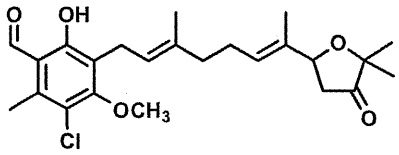
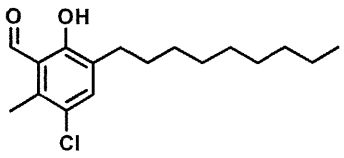
Table IX. TAO inhibition by 4-position substituted derivatives.

Compound	Structure	IC_{50} (nM)
29		4.0
30		30% inhibition at 50 μM

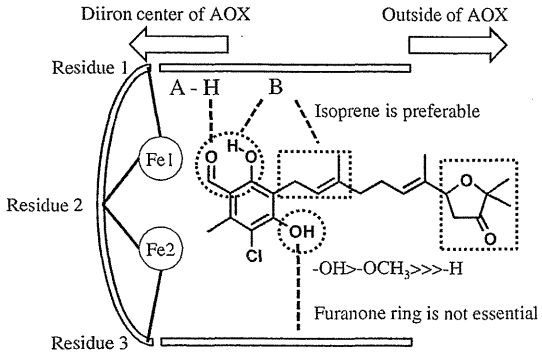
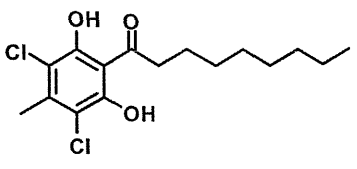
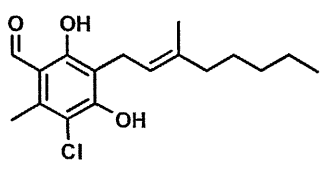


Fig. 2 Identification of pharmacophore of AF, which is a novel specific inhibitor of AOX. This figure illustrated the functional interaction between AF and AOX. The semicircular column and two rectangles represent the diiron catalytic centre of AOX and its substrate-binding cavity, in which AF interacts with AOX. The SAR study revealed that the hydrogen-bonding ability of 1-formyl and 6-hydroxyl group is responsible for potent inhibition of AOX (A–H, hydrogen-bonding donor; B, hydrogen-bonding acceptor). At 4-hydroxyl group, **-OH** is much preferable for potent inhibition rather than **–H**. Furanone ring is not essential for inhibition, suggesting this portion is oriented towards outside of AOX.

Table X. TAO inhibition by derivatives with various linker.

Compound	Structure	IC_{50} (nM)
23		200
24		0.06

and the manufacturing process after absorption, distribution, metabolism, excretion and toxicity study. The identification of the pharmacophore and the elucidation of the interaction between drug and drug target could open the door to a novel drug development of AF for HAT. This study should be highly advantageous to change the necessary physical properties, including optimizing water solubility (for effective absorption *in vivo*) and designing a compound with a simple structure (to reduce synthetic costs). The information from the current SAR study is expected to contribute to the synthesis of a promising candidate.

Supplementary Data

Supplementary data are available at *JB online*.

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Conflict of interest

Not declared.

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Supplementary data

Pharmacophore identification of ascofuranone, potent inhibitor of cyanide-insensitive alternative oxidase of *Trypanosoma brucei*

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Contents

1. Synthesis of ascofuranone derivatives

Synthesis of compound **1**

(2*E*,6*E*)-8-(3-Chloro-5-formyl-2,6-dihydroxy-4-methylphenyl)-2,6-dimethylocta-2,6-dienyl pivalate

According to the reported method (*1, 2*), geranyl acetate was treated with SeO_2 and $NaBH_4$ to give 8-hydroxy-3,7-dimethylocta-2,6-dienyl acetate (**31**), which was converted to 1-acetoxy-2,6-dimethylocta-2,6-dienyl pivalate (two steps, 28% yield). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 5.41 (t, *J*=7.0 Hz, 1H, C*H*=C), 5.35 (t, *J*=7.1 Hz, 1H, C*H*=C), 4.59 (d, *J*=7.0 Hz, 2H, AcOC*H*$_2$), 4.44 (s, 2H, C*H*$_2$OPiv), 2.21-2.15 (m, 2H, C*H*$_2$), 2.11-2.07 (m, 2H, C*H*$_2$), 1.71 (s, 3H, C*H*$_3$), 1.64 (s, 3H, C*H*$_3$), 1.20 (s, 9H, C(C*H*$_3$)$_3$). Acetate selective hydrolysis of the diester was performed by the guanidine method (*3*) to give 8-hydroxy-2,6-dimethylocta-2,6-dienyl pivalate (**32**) (90% yield). ^{1}H-NMR ($CDCl_3$) δ 5.47-5.34 (m, 2H, 2 x C*H*=C), 4.44 (s, 2H, C*H*$_2$OPiv), 4.15 (d, *J*=6.6 Hz, 2H, C*H*$_2$OH), 2.23-2.13 (m, 2H, C*H*$_2$), 2.11-2.03 (m, 2H, C*H*$_2$), 1.67 (s, 3H, C*H*$_3$), 1.64 (s, 3H, C*H*$_3$), 1.42 (br s, 1H, CH$_2$O*H*), 1.21 (s, 9H, C(C*H*$_3$)$_3$). The primary alcohol was brominated with CBr_4 (3.0 eq) and (n-C_8H_{17})$_3$P (3.0 eq) in ether at 0°C. Coupling of the bromide with 3-chloro-4,6-dihydroxy-2-methylbenzaldehyde (**33**) was performed by using a modification of the previously reported method (*4*) to afford **1** (two steps, 41% yield). Mp 60°C. ^{1}H-NMR ($CDCl_3$) δ 12.70 (s, 1H, Ar-O*H*), 10.14 (s, 1H, Ar-C*H*O), 6.54 (s, 1H, Ar-O*H*), 5.38 (t, *J*=6.8 Hz, 1H, C*H*=C), 5.22 (t, *J*=6.8 Hz, 1H, C*H*=C), 4.40 (s, 2H, C*H*$_2$OPiv), 3.39 (d, *J*=6.8 Hz, 2H, Ar-C*H*$_2$), 2.60 (s, 3H, Ar-C*H*$_3$), 2.16-2.11 (m, 2H, C*H*$_2$), 2.04-2.00 (m, 2H, C*H*$_2$), 1.78 (s, 3H, C*H*$_3$), 1.61 (s, 3H, C*H*$_3$), 1.20 (s, 9H, C(C*H*$_3$)$_3$). ^{13}C-NMR ($CDCl_3$) δ 193.3, 178.4, 162.2, 156.4, 137.7, 136.2, 130.3, 128.4, 121.2, 114.4, 113.6, 113.3, 69.9, 39.1, 38.9, 27.2, 26.1, 22.0, 16.1, 14.4, 13.8. IR (KBr) 3244, 2978, 2922, 1728, 1616, 1485, 1450, 1421, 1369, 1279, 1234, 1157, 1105, 1032, 959, 910, 876, 770, 718, 635, 604, 575, 536 cm^{-1}. Found: C, 65.07; H, 7.32; Cl, 8.44%. Calcd for $C_{23}H_{31}ClO_5$: C, 65.32; H, 7.39; Cl, 8.38%.

Synthesis of compound **2**

3-Chloro-4,6-dihydroxy-5-[(2*E*,6*E*)-8-hydroxy-3,7-dimethylocta-2,6-dienyl]-2-methylbenzaldehyde.

8-Hydroxy-3,7-dimethylocta-2,6-dienyl acetate (**31**) was treated with dihydropyrane (2.0 eq) and pyridinium *p*-toluenesulfonate (0.2 eq) in ether at 25°C to afford 3,7-dimethyl-8-(tetrahydropyran-2-yloxy)octa-2,6-dienyl acetate (93% yield), which was hydrolyzed with K_2CO_3 (2.0 eq) in MeOH/H_2O (8/10 v/v) at 25°C to give 3,7-dimethyl-8-(tetrahydropy

ran-2-yloxy)octa-2,6-dien-1-ol (60% yield). ^{1}H-NMR ($CDCl_3$) δ 5.35-5.45 (m, 2H, 2 x C*H*=C), 4.61 (t, *J*=3.4Hz, 1H, THP(2)-*H*), 4.16-4.08 (m, 3H, $HOCH_2$, *H*CHO), 3.9-3.84 (m, 2H, HC*H*O, THP(6)-*H*), 3.45-3.55 (m, 1H, THP(6)-*H*), 2.22-2.07 (m, 4H, CH_2CH_2), 1.83-1.53 (m, 12H, 2 x CH_3, THP(3,4,5)-H_2). The primary alcohol was brominated with CBr_4 (3.0 eq) and (n-C_8H_{17})$_3$P (3.0 eq) in ether at 0°C. Coupling of the bromide with aldehyde **33** was performed by using a modification of the previously reported method (*4*) to afford 3-chloro-4,6-dihydroxy-5-[(2*E*,6*E*)-8-(tetrahydropyran-2-yloxy)-3,7-dimethylocta -2,6-dienyl]-2-methylbenzaldehyde (two steps, 30% yield). Mp 44-45°C. ^{1}H-NMR ($CDCl_3$) δ 12.70 (s, 1H, Ar-O*H*), 10.14 (s, 1H, Ar-C*H*O), 6.66 (s, 1H, Ar-O*H*), 5.37 (t, *J*=6.8 Hz, 1H, C*H*=C), 5.22 (t, *J*=7.1 Hz, 1H, C*H*=C), 4.61 (t, *J*=3.5 Hz, 1H, THP(2)-*H*), 4.0 5 (d, *J*=11.9 Hz, 1H, HC*H*O), 3.83-3.90 (m, 1H, THP(6)-*H*), 3.83 (d, *J*=11.9 Hz, 1H, *H* CHO), 3.48-3.54 (m, 1H, THP(6)-*H*), 3.37-3.41 (m, 2H, Ar-CH_2), 2.61 (s, 3H, Ar-CH_3), 2.0-2.2 (m, 4H, CH_2CH_2), 1.6-1.9 (m + s (δ 1.77, CH_3) + s (δ 1.62, CH_3), 12H, THP(3,4,5)-H_2). IR (KBr) 3200-3500, 1613, 1424, 1281, 1250, 1233, 1111 cm^{-1}. Calcd for $C_{23}H_{31}ClO_5$: C, 65.32; H, 7.39; Cl, 8.38%. Found: C, 65.18; H, 7.36; Cl, 8.41%. Removal of the THP group with pyridinium *p*-toluenesulfonate (0.4 eq) in MeOH at 45°C for 1 h gave **2** (90% yield). Mp 99.0-99.7°C. ^{1}H-NMR ($CDCl_3$) δ 12.72 (s, 1H, Ar-O*H*), 10. 14 (s, 1H, Ar-C*H*O), 5.34 (t, *J*=6.6 Hz, 1H, CH_2C*H*=C), 5.22 (t, *J*=6.9 Hz, 1H, CH_2C*H* =C), 3.97 (d, *J*=6.9 Hz, 2H, Ar-CH_2), 2.61 (s, 3H, Ar-CH_3), 2.0-2.2 (m, 4H, C(CH_3)C*H* $_2$CH_2CH=C), 1.78 (s, 3H, CH_3), 1.64 (s, 3H, CH_3). HRMS (DART) calcd for $C_{18}H_{22}ClO$ $_3$ (M-OH) 321.1257, found 321.1235.

