plate from CH_2Cl_2 and hexane); $[\alpha]^{21}_D$=−29.5° (c 0.38, $CHCl_3$); IR (film) 3310, 1701, 1595, 1535, 1496, 1400, 1308, 1238, 1074 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 1.23 (3H, s), 1.34 (3H, s), 2.22 (3H, s), 2.50 (1H, m), 3.76 (1H, d, J=8.6 Hz), 3.77 (3H, s), 3.79 (1H, d, J=8.6 Hz), 6.24 (1H, m), 6.74 (1H, d, J=8.1 Hz), 6.96 (1H, dd, J=8.1, 1.6 Hz), 7.08 (1H, d, J=1.6 Hz), 7.18 (2H, d, J=8.8 Hz), and 7.37 (2H, d, J=8.8 Hz); EIMS m/z(rel. int.) 445[M^+] (30), 447[M+2] (29), 248 (13), 230 (22), 215 (17), 188 (26), 175 (52), and 149 (100); HREIMS found 445.1255[M^+] (Calcd 445.1253 for $C_{23}H_{28}O_3NBr$).

Crystal data: Triclinic, a=8.943 (2), b=9.5118 (9), c=13.241 (3) Å, α=105.53 (2), β= 90.3 (2), γ=92.39 (1)°, space group $P1$, D_{cal}=1.10, CuKα radiation, λ=1.54178 Å. Diffraction measurement was made on a Mac Science MXC18. The structure was solved by direct methods and refined out by full matrix least-squares. Final R factor=0.0546. Crystallographic data have been deposited at the CCDC (165776), 12 Union Road, Cambridge CB2 1EZ, UK.

4.2.10. 1,2-Dimethyl-1-formyl-2-(2-methoxy-5-methylphenyl)cyclopentane (14). To a solution of dimethyl sulfoxide (0.02 mL, 0.28 mmol) in CH_2Cl_2 (0.5 mL) was added oxalyl chloride (0.02 mL, 0.19 mmol) at −78°C, and stirring was continued for 10 min. A solution of **12** (23 mg, 0.1 mmol) in CH_2Cl_2 (1 mL) was added to this solution, and the reaction mixture was stirred for 1 h at the same temperature and then treated with Et_3N (0.09 mL, 0.65 mmol). After further stirring for 30 min at 0°C, the reaction mixture was treated with saturated NH_4Cl solution and extracted with ether. The organic layer was washed with water and saturated NaCl solution, dried over

$MgSO_4$. The solvent was removed in vacuo and the residue was chromatographed on silica gel (hexane/EtOAc=15:1) to afford **14** (22 mg, 96%) as an oil: $[\alpha]^{22}_D=-49.2°$ (*c* 0.75, $CHCl_3$); IR (film) 1718, 1606, 1585, 1499, 1464, 1244, and 1032 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 1.30 (3H, s), 1.33 (3H, s), 2.28 (3H, s), 2.38 (1H, m), 3.70 (3H, s), 6.71 (1H, d, *J*=8.1 Hz), 7.00 (1H, dd, *J*=8.1, 1.6 Hz), 7.06 (1H, d, *J*=1.6 Hz), and 9.05 (1H, s); EIMS *m/z*(rel. int.) 246[M^+] (23), 229 (4), 175 (100), 162 (16), 149 (47), and 135 (17); HREIMS *m/z* 246.1604 [M^+] (Calcd 246.1620 for $C_{16}H_{22}O_2$).

