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Design and synthesis of biologically active analogues of vitamin K_2: Evaluation of their biological activities with cultured human cell lines

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Abstract—Novel ω-oxygenated vitamin K_2 analogues were efficiently synthesized and their biological activities were evaluated. Some were biologically active and the side-chain played an important role in γ-carboxylation and apoptosis-inducing activity. The results provide useful information on the structure–activity relationship of vitamin K_2 analogues for the development of new drugs.

1. Introduction

Vitamin K is an essential cofactor required for the post-translational conversion of glutamic acid residues into γ-carboxyglutamic acid (Gla) via γ-glutamylcarboxylase within vitamin K-dependent proteins.[1] As Gla-containing proteins, a number of blood coagulation factors including coagulation factors II (prothrombin), VII, IX, and X, a bone-specific protein such as osteocalcin, and other proteins have been identified.[2,3] The physiological function and putative health roles of vitamin K-dependent proteins include bone mineralization,[4,5] inhibition of vascular calcification,[6] cell cycle regulation, and signal transduction.[7]

There are two naturally occurring forms of vitamin K: vitamin K_1 (**1**) (phylloquinone: PK) and vitamin K_2 (**2**) (menaquinone-*n*: MK-*n*) (Fig. 1). PK is synthesized by plants and is present in large amounts in various green vegetables, which are the major source of dietary vitamin K. On the other hand, MK-*n* are of bacterial origin and have a variable side-chain length of four to thirteen isoprene units. Among these homologues, menaquinone-4 (MK-4) shows strong coagulation activities in comparison with PK and other MK-*n*. Some groups recently reported that MK-4 exhibits anti-arteriosclerosis activity[8] and anti-tumor actions in various cancer cells,[9–11] and acts as an SXR-specific ligand for the regulation of gene transcription.[12] MK-4 is accumulated in various tissues at high concentrations after its biosynthesis by conversion from PK and other MK-*n* in the body.[13] However, its biologically essential roles other than as a coenzyme for γ-glutamylcarboxylase are still unknown.

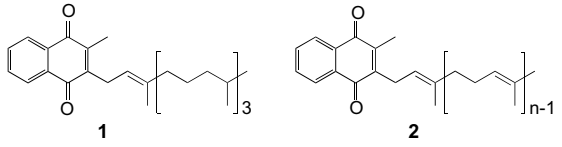

Figure 1. Structure of vitamin K homologues: phylloquinone (**1**) (PK) and menaquinones (**2**) (MK-*n*).

The aim of our study is to investigate the structure–activity relationship of vitamin K analogues. Various analogues have been synthesized and their biological activities investigated.[14–17] We have especially focused on modifications of the side-chain of vitamin K. Metabolic pathways are typical examples of modifications. In terms of the study of vitamin K homologues

Keywords: Vitamin K; Metabolite; Menaquinone-4; ω-Oxidation; K acid.
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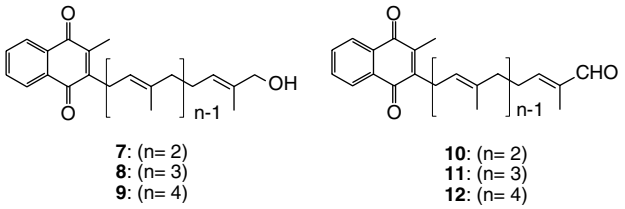
in vivo, major aglycone metabolites have been detected. They are urinary metabolites as glucuronides of ω-carboxylic acid (**3**) and (**4**),[18,19] K acid I (also called '7C-aglycon'; 2-methyl-3-(5′-carboxy-3′- methyl-2′-pentenyl)-1, 4-naphthoquinone) (**5**), and K acid II (or '5C-aglycon'; 2-methyl-3-(3′-3′-carboxymethylpropyl)-1, 4-naphthoquinone) (**6**)[20] as shown in Figure 2. In this catabolic pathway, **3** or **4** is formed by oxygen attacking hepatic metabolites at the ω-carbon of PK or MK-*n*, then **5** and **6** are successively generated by a side-chain shortening of β-oxidation.[21] The resultant aglycone metabolites are then conjugated with glucuronic acid to facilitate their biliary and urinary elimination.[22,23] Despite the metabolic study, biological activities of **3**–**6** have hardly been evaluated so far. Studying the derivatives in detail would provide insight into the biological significance of vitamin K and valuable information for the development of new drugs. In this study, we focused on MK homologues and synthesized six kinds of analogues because MK-4 has various biological activities. But the known metabolites such as ω-carboxylic acid (**4**) were quite unstable and consequently unable to be used for assays. Therefore, we synthesized new analogues, into which was introduced a terminal ω-hydroxyl or ω-aldehyde group instead of ω-carboxylic acid in the side-chain moiety of MK (menaquinone-2, 3, and 4) (**7**)–(**12**) as shown in Figure 3. We report here the design, synthesis, and structure–activity relationship of new vitamin K analogues based on metabolites.