Synthesis of compound **3**

(3*E*,7*E*)-9-(3-Chloro-5-formyl-2,6-dihydroxy-4-methyl)phenyl-3,7-dimethylnona-3,7-dienyl ac etate.

According to the reported method (*5*), geranyl acetate was treated with SeO_2 and MnO_2 to give 8-acetoxy-2,6-dimethylocta-2,6-dienal (**34**). Hydrolysis of the acetate **34** wi th K_2CO_3 (0.5 eq) in MeOH followed by treatment with *t*-butyldimethylsilyl chloride (3. 0 eq) gave 8-(*t*-butyldimethylsilyloxy)-2,6-dimethylocta-2,6-dienal (three steps, 25% yield). ^{1}H-NMR ($CDCl_3$) δ 9.37 (s, 1H, C*H*O), 6.46 (t, *J*=7.1 Hz, 1H, C*H*=CCHO), 5.34 (t, *J* =6.2 Hz, 1H, TBSOCH_2C*H*=C), 4.19 (d, *J*=6.2 Hz, 2H, TBSOCH_2CH), 2.47 (q, *J*=7.3 Hz, 2H, CH_2), 2.19 (t, *J*=7.3 Hz, 2H, CH_2), 1.74 (s, 3H, CH_3), 1.65 (s, 3H, CH_3), 0.89 (s, 9H, C(CH_3)$_3$), 0.06 (s, 6H, Si(CH_3)$_2$). Treatment of the aldehyde with methyl lithiu m (2.0 eq) in THF gave 9-(*t*-butyldimethylsilyloxy)-3,7-dimethylnona-3,7-dien-2-ol, which was acetylated with acetic anhydride and then desilylated with tetrabutylammonium fluo ride to give 9-hydroxy-3,7-dimethylnona-3,7-dien-2-yl acetate (three steps, 47% yield). ^{1}H

-NMR ($CDCl_3$) δ 5.39 (m, 2H, 2 x C*H*=C), 5.22 (q, *J*=6.6 Hz, 1H, C*H*OAc), 4.21 (d, *J*=6.2 Hz, 2H, HOC*H*$_2$CH), 2.19-2.13 (m, 2H, C*H*$_2$), 2.09-2.05 (m, 2H, C*H*$_2$), 2.03 (s, 3 H, COC*H*$_3$), 1.66 (s, 3H, C*H*$_3$), 1.61 (s, 3H, C*H*$_3$), 1.28 (d, *J*=6.6 Hz, 3H, CHCOC*H*$_3$).

The primary alcohol was brominated with CBr_4 (3.0 eq) and (n-C_8H_{17})$_3$P (3.0 eq) in e ther at 0°C. Coupling of the bromide with phenolic compound **33** was performed by usi ng a modification of the previously reported method (*4*) to afford **3** (two steps, 22% yi eld). Mp 101-102°C. ^{1}H-NMR ($CDCl_3$) δ 12.69 (s, 1H, Ar-O*H*), 10.14 (s, 1H, C*H*O), 6. 56 (s, 1H, Ar-O*H*), 5.36 (t, *J*=7.3 Hz, 1H, C*H*=C), 5.20 (m, 2H, C*H*(OAc)CH$_3$ & C*H*= C), 3.39 (d, *J*=7.3 Hz, 2H, Ar-C*H*$_2$), 2.61 (s, 3H, Ar-C*H*$_3$), 2.10 (m, 2H, C*H*$_2$), 2.02 (s, 3H, OC(O)C*H*$_3$), 2.03-2.00 (m, 2H, C*H*$_2$), 1.77 (s, 3H, C*H*$_3$), 1.58 (s, 3H, C*H*$_3$), 1.22 (d, *J*=6.6 Hz, 3H, CH(OAc)C*H*$_3$). IR (KBr) 3356, 2986, 2916, 1711, 1624, 1456, 1422, 1377, 1283, 1254, 1157, 1115, 1080, 1024, 959, 910, 841, 808, 708, 631, 583, 544, 523 cm^{-1}. Found: C, 63.85; H, 6.91; Cl, 8.95%. Calcd for $C_{21}H_{27}ClO_5$: C, 63.87; H, 6.89; Cl, 8.98%.

Synthesis of compound **4** (tetrahydroascofuranone)

3-Chloro-4,6-dihydroxy-5-[3,7-dimethyl-7-(5,5-dimethyl-4-oxotetrahydrofuran-2-yl)heptyl]-2-methylbenzaldehyde

An ethanol solution of ascofuranone was stirred under H_2 atmosphere in the presence of 5% Pd/C at 0°C to give **4** (25% yield). ^{1}H-NMR ($CDCl_3$) δ 0.89 (d, *J*=6.8 Hz, 1.1H, CHC*H*$_3$), 0.955, 0.960, 0.98 (three d, *J*=6.5 Hz, 4.9H, CHC*H*$_3$), 1.05-1.83 (m + s (δ 1.20, C*H*$_3$ of tetrahydrofuran moiety) + s (δ 1.27, C*H*$_3$ of tetrahydrofuran moiety), 16H), 2.11-2.32 (m + s (δ 2.17, Ar-C*H*$_3$), 3.1H), 2.39-2.50 (m, 1H), 2.59-2.78 (m + s (δ 2.60, Ar-C*H*$_3$), 2.9H), 3.94-4.05 (m, 1H, C(2)-*H* of tetrahydrofuran moiety), 6.40 (br s, 1H, Ar-O*H*), 10.14 (s, 1H, C*H*O), 12.65 (s, 1H, Ar-O*H*); IR (neat) 3200-3600, 2934, 1751, 1626, 1460, 1420, 1246 cm^{-1}; MS *m/z* 426 (M+2, 1), 424 (M$^+$, 3), 201 (39), 199 (100). Found: C, 64.72; H, 7.68; Cl, 8.42%. Calcd for $C_{23}H_{33}ClO_5$: C, 65.01; H, 7.83; Cl, 8.34%.

Synthesis of compound **5**

3-Chloro-4,6-dihydroxy-5-[(*E*)-7-(5,5-dimethyl-4-oxotetrahydrofuran-2-yl)hept-1-enyl]-2-methylbenzaldehyde

1,8-Octanediol was treated with dihydropyrane (0.95 eq) and pyridinium *p*-toluenesul fonate (0.2 eq) in chloroform at 25°C to afford 8-(tetrahydropyran-2-yloxy)octanol (54% yield), which was treated with $(COCl)_2$ (2.4 eq) in DMSO at -55°C and then with Et_3N (6.1 eq) to give 8-(tetrahydropyran-2-yloxy)octanal (**35**) (90% yield). ^{1}H-NMR ($CDCl_3$) δ 9.77 (t, *J*=1.8 Hz, 1H, C*H*O), 4.57 (dd, *J*=2.6, 4.8 Hz, 1H, OC*H*O), 3.90-3.84 (m, 1

H, C$\underline{H_2}$O), 3.73 (td, *J*=6.8, 9.6 Hz, 1H, C$\underline{H_2}$O), 3.53-3.48 (m, 1H, C$\underline{H_2}$O), 3.38 (td, *J*=6 .6, 9.5 Hz, 1H, C$\underline{H_2}$O), 2.42 (dt, *J*=1.8, 7.5 Hz, 2H, C$\underline{H_2}$CHO), 1.87-1.78 (m, 1H, OCH C$\underline{H_2}$), 1.75-1.68 (m, 1H, OCHC$\underline{H_2}$), 1.67-1.49 (m, 8H, 4 x C$\underline{H_2}$),1.43-1.28 (m, 6H, 3 x C$\underline{H_2}$). The aldehyde was transformed to 2,2-dimethyl-3-oxo-5-[7-(tertehydropyran-2-yloxy) heptyl]tetrahydrofuran (four steps, 55% yield) by using a modification of the previously reported method (*2*) . ^{1}H-NMR ($CDCl_3$) δ 4.57 (dd, *J*=2.8, 4.2 Hz, 1H, OC$\underline{H}$O), 4.20-4.14 (m, 1H, $CH_2C\underline{H}CH_2C$=O), 3.89-3.85 (m, 1H, C$\underline{H_2}$O), 3.73 (dt, *J*=6.9, 9.4 Hz, 1H, C$\underline{H_2}$O), 3.52-3.48 (m, 1H, C$\underline{H_2}$O), 3.38 (dt, *J*=6.7, 9.6 Hz, 1H, C$\underline{H_2}$O), 2.55 (dd, *J*=5.8, 18.1 Hz, 1H, C$\underline{H_2}$C=O), 2.20 (dd, *J*=10.1, 18.1 Hz, 1H, C$\underline{H_2}$C=O),1.86-1.80 (m, 1H, C $\underline{H_2}$CHO), 1.77-1.69 (m, 2H), 1.64-1.51 (m, 7H), 1.48-1.42 (m, 1H), 1.35 (br, 7H), 1.28 (s, 3H, C$\underline{H_3}$), 1.20 (s, 3H, C$\underline{H_3}$). IR (neat) 2922, 2854, 1757, 1462, 1443, 1369, 1350, 1177, 1119, 1070, 1032, 988, 905, 872, 814, 731 cm^{-1}. Removal of the tetrahydropyrany l group followed by oxidation with $(COCl)_2$ in DMSO gave 7-(5,5-dimethyl-4-oxotetrahy drofuran-2-yl)heptanal (**36**) (two steps, 80% yield). ^{1}H-NMR ($CDCl_3$) δ 9.60 (t, *J*=1.7 H z, 1H, C$\underline{H}$O), 4.01 (m, 1H, $CH_2C\underline{H}CO$), 2.39 (dd, *J*=5.7 Hz, 17.8 Hz, 1H, C$\underline{H_2}$C=O), 2. 27 (dt, *J*=1.6 Hz, 7.4 Hz, 2H, C$\underline{H_2}$CHO), 2.04 (dd, *J*=10.1 Hz, 17.8 Hz, 1H, C$\underline{H_2}$C=O), 1.62-1.52 (m, 1H, C$\underline{H_2}CHCH_2$C=O), 1.51-1.42 (m, 3H), 1.35-1.26 (m, 1H), 1.20 (br s, 5H), 1.09 (s, 3H, C$\underline{H_3}$), 1.03 (s, 3H, C$\underline{H_3}$). IR (neat) 2932, 2860, 2721, 1755, 1724, 14 62, 1375, 1360, 1177, 1113, 1011, 702, 534 cm^{-1}. HRMS (EI) found: 226.1569. Calcd. f or $C_{13}H_{22}O_3$: M^+ 226.1569. Coupling of the aldehyde **36** with phenolic compound **33** wa s performed by using a modification of the previously reported method (*6*) to afford 3-c hloro-4,6-dihydroxy-5-[7-(3,3-dimethyl-4-oxo-2-oxacyclopentyl)-1-hydroxyheptyl]-2-methylbe nzaldehyde, which was treated with 0.2 M H_3PO_4 in acetic acid at 120°C to give comp ound **5** (two steps, 14% yield). Mp 99-100°C. ^{1}H-NMR ($CDCl_3$) δ 13.07 (s, 1H, Ar-O$\underline{H}$), 10.15 (s, 1H, Ar-C$\underline{H}$O), 6.66 (dt, *J*=6.9, 16.3 Hz, 1H, Ar-CH=C$\underline{H}$), 6.59 (s, 1H, Ar-O$\underline{H}$), 6.53 (d, *J*=16.3 Hz, 1H, Ar-C$\underline{H}$=CH), 4.18 (m, 1H, C$\underline{H}CH_2$C=O), 2.62 (s, 3H, Ar-C$\underline{H_3}$), 2.57 (dd, *J*=5.7, 17.9 Hz, 1H, CHC$\underline{H_2}$C=O), 2.28 (q, *J*=6.9 Hz, 2H, CH=CHC$\underline{H_2}$), 2.21 (dd, *J*=10.1, 17.9 Hz, 1H, CHC$\underline{H_2}$C=O), 1.80-1.74 (m, 1H, C$\underline{H_2}CHCH_2$C=O), 1.66 -1.60 (m, 1H, C$\underline{H_2}CHCH_2$C=O), 1.55-1.48 (m, 2H, C$\underline{H_2}$), 1.44-1.35 (m, 4H, (C$\underline{H_2}$)$_2$), 1.2 7 (s, 3H, C$\underline{H_3}$), 1.20 (s, 3H, C$\underline{H_3}$). IR (neat) 3400, 2930, 2858, 1755, 1634, 1462, 1418 , 1375, 1285, 1256, 1175, 1113, 978, 910, 733, 675, 592 cm^{-1}. HRMS (EI) found: 394. 1552. Calcd. for $C_{21}H_{27}O_5Cl$: M^+ 394.1547.