4.2.11. 1,1,2-Trimethyl-2-(2-methoxy-5-methylphenyl)-cyclopentane (15). To a solution of **14** (96 mg, 0.39 mmol) in diethylene glycol (3 mL) was added NaOH (290 mg, 6.55 mmol) and hydrazine monohydrate (0.02 mL, 0.43 mmol). The reaction mixture was stirred at 150°C for 4 h and at 180°C for additional 3 h. The reaction mixture was extracted with ether, washed with water, saturated NaCl solution, dried over $MgSO_4$ and concentrated in vacuo. The residue was chromatographed on silica gel (hexane/EtOAc=19:1) to afford **15** (78 mg, 83%) as an oil: IR (film) 1606 1498, 1240, and 1035 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 0.68 (3H, s), 1.15 (3H, s), 1.35 (3H, s), 2.28 (3H, s), 2.53 (1H, m), 3.74 (3H, s), 6.75 (1H, d, *J*=8.2 Hz), 6.96 (1H, dd, *J*=8.2, 2.2 Hz), and 7.11 (1H, d, *J*=2.2 Hz); EIMS *m/z*(rel. int.) 232 [M^+] (81), 217 (12), 201 (5), 189 (1), 175 (43), 162 (73), 149 (100), 135 (40), and 119 (41); HREIMS *m/z* 232.1842 [M^+] (Calcd 232.1827 for $C_{16}H_{24}O$).

4.2.12. (−)-α-Herbertenol (16). To a solution of **14** (14 mg, 0.06 mmol) in CH_2Cl_2 (2 mL) was slowly added a solution of BBr_3 (1 M in CH_2Cl_2, 0.08 mL, 0.08 mmol) at −78°C for 40 min, and the mixture was stirred at 0°C for 40 min and at room temperature for 3 h. The reaction mixture was extracted with ether, washed with water and saturated NaCl solution, and dried over $MgSO_4$. After evaporation of the solvent, the residue was chromatographed on silica gel (hexane/EtOAc=19:1) to afford (−)-α-herbertenol (**16**) (9 mg, 66%) as an oil: $[\alpha]^{24}_D=-41.4°$ (*c* 0.43, $CHCl_3$); IR (film) 3531, 2959, 2874, 1606, 1506, 1462, 1406, 1373, 1253, 1168, 1143, and 810 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 0.76 (3H, s), 1.18 (3H, s), 1.41 (3H, s), 2.26 (3H, s), 2.59 (1H, m), 4.61 (1H, s), 6.57 (1H, d, *J*=8.0 Hz), 6.86 (1H, dd, *J*=8.0, 2.0 Hz), and 7.09 (1H, d, *J*=2.0 Hz); ^{13}C NMR (50 MHz, $CDCl_3$) δ 20.4, 20.9, 23.0, 25.6, 27.0, 39.5, 41.3, 44.7, 51.0, 116.7, 127.3, 129.0, 130.1, 133.1, and 152.3; EIMS *m/z*(rel. int.) 218 [M^+] (70), 203 (8), 175 (10), 161 (34), 148 (100), 135 (85), 121 (29), 105 (13), 91 (11), and 77 (8); HREIMS *m/z* 218.1664 [M^+] (Calcd 218.1671 for $C_{15}H_{22}O$).

4.2.13. 1,1,2-Trimethyl-2-(2-methoxymethyloxy-5-methylphenyl)cyclopentane (17). To a solution of (−)-herbertenol (**16**) (30 mg, 0.14 mmol) in CH_2Cl_2 (1.5 mL) was added diisopropylethylamine (0.09 mL, 0.50 mmol) and chloromethyl methyl ether (0.04 mL, 0.50 mmol). The mixture was stirred at room temperature for 23 h. The reaction mixture was extracted with ether, washed with water and saturated NaCl solution, dried over $MgSO_4$, and concentrated in vacuo. Evaporation of organic solvent gave the residue, which was chromatographed on silica gel (hexane/CH_2Cl_2=9:1) to afford **17** (16 mg, 71%) as an oil and **16** (recovery, 14 mg): $[\alpha]^{21}_D=-50.3°$ (*c* 1.57, $CHCl_3$); IR (film) 1497, 1371, 1229, 1146, 1078,1 015, 922, and 814 cm^{-1}; EIMS *m/z* (rel. int.) 262 [M^+] (44), 217 (62), 173 (23), 161 (47), 145 (32), 135 (100), and 121 (31); ^1H NMR (200 MHz, $CDCl_3$) δ 0.72 (3H, s), 1.16 (3H, s), 1.37 (3H, s), 2.28 (3H, s), 2.53 (1H, m), 3.48 (3H, s), 5.09 (1H, d, *J*=7.0 Hz), 5.16 (1H, d, *J*=7.0 Hz), 6.93 (1H, dd, *J*=1.8, 8.1 Hz), 7.03 (1H, d, *J*=8.1 Hz), and 7.13 (1H, d, *J*=1.8 Hz); ^{13}C NMR (50 MHz, $CDCl_3$) δ 20.5, 20.9, 23.2, 25.8, 27.4, 39.8, 41.8, 44.3, 51.3, 55.9, 94.6, 114.5, 127.3, 129.7, 130.0, 136.0, and 154.7; HREIMS *m/z* 262.1938 [M^+] (Calcd 262.1933 for $C_{17}H_{26}O_2$).