7: (n= 2)
8: (n= 3)
9: (n= 4)

10: (n= 2)
11: (n= 3)
12: (n= 4)

Figure 3. Chemical structures of our compounds, which are ω-hydroxyl analogues (**7**)–(**9**) and ω-aldehyde analogues (**10**)–(**12**).

2. Results and discussion

We planned to synthesize the requisite analogues through coupling of the naphthoquinone derivative and side-chain moiety. The outlines are shown in Schemes 1 and 2. For the synthesis of the side-chain, we chose geraniol (**13a**), farnesol (**13b**), and geranylgeraniol (**13c**) as the starting material (Scheme 1). The primary hydroxyl group of **13a**–**c** was protected with an acetyl group to give **14a**–**c** in quantitative yield. The selective terminal ω-oxygenation of **14a** and **14b** with SeO_2, 70% *t*-BuOOH solution, and salicylic acid in CH_2Cl_2 according to a reported method afforded the ω-oxygenated alcohols **15a** and **15b** with the ω-aldehyde analogue in good yield.[24] The conversion of **14c** to ω-oxygenated **15c** did not give a good yield under the same conditions. A possible reason for the low chemical yield is that selective reactivity of SeO_2 was reduced due to a longer alkyl chain and ω-1-oxygenated and diol derivatives were generated. The mixture was separated by silica gel column chromatography to give the desired **15c** in 40% yield. Protection of the hydroxyl group of **15a**–**c** with 3, 4-dihydro-2*H*-pyran (DHP) and *p*-toluenesulfonic acid (TsOH) in CH_2Cl_2 gave **16a**–**c**, which were protected by a tetrahydropyran (THP) group, in excellent yield. Removal of the acetyl group in **16a**–**c** gave the corresponding alcohol **17a**–**c** in 78–82%.

The naphthoquinone part **18** was prepared from 1, 4-diacetoxy-2-methylnaphthoquinone with selective hydrolysis of the acetyl group. Scheme 2 shows the synthesis of ω-oxygenated vitamin K analogues. 1-Acetoxy-2-methyl-4-naphthalenol (**18**)[25] was used as a naphthoquinone synthon for the coupling with side-chain analogues. Treatment of the monoacetate **18** with an alkyl side-chain alcohol **17a**–**c** in the presence of boron trifluoride etherate yielded **19a**–**c** in 45–51% yield. Although we first tried to obtain ω-alcohol compounds **7**–**9** directly by alkaline hydrolysis, the chemical yield was extremely low. Presumably, the quinone form of **19a**–**c** was degraded under alkaline conditions or a side-chain moiety reacted and polymerization occurred. Therefore, we employed a 2-step synthesis by way of dimethyl ether analogues **20a**–**c**. In short, monoacetate derivatives **19a**–**c** were treated with an excess amount of potassium hydride in anhydrous conditions. This was followed by the addition of methyl iodide to give **20a**–**c**. Finally, deprotection of the THP group of naphthohydroquinone methyl ether **20a**–**c** with $Ce(NH_4)_2(NO_3)_6$ in water gave the desired ω-oxygenated