Synthesis of compound **6**

3-Chloro-4,6-dihydroxy-2-methyl-5-[7-(3,3-dimethyl-4-oxo-2-oxacyclopentyl)heptyl]benz aldehyde

An ethyl acetate solution of compound **5** was stirred under H_2 atmosphere in the pr esence of 5% Pd/C at 0°C to give compound **6** (98% yield). Mp 70-71°C. ^{1}H-NMR (C DCl_3) δ 12.66 (s, 1, Ar-O*H*), 10.14 (s, 1H, Ar-C*H*O), 6.32 (br s, 1H, Ar-O*H*), 4.16 (m, 1H, C*H*CH_2C=O), 2.66 (t, *J*=7.7 Hz, 2H, Ar-CH_2), 2.61 (s, 3H, Ar-CH_3), 2.55 (dd, *J*= 5.8, 18.1 Hz, 1H, CHCH_2C=O), 2.20 (dd, *J*=10.1, 18.1 Hz, 1H, CHCH_2C=O), 1.78-1.71 (m, 1H, CH_2CHCH_2C=O), 1.63-1.56 (m, 2H, CH_2), 1.55-1.49 (m, 2H, CH_2), 1.47-1.40 (m, 1H, CH_2CHCH_2C=O), 1.34 (m, 6H, (CH_2)$_3$), 1.28 (s, 3H, CH_3), 1.20 (s, 3H, CH_3). HRMS (EI) found: 396.1690. Calcd. for $C_{21}H_{29}ClO_5$: 396.1704.

Synthesis of compound **7a-7f**

3-chloro-5-(1-dodecenyl)-4,6-dihydroxy-2-methylbenzaldehyde (**7f**) (a typical procedure)

According to the reported prucedure (*6*), coupling reaction of dodecanal with phenolic compound **33** was performed to give 3-chloro-4,6-dihydroxy-5-(1-hydroxydodecyl)-2-methylbenzaldehyde (86% yield). ^{1}H-NMR ($CDCl_3$) δ 0.88 (t, *J* = 6.6 Hz, 3H, $(CH_2)_{10}CH_3$), 1.15-1.55 (m, 18H, $CH_2(CH_2)_9CH_3$), 1.65-1.91 (m, 2H, Ar-CH(OH)CH_2), 2.59 (s, 3H, Ar-CH_3), 3.09 (br s, 1H, Ar-CH(O*H*)CH_2), 5.34 (dd, *J*=5.0, 7.7 Hz, 1H, C(5)-C*H*(OH)CH_2), 9.90 (br s, 1H, Ar-O*H*), 10.08 (s, 1H, C*H*O), 12.79 (s, 1H, C(6)-O*H*); IR (KBr) 3000-3600, 2928, 2860, 1624, 1460, 1373, 1285, 1225 cm^{-1}. The product was treated with 0.2 M H_3PO_4 in acetic acid at 120°C to give compound **7f** (76% yield). ^{1}H-NMR ($CDCl_3$) δ 0.88 (t, *J*=6.6 Hz, 3H, $(CH_2)_7CH_3$), 1.22-1.40 (m, 14H, $(CH_2)_7CH_3$), 1.43-1.55 (m, 2H, Ar-CH=CHCH_2CH_2), 2.22-2.30 (m, 2H, Ar-CH=CHCH_2-), 2.62 (s, 3H, Ar-CH_3), 6.52 (d, *J*=16.2 Hz, 1H, Ar-C*H*=CHCH_2), 6.57 (s, 1H, ArO*H*), 6.65 (dt, *J*=6.5, 16.2 Hz, 1H, Ar-CH=C*H*CH_2), 10.15 (s, 1H, C*H*O), 13.04 (s, 1H, Ar-O*H*); IR(KBr) 3200-3600, 2915, 2849, 1617, 1419, 1283, 1228, 1141, 975 cm^{-1}. Found: C, 67.79; H, 8.39; Cl, 9.83%. Calcd for $C_{20}H_{29}O_3Cl$: C, 68.07; H, 8.28; Cl, 10.05%.

This procedure applies to the synthesis of **7a-7e**.

3-Chloro-4,6-dihydroxy-2-methyl-5-(1-propenyl)benzaldehyde (**7a**)

Two steps, 55% yield. Mp 119-121°C; ^{1}H-NMR ($CDCl_3$) δ 1.96 (d, *J*=6.4 Hz, 3H, CH=CHCH_3), 2.62 (s, 3H, Ar-CH_3), 6.55 (d, *J*=16.1 Hz, 1H, Ar-C*H*=CHCH_3), 6.58 (s, 1H, Ar-O*H*), 6.67 (dq, *J*=6.4, 16.1 Hz, 1H, Ar-CH=C*H*CH_3), 10.15 (s, 1H, C*H*O), 13.05 (s, 1H, Ar-O*H*); IR (KBr) 3200-3600, 2926, 1620, 1415, 1286, 1258, 1130, 978, 793 cm^{-1}. Found: C, 58.28; H, 4.84; Cl, 15.44%. Calcd for $C_{11}H_{11}ClO_3$: C, 58.29; H, 4.89; Cl, 15.64%.

3-Chloro-4,6-dihydroxy-2-methyl-5-(1-pentenyl)benzaldehyde (**7b**)

Two steps, 57% yield. Mp 121-122°C; ^{1}H-NMR ($CDCl_3$) δ 0.97 (t, *J*=7.3 Hz, 3H,

$CH_2CH_2C\underline{H}_3$), 1.48-1.56 (m, 2H, $CH_2C\underline{H}_2CH_3$), 2.23-2.28 (m, 2H, Ar-CH=CHC$\underline{H}_2$), 2.62 (s, 3H, Ar-C$\underline{H}_3$), 6.53 (d, J=16.3 Hz, 1H, Ar-C$\underline{H}$=CHCH$_2$), 6.59 (s, 1H, Ar-O$\underline{H}$), 6.66 (dt, J=6.9, 16.3 Hz, 1H, Ar-CH=C$\underline{H}$CH$_2$), 10.15 (s, 1H, C$\underline{H}$O), 13.06 (s, 1H, Ar-O$\underline{H}$); IR (KBr) 3100-3500, 2957, 2928, 1622, 1414, 1283, 1231, 1138, 1117, 984, 843, 791 cm^{-1}. Found: C, 61.26; H, 5.90; Cl, 14.14%. Calcd for $C_{13}H_{15}ClO_3$: C, 61.30; H, 5.94; Cl, 13.92%.

3-Chloro-5-(1-heptenyl)-4,6-dihydroxy-2-methylbenzaldehyde (**7c**)

Two steps, 76% yield. Mp 96-97°C; ^{1}H-NMR ($CDCl_3$) δ 0.90 (t, J=7.1 Hz, 3H, $(CH_2)_2C\underline{H}_3$), 1.30-1.38 (m, 4H, $(C\underline{H}_2)_2CH_3$), 1.45-1.53 (m, 2H, Ar-CH=CHCH$_2$C$\underline{H}_2$), 2.24-2.29 (m, 2H, Ar-CH=CHC$\underline{H}_2$), 2.62 (s, 3H, Ar-C$\underline{H}_3$), 6.53 (d, J=16.3 Hz, 1H, Ar-C$\underline{H}$=CHCH$_2$), 6.59 (s, 1H, Ar-O$\underline{H}$), 6.66 (dt, J=6.9, 16.3 Hz, 1H, Ar-CH=C$\underline{H}$CH$_2$), 10.15 (s, 1H, C$\underline{H}$O), 13.06 (s, 1H, Ar-O$\underline{H}$); IR (KBr) 3100-3500, 2926, 2854, 1614, 1599, 1418, 1288, 1229, 1136, 980, 772 cm^{-1}. Found: C, 63.46; H, 6.66; Cl, 12.65%. Calcd for $C_{15}H_{19}Cl\ O_3$: C, 63.71; H, 6.77; Cl, 12.54%.

3-Chloro-4,6-dihydroxy-2-methyl-5-(1-nonenyl)benzaldehyde (**7d**)

Two steps, 70% yield. Mp 79.5-80.5°C; ^{1}H-NMR ($CDCl_3$) δ 0.88 (t, J=6.5 Hz, 3H, $(CH_2)_4C\underline{H}_3$), 1.23-1.40 (m, 8H, $(C\underline{H}_2)_4CH_3$), 1.42-1.55 (m, 2H, Ar-CH=CHCH$_2$C$\underline{H}_2$), 2.22-2.30 (m, 2H, Ar-CH=CHC$\underline{H}_2$), 2.62 (s, 3H, Ar-C$\underline{H}_3$), 6.52 (d, J=16.2 Hz, 1H, C(5)-C$\underline{H}$=CHCH$_2$), 6.57 (s, 1H, Ar-O$\underline{H}$), 6.65 (dt, J=6.5, 16.2 Hz, 1H, Ar-CH=C$\underline{H}$CH$_2$), 10.15 (s, 1H, C$\underline{H}$O), 13.04 (s, 1H, Ar-O$\underline{H}$); IR (KBr) 3200-3600, 2922, 2850, 1614, 1416, 1232, 1134, 980, 793 cm^{-1}; MS m/z 312 (M+2, 9), 310 (M$^+$, 25), 201 (35), 199 (100). Found: C, 65.95; H, 7.44; Cl, 11.35%. Calcd for $C_{17}H_{23}ClO_3$: C, 65.69; H, 7.46; Cl, 11.41%.