4.2.14. 1,1,2-Trimethyl-2-(3-hydroxy-2-methoxymethyloxy-5-methylphenyl)cyclopentane (18). To a solution of **17** (31 mg) and TMEDA (0.11 mL, 0.71 mmol) in THF (1 mL) was added dropwise *sec*-BuLi (1.08 M in cyclohexane, 0.66 mL, 0.71 mmol) at −78°C under argon atmosphere. After being stirred for 1 h at −78°C, (1*R*)-(−)-(10-camphorsulfonyl)oxaziridine (136 mg, 0.60 mmol) was added at −15°C and the reaction mixture was stirred for 50 min and then at room temperature. The reaction mixture was extracted with ether, washed with water and saturated NaCl solution, dried over $MgSO_4$, and concentrated in vacuo. The residue was chromatographed on silica gel (hexane/EtOAc=19:1) to afford **18** (12 mg, 51%) as an oil and **17** (recover, 8 mg): $[\alpha]^{22}_D=-93.1°$ (*c* 2.27, $CHCl_3$); IR (film) 3328, 1584, 1462, 1310, 1182, 1152, 1057, and 993 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 0.72 (3H, s) 1.14 (3H, s), 1.29 (3H, s), 2.25 (3H, s), 3.65 (3H, s), 4.86 (1H, d, *J*=6.3 Hz), 5.01 (1H, d, *J*=6.3 Hz), 6.67 (1H, d, *J*=2.6 Hz), 6.68 (1H, d, *J*=2.6 Hz), and 7.80 (1H, s); ^{13}C NMR (50 MHz, $CDCl_3$) δ 20.3, 21.3, 24.4, 25.3, 26.8, 39.3, 40.9, 45.0, 51.3, 57.0, 100.3, 115.8, 120.8, 133.5, 140.1, 143.9, and 149.1; EIMS *m/z* (rel. int.) 278 [M^+] (84), 246 (35), 233 (22), 217 (7), 203 (12), 189 (35), 175 (48), 164 (31), 151 (100); HREIMS *m/z* 278.1886 [M^+] (Calcd 278.1882 for $C_{17}H_{26}O_3$).

4.2.15. (−)-Herbertenediol (3). To a solution of **18** (20 mg, 0.07 mmol) in MeOH (2 mL) was added one drop of 48% hydrobromic acid and the mixture was stirred at 60° C for 15 min. After removal of solvent, the crude mixture was extracted with ethyl acetate, washed with water and saturated NaCl, dried over $MgSO_4$, and concentrated in vacuo. The residue was chromatographed on silica gel (hexane/EtOAc=4:1) to afford **3** (16 mg, 98%): mp 88–89.5°C (colorless plate from CH_2Cl_2 and hexane); $[\alpha]^{22}_D=-47.1°$ (*c* 1.0, $CHCl_3$); IR (film) 3512 and 1597 cm^{-1}; EIMS *m/z*(rel. int.) 234 [M^+] (78), 191 (7), 178 (11), 164 (52), 151 (100), and 137 (27); ^1H NMR (400 MHz, $CDCl_3$) δ 0.77 (3H, s), 1.19 (3H, s), 1.42 (3H, s), 2.23 (3H, s), 2.60 (1H, m), 6.56 (1H, s), and 6.69 (1H, s); ^{13}C NMR (100 MHz) δ 20.3, 21.2, 22.9, 25.4, 26.9, 39.0, 41.0, 44.9, 51.5, 113.5, 121.9, 128.3, 133.5, 141.0, and 143.4; HREIMS found 234.1618 [M^+] (Calcd 234.1620 for $C_{15}H_{22}O_2$).