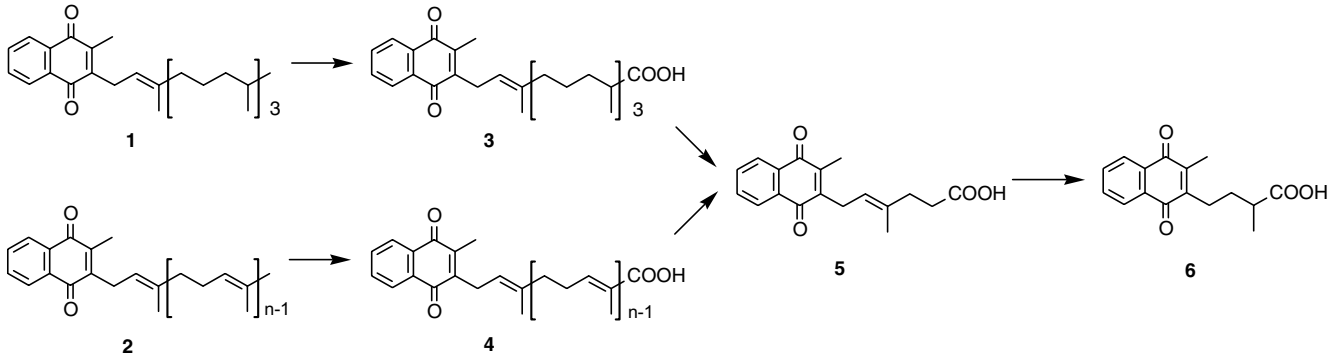
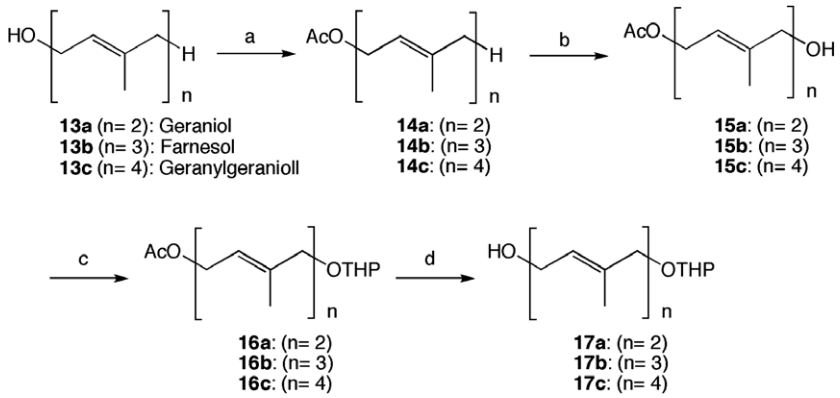
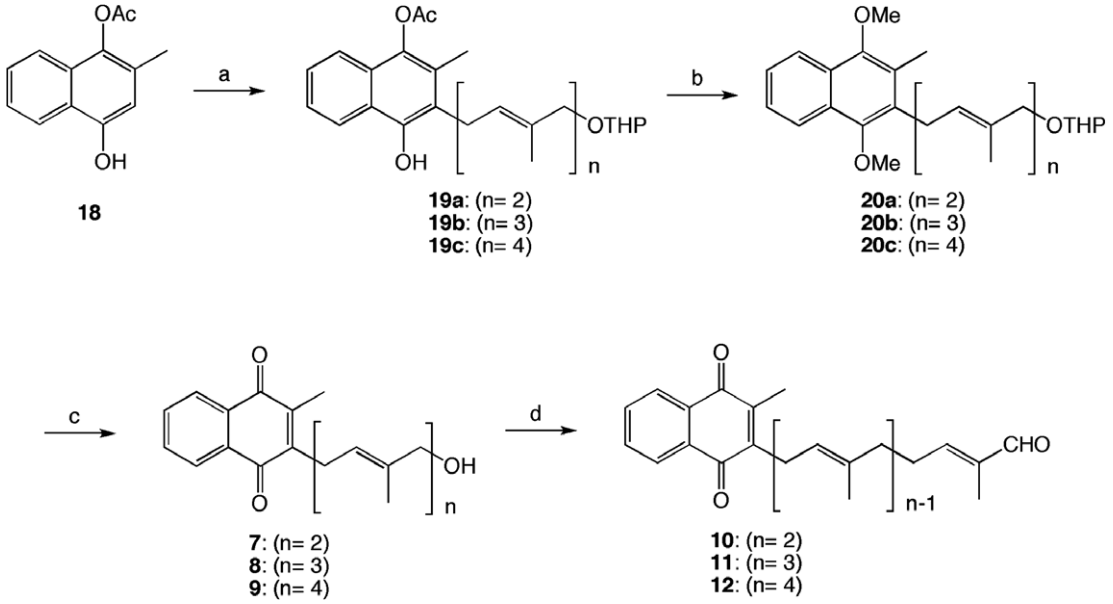


Figure 2. Metabolic pathway of vitamin K homologues and structural formulas of phylloquinone (**1**), menaquinones (**2**), ω-carboxylic acid (**3**) and (**4**), K acid I (**5**), and K acid II (**6**).



Scheme 1. Synthesis of side-chain moiety. Reagents and conditions: (a) pyridine, Ac_2O, 92–95%; (b) SeO_2, 70% *t*-BuOOH, salicylic acid, CH_2Cl_2, 40–75%; (c) DHP, TsOH, CH_2Cl_2, 75–81%; (d) 1 N NaOH aq, MeOH, 78–82%.