3-Chloro-5-(1-decenyl)-4,6-dihydroxy-2-methylbenzaldehyde (**7e**)

Two steps, 67% yield. Mp 83-84°C; ^{1}H-NMR ($CDCl_3$) δ 0.88 (t, J=6.8 Hz, 3H, $(CH_2)_5C\underline{H}_3$), 1.22-1.40 (m, 10H, $(C\underline{H}_2)_5CH_3$), 1.45-1.55 (m, 2H, Ar-CH=CHCH$_2$C$\underline{H}_2$), 2.22-2.30 (m, 2H, Ar-CH=CHC$\underline{H}_2$), 2.62 (s, 3H, Ar-C$\underline{H}_3$), 6.52 (d, J=16.2 Hz, 1H, Ar-C$\underline{H}$=CHCH$_2$), 6.57 (s, 1H, Ar-O$\underline{H}$), 6.65 (dt, J=6.5, 16.2 Hz, Ar-CH=C$\underline{H}$CH$_2$), 10.15 (s, 1H, C$\underline{H}$O), 13.04 (s, 1H, Ar-O$\underline{H}$); IR (KBr) 3200-3600, 2922, 2850, 1617, 1420, 1231, 1142, 975, 595 cm^{-1}. Found: C, 66.38; H, 7.60; Cl, 10.85%. Calcd for $C_{18}H_{25}ClO_3$: C, 66.55; H, 7.76; Cl, 10.91%.

Synthesis of compound **8** and **12**

9-Anthryl 8-(3-chloro-5-formyl-2,6-dihydroxy-4-methylphenyl)octanoate (**8**)

8-(3-Chloro-5-formyl-2,6-dihydroxy-4-methylphenyl)octanoic acid (**12**)

Oxidation of aldehyde **35** with $NaClO_2$ followed by treatment with ethanol/H_2SO_4 gave ethyl 8-hydroxyoctanoate (two steps, 25% yield). ^{1}H-NMR ($CDCl_3$) δ 4.12 (q, J=7.0 Hz, 2H,

$OC\underline{H_2}CH_3$), 3.64 (dd, *J*=6.6, 7.3 Hz, 2H, $C\underline{H_2}OH$), 2.29 (t, *J*=7.7 Hz, 2H, $C\underline{H_2}CO_2Et$), 1.66-1.53 (m, 5H, $C\underline{H_2}CH_2OH$, $C\underline{H_2}CH_2CO_2Et$, and $O\underline{H}$), 1.34 (m, 6H, $(C\underline{H_2})_3$), 1.26 (t, *J*=7.0 Hz, 3H, $OCH_2C\underline{H_3}$). The primary alcohol was oxidized with $(COCl)_2$ in DMSO to give the corresponding aldehyde. Similar to the transformation of aldehyde **36** to compound **6** in three steps, the aldehyde was subjected to the coupling reaction, dehydration, and reduction to give ethyl 8-(3-chloro-5-formyl-2,6-dihydroxy-4-methylphenyl)octanoate (2% yield from **35**). Mp 54-55°C. ^{1}H-NMR ($CDCl_3$) δ 12.66 (s, 1H, Ar-$O\underline{H}$), 10.14 (s,1H, Ar-$C\underline{H}O$), 6.34 (s, 1H, Ar-$O\underline{H}$), 4.11 (q, *J*=7.3 Hz, 2H, $CO_2C\underline{H_2}CH_3$), 2.66 (t, *J*=7.5 Hz, 2H, Ar-$C\underline{H_2}$), 2.61 (s, 3H, Ar-$C\underline{H_3}$), 2.28 (t, *J*=7.3 Hz, 2H, $C\underline{H_2}CO_2Et$) 1.65-1.49 (m, 4H, $ArCH_2C\underline{H_2}$ & $C\underline{H_2}CH_2CO_2Et$), 1.34 (br, 6H, $(C\underline{H_2})_3$) 1.26 (t, *J*=7.3 Hz, 3H, $CO_2CH_2C\underline{H_2}$). IR (KBr) 3321, 2930, 2847, 1728, 1612, 1421, 1285, 1244, 1140, 783, 590 cm^{-1}. HRMS (EI) found: 356.1381. Calcd. for $C_{18}H_{25}ClO_5$: 356.1391. Hydrolysis of the ester with NaOH in aqueous acetone gave compound **12** (66% yield). Mp 149-150°C. ^{1}H-NMR ($CDCl_3$) δ 12.66 (s, 1H, Ar-$O\underline{H}$), 10.14 (s, 1H, Ar-$C\underline{H}O$), 6.33 (br s, 1H, Ar-$O\underline{H}$), 2.66 (t, *J*=7.7 Hz, 2H, Ar-$C\underline{H_2}$), 2.61 (s, 3H, Ar-$C\underline{H_3}$), 2.35 (t, 2H, *J*=7.7 Hz, $C\underline{H_2}COOH$), 1.68-1.48 (m, 4H, $C\underline{H_2}CH_2COOH$ & Ar-$CH_2C\underline{H_2}$), 1.35 (br, 6H, $(C\underline{H_2})_3$). IR (KBr) 3350, 2930, 2850, 1710, 1620, 1420, 1370, 1280, 1245, 1135, 1120, 940, 775, 590 cm^{-1}. HRMS (EI) found: 328.1057. Calcd. for $C_{16}H_{21}ClO_5$: 328.1078.

Conpound **12** was treated with 9-anthracenemethanol in the presence of dicyclohexylcarbodiimide and 4-(dimethylamino)pyridine in tetrahydrofuran to give compound **8** (53% yield). Mp 150-151°C. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 12.64 (s, 1H, Ar-$O\underline{H}$), 10.13 (s, 1H, Ar-$C\underline{H}O$), 8.51 (s, 1H, Ar-$\underline{H}$), 8.33 (d, *J*=8.8 Hz, 2H, Ar-$\underline{H}$), 8.03 (d, *J*=8.4 Hz, 2H, Ar-$\underline{H}$), 7.57 (t, *J*=7.7 Hz, 2H, Ar-$\underline{H}$), 7.49 (t, *J*=7.4 Hz, 2H, Ar-$\underline{H}$), 6.29 (s, 1H, Ar-$O\underline{H}$), 6.15 (s, 2H, $CO_2C\underline{H_2}Ar$), 2.62 (t, *J*=7.3 Hz, 2H, Ar-$C\underline{H_2}$), 2.60 (s, 3H, Ar-$C\underline{H_3}$), 2.32 (t, *J*=7.5 Hz, 2H, $C\underline{H_2}CO_2CH_2Ar$), 1.62-1.55 (m, 2H, $C\underline{H_2}$), 1.50-1.42 (m, 2H, $C\underline{H_2}$), 1.27 (br, 6H, $(C\underline{H_2})_3$). IR (KBr) 3356, 2916, 2853, 1717, 1634, 1468, 1421, 1391, 1373, 1296, 1252, 1182, 1126, 1094, 949, 889, 795, 733, 638, 590 cm^{-1}. HRMS (EI) found: 518.1859. Calcd. for $C_{31}H_{31}ClO_5$: 518.1860.

Synthesis of compound **9** and **10**

2,2-Dimethyl-1,3-dioxolan-4-ylmethyl 10-(3-chloro-5-formyl-2,6-dihydroxy-4-methylphenyl)decanoate (**9**)

2-Oxo-1,3-dioxolan-4-ylmethyl 10-(3-chloro-5-formyl-2,6-dihydroxy-4-methylphenyl)decanoate (**10**)

Methyl 10-hydroxydecanoate was oxidized with $(COCl)_2$ in DMSO to give the corresponding aldehyde. Similar to the transformation of aldehyde **36** to compound **6** in three steps, the aldehyde was subjected to the coupling reaction, dehydration, and reduction to give

methyl 10-(3-chloro-5-formyl-2,6-dihydroxy-4-methylphenyl)decanoate (four steps, 17% yield). Mp 87-88°C. ^{1}H-NMR ($CDCl_3$) δ 12.65 (s, 1H, Ar-O$\underline{H}$), 10.14 (s, 1H, Ar-C$\underline{H}$O), 6.37 (br s, 1H, Ar-O$\underline{H}$), 3.67 (s, 3H, COOC$\underline{H_3}$), 2.66 (t, *J*=8.0 Hz, 2H, Ar-C$\underline{H_2}$), 2.60 (s, 3H, Ar-C$\underline{H_3}$), 2.30 (t, *J*=7.7 Hz, 2H, C$\underline{H_2}$COOCH$_3$), 1.65-1.57 (m, 2H, C$\underline{H_2}$), 1.57-1.47 (m, 2H, C$\underline{H_2}$), 1.28 (br, 10H, (C$\underline{H_2}$)$_5$). IR (KBr) 3358, 2928, 2853, 1736, 1611, 1421, 1250, 1171, 1132, 777, 590 cm^{-1}. HRMS (EI) Found: 370.1533. Calcd. for $C_{19}H_{27}ClO_5$: 370.1547. Found: C, 61.41; H, 7.32; Cl, 9.43%. Calcd. for C, 61.53; H, 7.34; Cl, 9.67%. Hydrolysis of the ester with NaOH in aqueous acetone gave 10-(3-chloro-5-formyl-2,6-dihydroxy-4-methylphenyl)decanoic acid (89% yield). Mp 154-156°C. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 12.66 (s, 1H, Ar-O$\underline{H}$), 10.14 (s, 1H, Ar-C$\underline{H}$O), 6.34 (br s, 1H, Ar-O$\underline{H}$), 2.66 (t, *J*=7.7 Hz, 2H, Ar-C$\underline{H_2}$), 2.61 (s, 3H, Ar-C$\underline{H_3}$), 2.35 (t, *J*=7.5 Hz, 2H, C$\underline{H_2}$COOH), 1.67-1.47 (m, 4H, C$\underline{H_2}$CH$_2$COOH & Ar-CH$_2$C$\underline{H_2}$), 1.35 (br, 10H, (C$\underline{H_2}$)$_5$). IR (KBr) 3360, 2920, 2853, 1715, 1614, 1470, 1418, 1371, 1236, 1184, 1126, 934, 847, 773, 588 cm^{-1}. HRMS (EI) found: 356.1408. Calcd. for $C_{18}H_{25}ClO_5$: 356.1391. The carboxylic acid was treated with glycerol 1,2-carbonate in the presence of dicyclohexylcarbodiimide and 4-(dimethylamino)pyridine in tetrahydrofuran to give compound **9** (28% yield). Mp 55-56°C. ^{1}H-NMR ($CDCl_3$) δ 12.65 (s, 1H, Ar-O$\underline{H}$), 10.14 (s, 1H, Ar-C$\underline{H}$O), 6.38 (br s, 1H, Ar-O$\underline{H}$), 4.32 (m, 1H, C$\underline{H}$OC(CH$_3$)$_2$OCH$_2$-), 4.17 (dd, *J*=4.8, 11.7 Hz, 1H, C(O)OC$\underline{H_2}$CH), 4.11-4.06 (m, 2H, CHOC(CH$_3$)$_2$OC$\underline{H_2}$ & C(O)OC$\underline{H_2}$CH), 3.74 (dd, *J*=6.2, 8.4 Hz, 1H, CHOC(CH$_3$)$_2$OC$\underline{H_2}$), 2.66 (t, *J*=7.7 Hz, 2H, Ar-C$\underline{H_2}$), 2.60 (s, 3H, Ar-C$\underline{H_3}$), 2.33 (t, *J*=7.7 Hz, 2H, CH$_2$C$\underline{H_2}$C(O)O), 1.65-1.58 (m, 2H, C$\underline{H_2}$), 1.54-1.48 (m, 2H, C$\underline{H_2}$), 1.43 (s, 3H, C$\underline{H_3}$), 1.39 (s, 3H, C$\underline{H_3}$), 1.28 (br, 10H, (C$\underline{H_2}$)$_5$). ^{13}C-NMR ($CDCl_3$) δ 193.26, 173.69, 162.42, 156.20, 137.24, 115.74, 113.47, 113.04, 109.81, 73.60, 66.35, 64.50, 34.10, 29.47, 29.27, 29.25, 29.14, 29.03, 28.29, 26.67, 25.38, 24.82, 22.82, 14.44. IR (KBr) 3265, 2922, 2853, 1745, 1620, 1526, 1460, 1425, 1369, 1331, 1244, 1219, 1171, 1132, 1092, 1045, 1007, 980, 932, 851, 795, 712, 625, 596, 534 cm^{-1}. HRMS (EI) found: 470.2047. Calcd. for $C_{24}H_{35}ClO_7$: 470.2071.