4.3. Synthesis of mastigophrenes A and B

4.3.1. Oxidative dimerization of 18 with (*tert*-BuO)$_2$. To a solution of **18** (90 mg, 0.32 mmol) in chlorobenzene (3 mL) was added di-*tert*-butylperoxide (0.12 mL, 0.65 mmol)

under an argon atmosphere and the mixture was refluxed for 4 h. The solvent was removed and the residue was purified on silica gel (hexane/CH_2Cl_2=2:1) to afford **20** (24 mg, 13.5%) as an oil and **18** (recovery, 70 mg, 77.8%): EIMS *m/z* (rel. int.) 554 [M^+] (21), 522 (21), 490 (10), 478 (100), 408 (12), 396 (26), 261 (8), 111 (9); HREIMS *m/z* 554.3610 [M^+] (Calcd 554.3608 for $C_{34}H_{50}O_6$); ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.74 (3H, s), 0.75 (3H, s), 1.15 (3H, s), 1.16 (3H, s), 1.33 (3H, s), 1.35 (3H, s), 1.93 (3H, s), 1.95 (3H, s), 3.59 (6H, s), 4.90 (1H, d, *J*=6.2 Hz), 4.93 (1H, d, *J*=6.2 Hz), 5.02 (1H, d, *J*=6.2 Hz), 5.20 (1H, d, *J*=6.2 Hz), 6.78 (1H, s), 6.80 (1H, s), 7.31 (1H, s), and 7.51 (1H, s). To a solution of **20** (20 mg, 0.036 mmol) in MeOH (3.5 mL) was added two drop of 48% hydrobromic acid and the mixture was stirred at 60°C for 15 min. After removal of solvent, the crude mixture was extracted with ethyl acetate, washed with water and saturated NaCl, dried over $MgSO_4$, and concentrated in vacuo. The residue was chromatographed on silica gel (hexane/CH_2Cl_2=1:1) to afford **1** (6 mg, 34%) and **2** (5 mg, 29%) as needles.

4.3.2. Oxidative dimerization of 19 with (*tert*-BuO)$_2$. To a solution of **19** (47 mg, 0.19 mmol) in chlorobenzene (1.5 mL) was added di-*tert*-butylperoxide (0.06 mL, 0.34 mmol) under an argon atmosphere and the mixture was refluxed for 4 h. The solvent was removed and the residue was purified on silica gel (hexane/CH_2Cl_2=2:1) to afford **21** (25 mg, 56%) as a mixture, which was separated by chromatography on silica gel (hexane/CH_2Cl_2=3:2) to afford **21a** (14 mg) and **21b** (10 mg), each of which was treated with BBr_3 (1 M in CH_2Cl_2,) at −78°C for 30 min and then at 0°C for 1 h giving rise to **1** (9.4 mg, 70%) and **2** (4 mg, 41%), respectively.

4.4. Oxidative dimerization of (−)-herbertenediol (3) with HRP

To a solution of **3** (23 mg, 0.1 mmol) in acetonitrile (0.3 mL) and phosphate buffer (0.1 M, pH 6.0, 0.5 mL) was added 0.45% hydrogen peroxide (0.55 mL, 0.75 eq.) and a solution of HRP (1.0 mg, 200 units, type II from Sigma, USA) in phosphate buffer (0.1 M, pH 6.0, 0.5 mL). The reaction mixture was stirred at 35°C for 6 days. The reaction was quenched by adding 1 M $NaHSO_3$ and then adjusted to pH 7.0 with 1 M NaOH, finally to pH 3.0 with 1 M $NaHSO_3$. The reaction mixture was extracted with ethyl acetate, washed with water and saturated NaCl, dried over $MgSO_4$, and concentrated in vacuo. The residue (23 mg) was chromatographed on silica gel (hexane/CH_2Cl_2=1:1) to afford **1** (2.2 mg, 10%) and **2** (4.2 mg, 18%) and recovery **3** (16.5 mg, 72%).