Scheme 2. Synthesis of vitamin K analogues. Reagents and conditions: (a) **17a**–**c**, BF_3Et_2O, EtOAc/dioxane (1:1), 45–51%; (b) KH, CH_3I, THF, 60–65%; (c) CAN, CH_3CN–H_2O, 71–76%; (d) PDC, CH_2Cl_2 35–55%.

vitamin K homologues **7**–**9** in good yield. These analogues were further converted to ω-aldehyde analogues **10**–**12** with PDC treatment in good yield. Thus, six kinds of ω-oxygenated analogues were prepared.

To investigate the biological activities of those analogues, we compared coenzyme activity for γ-glutamylcarboxylase (GGCX), which is a classical role of vitamin K homologues, between natural vitamin K and our new analogues. This in vitro assay was conducted using previously described methods.[26–29] In short, the amount of $^{14}CO_2$ incorporated into the exogenous substrate peptide 'FLEEL' was measured in reaction mixtures containing reduced vitamin K, mouse liver microsome, and $NaH^{14}CO_3$. We investigated whether the GGCX activity increases or not if functional groups are introduced into the vitamin K molecule. We chose MK-4 and ω-hydroxylated MK-4 (**9**) because MK-4 is now used as a therapeutic agent for osteoporosis in Japan.[30] ω-Aldehyde MK-4 (**12**) was not assayed because **12** should be converted to **9** during the reduction procedure for the conversion of quinone to hydroquinone in this assay. The K_m and V_{max} values for MK-4 and ω-hydroxylated MK-4 (**9**) (both in their hydroquinone form) were determined from the initial carboxylation rates at 8 different vitamin concentrations and the data are presented in Figure 4. We have also determined the kinetic constants for the hydroquinone forms of the vitamins and summarized the data in Table 1. The K_m for MK-4 was 6 times higher than that for MK-4 ω-OH (**9**), whereas the V_{max} value for MK-4 was also 1.3 times higher than that for MK-4 ω-OH (**9**). The V_{max}/K_m values were 5 times higher for **9** than for MK-4. This result means that **9** showed strong activity toward GGCX compared to MK-4. The hydrophobicity as well as the terminal hydrophilicity for the side chain of vitamin K might be related to the interaction with the vitamin K-binding site of GGCX. There are reports that vitamin K promotes the calcification of bone in cooperation with vitamin D.[31] Therefore, if potent vita-

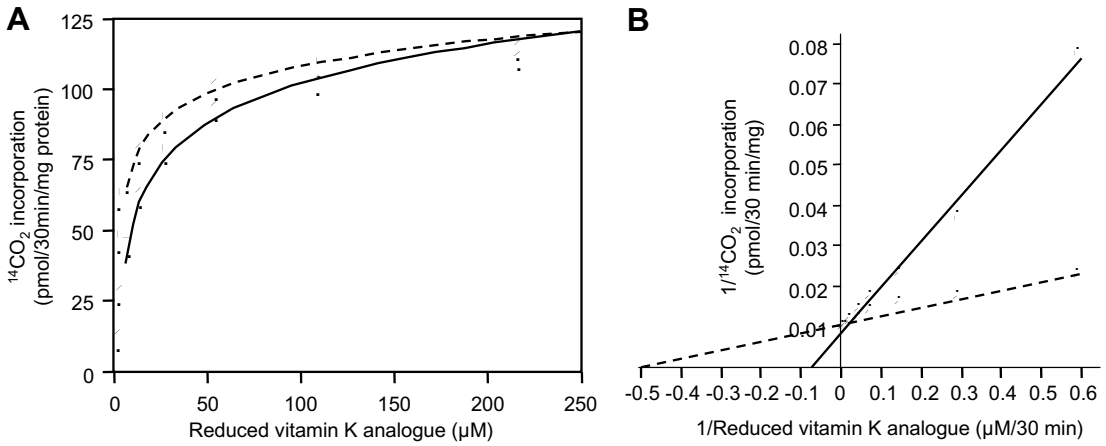


Figure 4. Kinetic analysis of vitamin K hydroquinone analogues. (A) The CO_2 incorporation as a function of the concentration of MK-4 hydroquinone (——) and MK-4 ω-OH hydroquinone (- - - - -). (B) Double-reciprocal plots of v vs. [S] for MK-4 hydroquinone (——) and MK-4 ω-OH hydroquinone (- - - - -).