Treatment of the carboxylic acid with 2,2-dimethyl-1,3-dioxolane-4-mathanol in the presence of dicyclohexylcarbodiimide and 4-(dimethylamino)pyridine in tetrahydrofuran to give compound **10** (33% yield). Mp 70-72°C. ^{1}H-NMR ($CDCl_3$) δ 12.65 (s, 1H, Ar-O$\underline{H}$), 10.13 (s, 1H, Ar-C$\underline{H}$O), 6.49 (br s, 1H, Ar-O$\underline{H}$), 4.93 (m, 1H, CO$_2$CH$_2$C$\underline{H}$OC(O)OCH$_2$), 4.56 (dd, *J*=8.4, 8.8 Hz, 1H, CO$_2$C$\underline{H_2}$CHOC(O)OCH$_2$), 4.37 (dd, *J*=3.3, 1H, 12.6 Hz, CO$_2$CH$_2$CHOC(O)OC$\underline{H_2}$), 4.31 (dd, *J*=5.8, 8.8 Hz, 1H, CO$_2$C$\underline{H_2}$CHOC(O)OCH$_2$), 4.26 (dd, *J*=4.2, 12.6 Hz, 1H, CO$_2$CH$_2$CHOC(O)OC$\underline{H_2}$), 2.66 (t, *J*=7.7 Hz, 1H, Ar-C$\underline{H_2}$), 2.60 (s, 3H, Ar-C$\underline{H_3}$), 2.37 (t, *J*=7.5 Hz, 2H, CH$_2$C$\underline{H_2}$C(O)O), 1.65-1.58 (m, 2H, C$\underline{H_2}$), 1.55-1.48 (m, 2H, C$\underline{H_2}$), 1.29 (br, 10H, (C$\underline{H_2}$)$_5$). ^{13}C-NMR ($CDCl_3$) δ 193.24, 173.27, 162.37, 156.26, 154.36, 137.27, 115.68, 113.41, 113.06, 73.75, 66.96, 62.78, 33.83, 29.41, 29.23, 29.18, 29.05, 28.94, 28.25, 24.66, 22.77, 14.40. IR (KBr) 3362, 2922, 2853, 1788, 1736, 1620, 1599, 1468, 1416, 1398, 1283, 1248, 1165, 1136,

1092, 1040, 878, 752, 586 cm^{-1}. HRMS (EI) found: 456.1546. Calcd. for $C_{22}H_{29}ClO_8$: 456.1551.

Synthesis of compound **11**

5-Chloro-2,4-dihydroxy-3-(8-hydoroxyoctyl)-6-methylbenzaldehyde.

Similar to the transformation of aldehyde **36** to compound **6** in three steps, aldehyde **35** was subjected to the coupling reaction, dehydration, and reduction. In this case, the tetrahydropyranyl group in **35** was exchanged to acetyl group under acidic conditions in the second step. Therefore, 8-(3-chloro-5-formyl-2,6-dihydroxy-4-methylphenyl)octyl acetate was obtained. Hydrolysis of the ester with NaOH in aqueous acetone gave compound **11** (four steps, 16% yield). Mp 129-130°C. ^{1}H-NMR ($CDCl_3$) δ 12.66 (s, 1H, Ar-O*H*), 10.14 (s, 1H, Ar-C*H*O), 6.33 (s, 1H, Ar-O*H*), 3.64 (t, *J*=6.2 Hz, 2H, C*H*$_2$OH), 2.67 (t, *J*=7.3 Hz, 2H, Ar-C*H*$_2$), 2.61 (s, 3H, Ar-C*H*$_3$), 1.64-1.47 (m, 4H, C*H*$_2$CH$_2$OH & Ar-CH$_2$C*H*$_2$), 1.34 (br, 8H, (C*H*$_2$)$_4$). IR (KBr) 3539, 2924, 1627, 1421, 1296, 1257, 1132, 1016, 812 cm^{-1}. HRMS (EI) found: 314.1265. Calcd. for $C_{16}H_{23}ClO_4$: 314.1285.

Synthesis of compound **13**

12-(3-Chloro-5-formyl-2,6-dihydroxy-4-methylphenyl)dodecanoic acid

The Baeyer-Villiger oxidation of cyclododecanone followed by treatment with ethanol/H_2SO_4 gave ethyl 12-hydroxydodecanoate (two steps, 55% yield). ^{1}H-NMR ($CDCl_3$) δ 4.14 (q, *J*=7.3 Hz, 2H, CO$_2$C*H*$_2$CH$_3$), 3.64 (t, *J*=6.4 Hz, 2H, C*H*$_2$OH), 2.26 (t, *J*=7.5 Hz, 2H, C*H*$_2$CO$_2$Et), 1.71 (br s, 1H, O*H*), 1.68-1.53 (m, 4H, C*H*$_2$CH$_2$OH & C*H*$_2$CH$_2$CO$_2$Et), 1.28 (m, 14H, (C*H*$_2$)$_7$), 1.26 (t, *J*=7.3 Hz, 3H, CO$_2$CH$_2$C*H*$_3$). The primary alcohol was oxidized with $(COCl)_2$ in DMSO to give the corresponding aldehyde (95% yield). Mp 60-61°C. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 9.77 (t, *J*=1.8 Hz, 1H, C*H*O), 4.12 (q, *J*=7.1 Hz, 2H, CO$_2$C*H*$_2$CH$_3$), 2.42 (dt, *J*=1.8, 7.3 Hz, 2H, CH$_2$C*H*$_2$CHO), 2.28 (t, *J*=7.6 Hz, 2H, C*H*$_2$CO$_2$Et), 1.65-1.58 (m, 4H, C*H*$_2$CH$_2$CHO & C*H*$_2$CH$_2$CO$_2$Et), 1.28 (br, 12H, (C*H*$_2$)$_6$), 1.25 (t, *J*=7.1 Hz, 3H, CO$_2$CH$_2$C*H*$_3$). Similar to the transformation of aldehyde **36** to compound **6** in three steps, the aldehyde was subjected to the coupling reaction, dehydration, and reduction to give ethyl 12-(3-chloro-5-formyl-2,6-dihydroxy-4-methylphenyl)dodecanoate (three steps, 7% yield). Mp 59-60°C. ^{1}H-NMR ($CDCl_3$) δ 12.65 (s,1H, Ar-O*H*), 10.14 (s, 1H, Ar-C*H*O), 6.33 (s, 1H, Ar-O*H*), 4.12 (q, *J*=7.2 Hz, 2H, CO$_2$C*H*$_2$CH$_3$), 2.66 (t, *J*=7.7 Hz, 2H, Ar-C*H*$_2$), 2.61 (s, 3H, Ar-C*H*$_3$), 2.28 (t, *J*=7.3 Hz, 2H, C*H*$_2$CO$_2$Et), 1.63-1.56 (m, 2H, C*H*$_2$), 1.54-1.49 (m, 2H, C*H*$_2$), 1.38-1.24 (m, 17H, (C*H*$_2$)$_7$ & CO$_2$CH$_2$C*H*$_3$). IR (KBr) 3348, 2930, 2853, 1736, 1610, 1452, 1416, 1377, 1327, 1279, 1240, 1167, 1128, 1020, 916, 860, 785, 708, 590 cm^{-1}. HRMS (EI) calcd for $C_{22}H_{33}ClO_5$: 412.2018, found 412.2032. Hydrolysis of the ester with NaOH in aqueous acetone gave compound **13** (80% yield). Mp 130-131°C. ^{1}H-NMR ($CDCl_3$) δ 12.66 (s, 1H,Ar-O*H*), 10.14 (s,

1H, Ar-C*H*O), 6.34 (br s, 1H, Ar-O*H*), 2.66 (t, *J*=7.7 Hz, 2H, Ar-C*H*$_2$), 2.61 (s, 3H, Ar-C*H*$_3$), 2.35 (t, *J*=7.3 Hz, 2H, C*H*$_2$COOH), 1.65-1.46 (m, 4H, C*H*$_2$CH$_2$COOH & Ar-CH$_2$C*H*$_2$), 1.35 (br, 14H, (C*H*$_2$)$_7$). IR (KBr) 3360, 2920, 2855, 1715, 1612, 1472, 1420, 1283, 1246, 1180, 1126, 937, 853, 785, 588 cm^{-1}. HRMS (EI) found: 384.1712. Calcd. for $C_{20}H_{29}ClO_5$: 384.1704.