4.4.1. Mastigophorene A (1). Needles, mp 217–220°C; $[\alpha]^{20}_D$=−69.0° (*c* 0.38, $CHCl_3$); IR (film) 3530, 2955, 1431, 1404, 1365, 1282, and 1221 cm^{-1}; UV λ_{max} (EtOH) 285 (ϵ 8400) and 230 (ϵ 20500) nm; CD (EtOH) $\Delta\epsilon$ (220 nm) +7.1, $\Delta\epsilon$ (206 nm) −12.1; EIMS *m/z* (rel. int.) 466 [M^+] (100), 423 (13), 396 (10), 384 (56), 356, (4), 300 (6), and 273 (5); HREIMS 466.3076 (Calcd 466.3083 for $C_{30}H_{42}O_4$); ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.79 (6H, s), 1.20 (6H, s), 1.46 (6H, s), 1.94 (6H, s), 4.69 (2H, s), 5.57 (2H, s), and 6.86 (2H, s); Anal. Calcd for $C_{30}H_{42}O_4$ H_2O (484.66736): C, 74.70; H, 8.71. Found C, 74.34, H 9.15.

4.4.2. Mastigophorene B (2). Needles, mp 167–170°C; $[\alpha]^{20}_D$=−47.0° (*c* 1.85, $CHCl_3$); IR (film) 3535, 2959, 1433, 1408, 1365, 1277, and 1226 cm^{-1}; UV λ_{max} (EtOH) 284 (ϵ 4300) and 238 (ϵ 7500) nm; CD (EtOH) $\Delta\epsilon$ (217 nm) −13.4, $\Delta\epsilon$ (203 nm) +12.3; EIMS *m/z* (rel. int.) 466 [M^+] (100), 423 (14), 396 (9), 384 (59), 299 (7), 234 (3); HREIMS 466.3084 (Calcd 466.3083 for $C_{30}H_{42}O_4$); ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.78 (6H, s), 1.21 (6H, s), 1.47 (6H, s), 1.92 (6H, s), 4.71(2H, s), 5.57 (2H, s), and 6.85 (2H, s); Anal. Calcd for $C_{30}H_{42}O_4$ $2H_2O$ (502.68264): C, 71.31; H, 8.92. Found C, 71.68, H 9.22.

4.5. Biological activity

4.5.1. Cell culture. Primary cell cultures were prepared as described.[17] All operations were carried out under sterile conditions. The neuronal cells were separated from the cerebral hemispheres of fetal 18-day SD rat (Japan SLC, Inc.) and suspended in 10% FAB/MEM, then seeded at 5×10^5 cells cm^{-2} into poly-L-lysine-coated 24 well-culture plates. After 48 h, the medium was changed into the serum-free medium, Dulbeco's modified Eagle's medium (DMEM) supplemented with N2, in the presence or absence of the compounds at the concentrations of 0.01, 0.1, 1 and 10 μM. After being incubated for 5 days, the cells are fixed with 4% paraformaldehyde/PBS for anti-MAP-2 immuno-histochemical stain. The neurite outgrowths affected by samples were analyzed under microscope and photographs taken with 150 magnifications.

4.5.2. Assay of neuronal survival. Neuronal survival was determined by the WST-8 reduction assay, which was performed using the cell counting kit-8 (Dojindo Co. Ltd.) according to the manifucture's procedure.

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16. High concentration (1000 units) of HRP completed the oxidative reaction of **3** within 2 h, giving rise to orthoquinone instead of dimers. On the other hand, it took 6 days to get dimers **1** and **2** rather than orthoquinone using low concentration (200 units) of HRP.
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