Synthesis of compound **14**

5'-Chloro-2',4'-dihydroxy-6'-methyl-3'-[(2*E*,6*E*)-7-(5,5-dimethyl-4-oxotetrahydrofuran-2-yl)-3,7-dimethylhepta-2,6-dienyl]acetophenone

Acetylation of orcinol with acetic acid/BF_3·OEt_2 at 80°C for 18 h gave 2',4'-dihydroxy-6'-methylacetophenone (65% yield). ^{1}H-NMR ($CDCl_3$) δ 13.44 (s, 1H, Ar-O*H*), 6.26 (d, *J*=2.6 Hz, 1H, Ar-*H*), 6.24 (d, *J*=2.6 Hz, 1H, Ar-*H*), 5.43 (s, 1H, Ar-O*H*), 2.63 (s, 3H, Ar-C*H*$_3$), 2.56 (s, 3H, Ar-COC*H*$_3$). The acetophenone was chlorinated with *N*-chlorosuccinimide in acetic acid to afford 3'-chloro-4',6'-dihydroxy-2'-methylacetophenone (**37**) (65% yield). ^{1}H-NMR ($CDCl_3$) δ 12.37 (s, 1H, Ar-O*H*), 6.52 (s, 1H, Ar-*H*), 6.09 (s, 1H, Ar-O*H*), 2.63 (br s, 6H, Ar-C*H*$_3$ and ArCOC*H*$_3$). According to the reported procedure (*4*), 4,5-dihydro-5-[(*1E*, *5E*)-7-hydroxy-1,5-dimethylhepta-1,5-dienyl]-2,2-dimethyl-3(2*H*)-furanone (**38**), prepared from aldehyde **34** in four steps, was subjected to bromination and coupling reaction with phenolic substrate **37** to give compound **14** (two steps, 22% yield). ^{1}H-NMR ($CDCl_3$) δ 12.64 (s, 1H, Ar-O*H*), 6.26 (s, 1H, Ar-O*H*), 5.50 (t, *J*=7.0 Hz, 1H, Ar-CH$_2$C*H*=C), 5.21 (t, *J*=6.8 Hz, 1H, C*H*=C), 4.52 (dd, *J*=6.4, 10.0 Hz, 1H, C*H*CH$_2$C=O), 3.40 (d, *J*=7.0 Hz, 2H, Ar-C*H*$_2$CH), 2.61 (s, 3H, Ar-COC*H*$_3$), 2.59 (s, 3H, Ar-C*H*$_3$), 2.40 (dd, *J*=6.4, 18.3 Hz, 1H, CHC*H*$_2$C=O), 2.34 (dd, *J*=10.0, 18.3 Hz, 1H, CHC*H*$_2$C=O), 2.19-2.13 (m, 2H, C*H*$_2$), 2.07-2.01 (m, 2H, C*H*$_2$), 1.79 (s, 3H, C*H*$_3$), 1.62 (s, 3H, C*H*$_3$), 1.28 (s, 3H, C*H*$_3$), 1.22 (s, 3H, C*H*$_3$). Found: C, 66.01; H, 7.28; Cl, 8.18%. Calcd for $C_{24}H_{31}ClO_5$: C, 66.27; H, 7.18; Cl, 8.15%.

Synthesis of compound **15**

(2*E*,6*E*)-8-(5-Acetyl-3-chloro-2,6-dihydroxy-4-methylphenyl)-2,6-dimethylocta-2,6-dienyl pivalate

According to the reported procedure (*4*), 8-hydroxy-2,6-dimethylocta-2,6-dienyl pivalate **32** was subjected to bromination and coupling reaction with phenolic substrate **37** to give compound **15** (two steps, 10% yield). ^{1}H-NMR ($CDCl_3$) δ 12.62 (s, 1, Ar-O*H*), 6.31 (s, 1H, Ar-O*H*), 5.38 (t, *J*=7.0 Hz, 1H, C*H*=C), 5.23 (t, *J*=6.2 Hz, 1H, C*H*=C), 4.39 (s, 2H, C*H*$_2$OPiv), 3.40 (d, *J*=7.0 Hz, 2H, Ar-C*H*$_2$), 2.61 (s, 3H, Ar-C*H*$_3$), 2.59 (s, 3H, C*H*$_3$C=O), 2.17-2.10 (m, 2H, C*H*$_2$), 2.06-1.98 (m, 2H, C*H*$_2$), 1.79 (s, 3H, C*H*$_3$), 1.60 (s, 3H, C*H*$_3$), 1.19 (s, 9H, C(C*H*$_3$)$_3$). IR (KBr) 3412, 2978, 2922, 1728, 1610, 1464, 1416, 1360, 1279, 1157, 1094, 1036, 984, 951, 841, 768, 600 cm^{-1}. HRMS (EI) found: 436.2024. Calcd. for $C_{24}H_{33}ClO_5$: 436.2017.

Synthesis of compound **16**

(*E*)-3-Chloro-4,6-dihydroxy-5-(3,7-dimethylocta-2,6-dienyl)-2-methylacetophenone

According to the reported procedure (*4*), coupling reaction of phenolic substrate **37** with geranyl bromide was performed to give compound **16** (3% yield). Mp 57-58°C. ^{1}H-NMR ($CDCl_3$) δ 12.56 (s, 1H, Ar-O*H*), 6.25 (s, 1H, Ar-O*H*), 5.23 (t, *J*=7.0 Hz, 1H, Ar-CH_2CH=C), 5.06 (t , *J*=6.7 Hz, 1H, C*H*=$C(CH_3)_2$), 3.41 (d, *J*=7.0 Hz, 2H, Ar-CH_2), 2.61 (s, 3H, Ar-CH_3), 2.58 (s, 3H, CH_3C=O), 2.10-2.03 (m, 2H, CH_2), 2.01-1.95 (m, 2H, CH_2), 1.79 (s, 3H, CH_3), 1.65 (s, 3H, CH_3), 1.57 (s, 3H, CH_3). IR (KBr) 3460, 2922, 2866, 1595, 1468, 1421, 1381, 1360, 1275, 1236, 1209, 1175, 1094, 993, 916, 826, 785, 638, 621, 600 cm^{-1}. Found: C, 67.80; H, 7.59 %. Calcd. for $C_{19}H_{25}ClO_3$: C, 67.75; H, 7.48 %.

Synthesis of compound **17**

5-[(*E,E*)-7-(3-Chloro–2,6-dihydroxy-5-hydroxyiminomethyl-4-methylphenyl)-1,5-dimethylhepta-1,5-dienyl]-4,5-dihydro-2,2-dimethyl-3(2*H*)-furanone (ascofuranone aldoxime)

Ascofuranone was treated with hydroxylamine hydrochloride in pyridine at 25°C to give compound **17** (20% yield). Mp 102-103°C; ^{1}H-NMR ($CDCl_3$) δ 1.23 (s, 3H, CH_3), 1.29 (s, 3H, CH_3), 1.64 (s, 3H, CH_3), 1.78 (s, 3H, CH_3), 1.99-2.10 (m, 2H), 2.14-2.20 (m, 2H), 2.42 (s, 3H, Ar-CH_3), 2.43 (dd, *J*=9.4, 18.2 Hz, 1H, H-C(4)-*H*), 2.46 (dd, *J*=6.8, 18.2 Hz, 1H, *H*-C(4)-H), 3.41 (d, *J*=6.9 Hz, 2H, Ar-CH_2), 4.52 (dd, *J*=6.8, 9.4 Hz, 1H, C(5)-*H*), 5.19 (t, *J*=6.5 Hz, 1H), 5.51 (t, *J* = 6.9 Hz, 1H), 6.97 (s, 1H, Ar-O*H*), 7.65 (s, 1H, N-O*H*), 8.53 (s, 1H, C*H*=N), 10.72 (s, 1H, Ar-O*H*). Found: C, 63.08; H, 6.98; N, 3.06; Cl, 8.33%. Calcd for $C_{23}H_{30}ClNO_5$: C, 63.37; H, 6.94; N, 3.21; Cl, 8.13%.

Synthesis of compound **18**

Methyl (*E*)-3-Chloro-4,6-dihydroxy-5-(3,7-dimethylocta-2,6-dienyl)-2-methylbenzoate

Oxidation of aldehyde **33** with $NaClO_2$ in DMSO followed by esterification with PPh_3/diethyl azodicarboxylate/methanol gave methyl 3-chloro-4,6-dihydroxy-2-methylbenzoate (two steps, 60% yield). ^{1}H-NMR ($CDCl_3$) δ 11.42 (s, 1H, Ar-O*H*), 6.54 (s, 1H, Ar-*H*), 6.06 (s, 1H, Ar-O*H*), 3.95 (s, 3H, CO_2CH_3), 2.63 (s, 3H, Ar-*H*). According to the reported procedure (*4*), coupling reaction of the benzoate with geranyl bromide was performed to give compound **18** (3% yield). ^{1}H-NMR (500 MHz, $CDCl_3$) δ 11.65 (s, 1H, Ar-O*H*), 6.20 (s, 1H, Ar-O*H*), 5.23 (t, *J*=7.1 Hz, 1H, Ar-CH_2CH=C), 5.06 (t, *J*=6.9 Hz, 1H, C*H*=$C(CH_3)_2$), 3.94 (s, 3H, CO_2CH_3), 3.44 (d, *J*=7.1 Hz, 2H, Ar-CH_2CH), 2.59 (s, 3H, Ar-CH_3), 2.09-2.03 (m, 2H, CH_2), 2.00-1.96 (m, 2H, CH_2), 1.79 (s, 3H, CH_3), 1.65 (s, 3H, CH_3), 1.57 (s, 3, CH_3). IR (KBr) 3508, 2935, 1655, 1603, 1464, 1439, 1415, 1383, 1313, 1292, 1258. 1202, 1196, 1161, 1088, 978, 799, 700 cm^{-1}. HRMS

(EI) Found: m/z, 338.1277. Calcd for $C_{18}H_{23}O_4Cl$: M^+, 338.1285.

Synthesis of compound **19**

4,6-dichloro-5-methyl-2-nonylresorcinol

The mixture of 5-methylresorcinol and nonanoyl chloride (1.2 eq) heated at 140°C was added $AlCl_3$ (1.2 eq) to promote the acylation of benzene ring. From the mixture of mono- and di- acylated products, 5-methyl-2-nonanoylresorcinol was isolated (30% yield). The ketone was reduced to secondary alcohol by $NaBH_4$ (1.0 eq) in EtOH (74% yield). The secondary alcohol was reduced to 5-methyl-2-nonylresorcinol, in the presence 10% Pd/C in EtOH with catalytic amount of HCl under H_2 atmosphere (56% yield). The chlorination was performed with SO_2Cl_2 (1.5 eq) in Et_2O at 4°C. Mono- and di- chlorinated products were purified by silica gel chromatography (hexane/AcOEt, 95:5), yielding 4-chloro-5-methyl-2-nonylresorcinol (48%) and 4,6-dichloro-5-methyl-2-nonylresorcinol (31%). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 0.88 (t, *J*=6.9 Hz, 3H, -C*H*$_3$), 1.25-1.35 (m, 12H, (C*H*$_2$)$_6$), 1.53 (m, 2H, Ar-CH$_2$C*H*$_2$), 2.42 (s, 3H, Ar-C*H*$_3$), 2.69 (t, *J*=7.7 Hz, Ar-C*H*$_2$), 5.61 (s, 2H, Ar-O*H*)

Synthesis of compound **20**

4-chloro-5-methyl-6-nitro-2-nonylresorcinol

Chloroform solution of 4-chloro-5-methyl-2-nonylresorcinol was stirred with nitric acid (2.0 eq) containing catalytic amount of H_2SO_4 at R.T. to afford 4-chloro-5-methyl-6-nitro-2-nonylresorcinol. The product was purified by silica gel chromatography (hexane/AcOEt, 95:5, 49% yield) ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 0.89 (t, *J*=6.9 Hz, 3H, -C*H*$_3$), 1.25-1.35 (m, 12H, (C*H*$_2$)$_6$), 1.53 (m, 2H, Ar-CH$_2$C*H*$_2$), 2.65 (s, 3H, Ar-C*H*$_3$), 2.72 (t, *J*=7.8 Hz, Ar-C*H*$_2$) , 6.31 (s, 1H, Ar-O*H*), δ 10.8 (s, 1H, Ar-O*H*)

Synthesis of compound **21**

7-(3-Chloro-5-cyano-2,6-dihydroxy-4-methylphenyl)heptyl pivalate

Aldehyde **33** was treated with hydroxylamine hydrochloride and AcONa in acetic ac id to give the corresponding aldoxime, which was stirred in acetic anhydride at reflux t emperature to afford 4,6-diacetoxy-3-chloro-2-methylbenzonitrile (two steps, 74% yield). ^{1} H-NMR ($CDCl_3$) δ 7.06 (s, 1H, Ar-*H*), 2.64 (s, 3H, Ar-C*H*$_3$), 2.39 (s, 3H, OCOC*H*$_3$), 2 .37 (s, 3H, OCOC*H*$_3$). ^{13}C-NMR ($CDCl_3$) 167.9, 167.5, 151.2, 150.7, 142.7, 125.7, 116.4 , 113.6, 106.8, 20.8, 20.6, 19.4. Similar to the transformation of aldehyde **36** to compou nd **6** in three steps, 7-pivaloyloxyheptanal was subjected to the coupling reaction with th e benzonitrile, dehydration, and reduction to afford compound **21** (three steps, 9% yield). Mp 67-68°C. ^{1}H-NMR ($CDCl_3$) δ 6.21 (br s, 1H, Ar-O*H*), 6.17 (s, 1H, Ar-O*H*), 4.05 (

t, J=6.6 Hz, 2H, C$\underline{H_2}$OPiv), 2.66 (t, J=7.7 Hz, 2H, Ar-C$\underline{H_2}$), 2.51 (s, 3H, Ar-C$\underline{H_3}$), 1.66 -1.58 (m, 2H, C$\underline{H_2}$), 1.56-1.48 (m, 2H, C$\underline{H_2}$), 1.35 (br, 6H, (C$\underline{H_2}$)$_3$), 1.20 (s, 9H, C(C$\underline{H_3}$)$_3$). ^{13}C-NMR ($CDCl_3$) 178.8, 156.3, 154.1, 137.2, 115.8, 115.3, 113.4, 93.9, 64.4, 38.8, 29.3, 28.9, 28.6, 28.3, 27.2, 25.8, 23.7, 18.9. IR (KBr) 3383, 2926, 2853, 2232, 1715, 1593, 1468, 1416, 1366, 1325, 1286, 1244, 1171, 1119, 1057, 1036, 980, 847, 799, 690, 627, 590 cm^{-1}. Found: C, 62.79; H, 7.31; Cl, 9.33; N, 3.70%. Calcd for $C_{20}H_{28}ClNO_4$: C, 62.90; H, 7.39; Cl, 9.28; N, 3.67%.

Synthesis of compound **22**

7-(5-Acetyl-3-chloro-2,6-dihydroxy-4-methylphenyl)heptyl pivalate

Similar to the transformation of aldehyde **36** to compound **6** in three steps, 7-pivaloyloxyheptanal was subjected to the coupling reaction with acetophene **37**, dehydration, and reduction to afford compound **22** (three steps, 24% yield). ^{1}H-NMR ($CDCl_3$) δ 12.64 (s, 1H, Ar-O$\underline{H}$), 6.15 (br s, 1, Ar-O$\underline{H}$), 4.04 (t, J=6.6 Hz, 2H, C$\underline{H_2}$OPiv), 2.67 (t, J=7.7 Hz, 2H, Ar-C$\underline{H_2}$), 2.61 (s, 3H, Ar-C$\underline{H_3}$), 2.59 (s, 3H, C$\underline{H_3}$C=O), 1.66-1.58 (m, 2H, C$\underline{H_2}$), 1.55-1.48 (m, 2H, C$\underline{H_2}$), 1.36 (br, 6H, (C$\underline{H_2}$)$_3$), 1.19 (s, 9H, C(C$\underline{H_3}$)$_3$). IR (KBr) 3412, 2943, 2866, 1720, 1607, 1464, 1416, 1366, 1273, 1161, 1115, 1074, 1036, 984, 860, 770, 596 cm^{-1}. HRMS (EI) found: 398.1870. Calcd for $C_{21}H_{31}ClO_5$: 398.1860.

Synthesis of compound **23**

4,6-dichloro-2-nonanoyl-5-methylresorcinol

5-methyl-2-nonanoylresorcinol was chlorinated with SO_2Cl_2 (1.2 eq) in Et_2O at 4°C. 4,6-dichloro-2-nonanoyl-5-methylresorcinol was purified by silica gel chromatography (hexane/AcOEt, 95:5, 30% yield). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 0.88 (t, J=6.9 Hz, 3H, -C$\underline{H_3}$), 1.25-1.35 (m, 10H, (C$\underline{H_2}$)$_5$), 1.70 (m, 2H, COCH$_2$C$\underline{H_2}$), 2.53 (s, 3H, Ar-C$\underline{H_3}$), δ 3.14 (t, J=7.4 Hz, 2H, COC$\underline{H_2}$), δ 10.2 (br s, 2H, Ar-O$\underline{H}$)

Synthesis of compound **24**

3-Chloro-4,6-dihydroxy-2-methyl-5-[(*E*)-3-methyloct-2-enyl]benzaldehyde

The Horner-Wadsworth-Emmons reaction of 2-heptanone with triethyl phosphonoacetate followed by DIBAL reduction in toluene at -85°C gave (*E*)-3-methyloct-2-enol (two steps, 41% yield). ^{1}H-NMR ($CDCl_3$) δ 5.40 (dt, J=1.1, 7.0 Hz, 1H, C$\underline{H}$=C), 4.15 (d, J=7.0 Hz, 2H, C$\underline{H_2}$OH), 2.01 (t, J=7.7 Hz, 2H, CH=C(CH$_3$)C$\underline{H_2}$), 1.67 (s, 3H, CH=C(C$\underline{H_3}$)CH$_2$), 1.45-1.38 (m, 2H, CH$_2$CH$_2$CH$_2$C$\underline{H_2}$CH$_3$), 1.36-1.21 (m, 5H, CH$_2$O$\underline{H}$ & CH$_2$C$\underline{H_2}$C$\underline{H_2}$CH$_2$CH$_3$), 0.89 (t, J=7.0 Hz, 3H, (CH$_2$)$_4$C$\underline{H_3}$). By using a modification of the reported method (*4*), the primary alcohol was brominated with CBr_4/Ph_3P and subjected to the coupling reaction with aldehyde **33** to give

compound **24** (two steps, 23% yield). Mp 99-101°C. ^{1}H-NMR ($CDCl_3$) δ 12.70 (s, 1H, Ar-O*H*), 10.14 (s, 1H, Ar-C*H*O), 6.42 (s, 1H, Ar-O*H*), 5.21 (tq, *J*=1.1, 7.0 Hz, 1H, C*H*=C), 3.40 (d, *J*=7.0 Hz, 2H, Ar-CH_2), 2.60 (s, 3H, Ar-CH_3), 1.96 (t, *J*=7.5 Hz, 2H, CH=C(CH_3)CH_2), 1.78 (s, 3H, CH=C(CH_3)CH_2), 1.41-1.34 (m, 2H, $CH_2(CH_2)_2CH_2CH_3$), 1.31-1.18 (m, 4H, $CH_2(CH_2)_2CH_2CH_3$), 0.86 (t, *J*=7.1 Hz, 3H, $CH_2(CH_2)_3CH_3$). IR (KBr) 3341, 2922, 2860, 1620, 1525, 1464, 1421, 1373, 1330, 1279, 1234, 1165, 1111, 955, 907, 876, 787, 715, 625, 592, 561 cm^{-1}. Found: C, 65.43; H, 7.44; Cl, 11.43%. Calcd for $C_{17}H_{23}ClO_3$: C, 65.69; H, 7.46; Cl, 11.41%.

Synthesis of compound **25** (demethyl AF)

5-Chloro-2,4-dihydroxy-3-[(2*E*,6*E*)-7-(5,5-dimethyl-4-oxotetrahydrofuran-2-yl)-3,7-dimethylhepta-2,6-dienyl]benzaldehyde

According to the reported procedure (*4*), 4,5-dihydro-5-[(*1E*, *5E*)-7-hydroxy-1,5-dimethylhepta-1,5-dienyl]-2,2-dimethyl-3(2*H*)-furanone (**38**) was subjected to bromination and coupling reaction with 5-chloro-2,4-dihydroxybebzaldehyde to give compound **25** (two steps, 11% yield). Mp 70-72°C. ^{1}H-NMR ($CDCl_3$) δ 11.54 (s, 1H, Ar-O*H*), 9.67 (s, 1H, C*H*O), 7.40 (s, 1H, Ar-*H*), 6.39 (s, 1H, Ar-O*H*), 5.51 (t, *J*=6.8 Hz, 1H, CH_2CH_2CH=C), 5.22 (t, *J*=7.1 Hz, 1H, $ArCH_2CH$=C), 4.53 (dd, *J*=6.2, 9.9 Hz, 1H, C(O)CH_2CH), 3.42 (d, *J*=7.1 Hz, 2H, $ArCH_2CH$=C), 2.46 (dd, *J*=6.2, 18.0 Hz, 1H, C(O)CH_2CH), 2.38 (dd, *J*=9.9, 18.0 Hz, 1H, C(O)CH_2CH), 2.20-2.14 (m, 2H, CH_2), 2.08-2.02 (2H, m, CH_2), 1.79 (s, 3H, CH_3), 1.63 (s, 3H, CH_3), 1.29 (s, 3H, CH_3), 1.23 (s, 3H, CH_3). IR (KBr) 3327, 2986, 2921, 2853, 1753, 1649, 1620, 1473, 1433, 137, 1331, 1290, 1252, 1205, 1167, 1111, 1084, 993, 916, 876, 820, 743, 610, 561, 523 cm^{-1}. HRMS (EI) found: 406.1537. Calcd for $C_{22}H_{27}ClO_5$: 406.1547.

Synthesis of compound **26**

(*E*)-5-Chloro-2,4-dihydroxy-3-(3,7-dimethylocta-2,6-dienyl)benzaldehyde

According to the reported procedure (*4*), $CaCl_2$/KOH mediated reaction of 5-chloro-2,4-dihydroxybebzaldehyde with geranyl bromide was performed in methanol to give compound **26** (10% yield). Mp 94-95°C. ^{1}H-NMR ($CDCl_3$) δ 11.53 (s, 1H, Ar-O*H*), 9.67 (s, 1H, Ar-C*H*O), 7.40 (s, 1H, Ar-*H*), 6.33 (s, 1H, Ar-O*H*), 5.23 (t, *J*=7.3 Hz, 1H, Ar-CH_2C*H*=C), 5.05 (t, *J*=7.0 Hz, 1H, C*H*=C(CH_3)$_2$), 3.44 (d, *J*=7.3 Hz, 2H, Ar-CH_2CH), 2.10-2.04 (m, 2H, CH_2), 2.02-1.98 (m, 2H, CH_2), 1.80 (s, 3H, CH_3), 1.65 (s, 3H, CH_3), 1.57 (s, 3H, CH_3). IR (KBr) 3231, 2916, 1628, 1576, 1464, 1425, 1387, 1331, 1275, 1240. 1202, 1157, 1088, 912, 876, 750, 715, 604 cm^{-1}. HRMS (EI) found: 308.1173. Calcd for $C_{17}H_{21}O_3Cl$: 308.1179.

Synthesis of compound **27**

(*E*)-2,4-Dihydroxy-3-(3,7-dimethylocta-2,6-dienyl)-6-methylbenzaldehyde

According to the reported procedure (*4*), known compound **27** (colletrin B) (*7*) was synthesized from 2,4-dihydroxy-6-methylbebzaldehyde and geranyl bromide (11% yield). Mp 120-121°C. ^{1}H-NMR ($CDCl_3$) δ 12.78 (s, 1H, Ar-O*H*), 10.08 (s, 1H, Ar-C*H*O), 6.21 (s, 1H, Ar-*H*), 6.15 (s, 1H, Ar-O*H*), 5.26 (t, *J*=7.1 Hz, 1H, Ar-CH_2C*H*=C), 5.04 (t, *J*=6.8 Hz, 1H, C*H*=C(CH_3)$_2$), 3.41 (d, *J*=7.1 Hz, 2H, ArCH_2CH), 2.50 (s, 3H, Ar-CH_3), 2.14-2.05 (m, 4H, CH_2CH_2),1.81 (s, 3H, CH_3), 1.68 (s, 3H, CH_3), 1.59 (s, 3H, CH_3). IR (KBr) 3132, 2908, 1610, 1491, 1435, 1327, 1254, 1217, 1171, 1101, 1003, 829, 750, 644, 569 cm^{-1}.

Synthesis of compound **28**

(*E*)-2,4-Dihydroxy-3-(3,7-dimethylocta-2,6-dienyl)benzaldehyde

According to the reported procedure (*4*), $CaCl_2$/KOH mediated reaction of 2,4-dihyd roxybebzaldehyde with geranyl bromide was performed in methanol to give compound **28** (10% yield). Mp 85°C. ^{1}H-NMR ($CDCl_3$) δ 11.79 (s, 1H, Ar-O*H*), 9.69 (s, 1H, Ar-C*H*O), 7.32 (d, *J*=8.6 Hz, 1H, Ar-*H*), 6.48 (d, *J*=8.6 Hz, 1H, Ar-*H*), 6.21 (s, 1H, Ar-O *H*), 5.27 (t, *J*=7.0 Hz, 1H, Ar-CH_2C*H*=C), 5.05 (m, 1H, C*H*=C(CH_3)$_2$), 3.45 (d, *J*=7.0 H z, 2H, Ar-CH_2), 2.16-2.05 (m, 4H, CH_2CH_2), 1.82 (s, 3H, CH_3), 1.68 (s, 3H, CH_3). IR (KBr) 3145, 2922, 1620, 1487, 1443, 1383, 1313, 1248, 1213, 1150, 1059, 787, 718, 64 2, 530 cm^{-1}. Anal. Found: C, 74.41; H, 8.14 %. Calcd for $C_{17}H_{22}O_3$: C, 74.42; H, 8.08 %.

Synthesis of compound **29**

3-Chloro–6-hydroxy-4-methoxy-2-methyl-5-[(*E,E*)-3-methyl-7-(tetrahydro-5,5-dimethyl-4-oxo-2-furan-2-yl) octa-2,6-dienyl]benzaldehyde (4-*O*-methylascofuranone)

Acsofuranone was treated with dimethyl sulfate/K_2CO_3 in acetone to give compound **29** (93% yield). ^{1}H-NMR ($CDCl_3$) δ 1.22 (s, 3H, CH_3), 1.28 (s, 3H, CH_3), 1.62 (s, 3H, CH_3), 1.79 (s, 3H, CH_3), 2.00-2.07 (m, 2H), 2.09-2.20 (m, 2H), 2.35 (dd, *J*=10.2, 18.2 Hz, 1H, H-C(3)-*H* of tetrahydrofuran moiety), 2.41 (dd, *J*=6.4, 18.2 Hz, 1H, *H*-C(3)-H of tetrahydrofuran moiety), 2.64 (s, 3H, Ar-CH_3), 3.38 (d, *J*=6.9 Hz, 2H, Ar-CH_2-), 3.86 (s, 3H, OCH_3), 4.50 (dd, *J*=6.4, 10.2 Hz, 1H, C(2)-*H* of tetrahydrofuran moiety), 5.18 (t, *J*=6.3 Hz, 1H), 5.51 (t, *J*=6.9 Hz, 1H), 10.26 (s, 1H, C*H*O), 12.52 (s, 1H, Ar-O*H*).

Synthesis of compound **30**

5-Chloro-2-hydroxy-3-nonyl-6-methylbenzaldehyde

The mixture of *m*-cresol and nonanoyl chloride (1.2 eq) was heated at 140°C, $AlCl_3$ (1.2 eq) was added to promote acylation (80% yield). Resulted 2-nonanoyl-5-methylphenol was reduced to 2-nonyl-5-methylphenol in the presence of Pd/C (10%) in EtOH with catalytic amount of HCl under H_2 atmosphere (97% yield). The phenol was formylated by refluxing with hexamethylenetetramine (1.0 eq) in TFA. 2-Hydroxy-3-nonyl-6-methylbenzaldehyde was obtained as minor product (6.3% yield). 5-Chloro-2-hydrozy-3-nonyl-6-methylbenzaldehyde (**30**) was produced by chlorination with SO_2Cl_2 (1.0 eq) in Et_2O at 4°C. The final product was purified by silica gel column chromatography (hexane/AcOEt, 99:1, 75% yield). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 0.86 (t, *J*=6.9 Hz, 3H, -C$\underline{H_3}$), 1.25-1.35 (m, 12H, (C$\underline{H_2}$)$_6$), 1.58 (m, 2H, Ar-CH_2C$\underline{H_2}$), 2.58 (t, *J*=7.9 Hz, 2H, Ar-C$\underline{H_2}$), 2.61 (s, 3H, Ar-C$\underline{H_3}$), 7.34 (*s*, 1H, Ar-$\underline{H}$), 10.3 (s, 1H, C$\underline{H}$O), δ 12.3 (s, 1H, Ar-O$\underline{H}$)

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Cloning and characterization of hypoxia-inducible factor-1 subunits from *Ascaris suum* — A parasitic nematode highly adapted to changes of oxygen conditions during its life cycle

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ABSTRACT

The parasitic nematode *Ascaris suum* successfully adapts to a significant decrease in oxygen availability during its life cycle by altering its metabolic system dramatically. However, little is known about the regulatory mechanisms of adaptation to hypoxic environments in *A. suum*. In multicellular organisms, hypoxia-inducible factor-1 (HIF-1), a heterodimeric transcription factor composed of HIF-1α and HIF-1β subunits, is a master regulator of genes involved in adaptation to hypoxia. In the present study, cDNAs encoding HIF-1α and HIF-1β were cloned from *A. suum* and characterized. The full-length *A. suum hif-1α* and *hif-1β* cDNAs contain open reading frames encoding proteins with 832 and 436 amino acids, respectively. In the deduced amino acid sequences of *A. suum* HIF-1α and HIF-1β, functional domains essential for DNA-binding, dimerization, and oxygen-dependent prolyl hydroxylation were conserved. The interaction between *A. suum* HIF-1α and HIF-1β was confirmed by the yeast two-hybrid assay. Both *A. suum hif-1α* and *hif-1β* mRNAs were expressed at all stages examined (fertilized eggs, third-stage larvae, lung-stage larvae, young adult worms, and adult muscle tissue), and most abundantly in the aerobic free-living third-stage larvae, followed by a gradual decrease after infection of the host. *hif-1* mRNA transcription was not sensitive to the oxygen environment in either third-stage larvae or adult worms (muscle tissue), and was regulated in a stage-specific manner. High expression of *hif-1* mRNAs in third-stage larvae suggests its contribution to pre-adaptation to a hypoxic environment after infection of their host. Sequence analysis of 5′-upstream regions of mitochondrial complex II (succinate–ubiquinone reductase/quinol-fumarate reductase) genes, which show stage-specific expression and play an important role in oxygen adaptation during the life cycle, revealed that all subunits except for the adult-type flavoprotein subunit (Fp) possess putative hypoxia-responsive elements (HREs), suggesting that they are *hif-1* target genes.

1. Introduction

Multicellular organisms have developed cellular and systemic responses to low oxygen levels to meet metabolic demands. The parasitic nematode *Ascaris suum* experiences extreme changes in oxygen conditions during its life cycle and possesses unique metabolic mechanisms for survival in hypoxic environments within the host (Fig. 1) (Kita and Takamiya, 2002; Komuniecki and Harris, 1995; Sakai et al., 2012; Tielens and Van Hellemond, 1998).

A. suum fertilized eggs develop into infectious third-stage larvae (L3) under normoxic conditions outside of the host. After infection of the host, L3 larvae penetrate the intestinal wall and reach the lung (LL3), migrating through the liver and the heart. Afterwards, the larvae migrate back to the small intestine via the trachea and become adult worms under hypoxic conditions (Sakai et al., 2012; Takamiya et al., 1993).

To cope with decreased oxygen availability, *A. suum* alters its energy metabolism from a mammalian-type aerobic pathway in the larval stage to a unique anaerobic pathway, the phosphoenolpyruvate carboxykinase (PEPCK)-succinate pathway, in the adult stage (Kita et al., 2002). For the establishment of this anaerobic metabolism, quinol-fumarate reductase activity of mitochondrial respiratory chain complex II plays a crucial role to produce succinate as an end product. Complex II is generally composed of four peptides: the flavoprotein (Fp), the iron-sulfur cluster (Ip), the hydrophobic membrane-anchoring cytochrome *b* large subunit

Abbreviations: ARNT, aryl hydrocarbon receptor nuclear translocator; bHLH, basic helix-loop-helix; CAD, C-terminal activation domain; C-TAD, C-terminal transactivation domain; FIH, factor inhibiting HIF; HIF, hypoxia-inducible factor; HRE, hypoxia-responsive element; N-TAD, N-terminal transactivation domain; ODD, oxygen-dependent degradation domain; PAS, Per-Arnt-Sim; PHD, prolyl hydroxylase; SL1, spliced leader sequence 1; VHL, von Hippel–Lindau tumor suppressor.

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