Table 1. Comparison of the kinetic parameters of vitamin K analogues

Substrate	K_m (μM)	V_{max} (pmol/30 min)	V_{max}/K_m (pmol/30 min/μM)
MK-4	13.3	126.4	9.5
MK-4 ω-OH (**9**)	2.0	99.9	50.0

min K analogues, which have coenzyme activity, were synthesized, they might be expected to have synergic effects on bone formation in the presence of vitamin D. However, further experimentation is necessary to study the biological activities involved.

We next evaluated the apoptosis-inducing activity of these analogues in cancer cell lines. As MK-4 is expected to become a therapeutic agent against hepatic cancer, vitamin K analogues have been studied for use as anticancer drugs.[32] We have already reported that some vitamin K analogues exhibited apoptosis-inducing activity toward HL-60 cells (human leukemia cells), and the introduction of a ω-hydroxyl group increased the activity in the case of MK-4 analogues.[33] We further explored the possibility of using our compounds against other cancer cells such as the osteosarcoma cell line MG-63, and human hepatoma cell lines HepG2 and Huh-7. Samples of analogues were added at 5 and 10 μM to cells. After 72 h, the cells were collected and treated with propidium iodide, and then apoptosis-inducing activity was assessed with a fluorescence-activated cell sorter (FACS) as previously reported.[34] In the case of MG-63 cells, the activity of most analogues increased in a dose-dependent manner as shown in Figure 5. The activity increased in the order MK-2, MK-3, and MK-4 analogues at 10 μM. The ω-aldehyde analogues are likely to be more active than the ω-hydroxylated analogues in these cells. On the other hand, HepG2 and Huh-7 cells showed no significant apoptosis as reported previously.[32] Thus, the apoptosis-inducing activ-

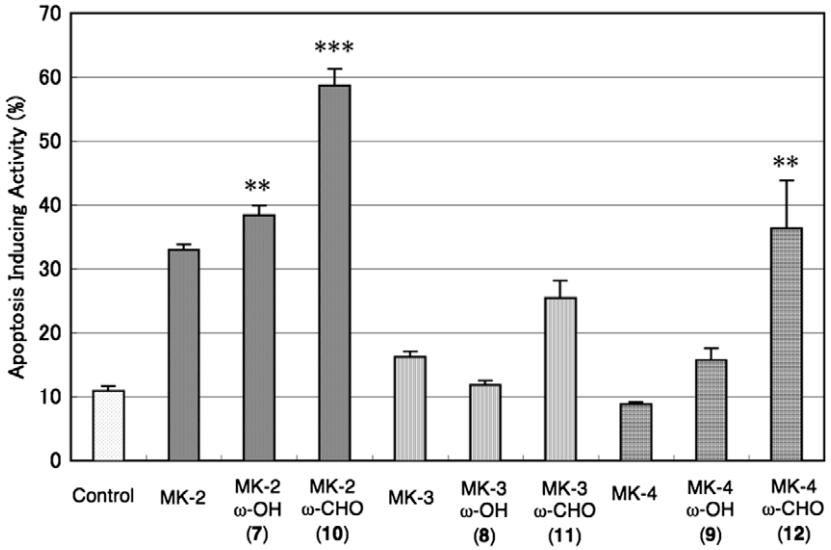


Figure 5. Apoptosis-inducing activity of menaquinone derivatives in MG-63 cells. MG-63 cells were treated with vitamin K_2 analogues at 10 μM for 3 days. Apoptosis-inducing activity was identified with flow cytometric analysis of DNA content. Columns, mean obtained from three independent experiments; bars, SD. Significant difference: $^{**}p < 0.01$, $^{***}p < 0.001$, compared with cells untreated with vitamin K_2 analogues.

ity of our analogues as well as natural vitamin K homologues exhibited was selective of cancer cells. In addition, increasing hydrophilicity through the introduction of an aldehyde or hydroxyl group into the side-chain moiety might enhance the apoptosis-inducing activity. Therefore, the physiological activity of the vitamin K changed with the length of the side-chain and the functional group of the terminal alkyl group.

In summary, our results indicated that vitamin K analogues might have potential as biologically active compounds and can provide information with which to develop new drugs based on vitamin K.

3. Experimental

3.1. (*E*)-3,7-Dimethylocta-2,6-dienyl acetate (Geranyl acetate) (14a)

To a suspension of geraniol (**13a**) (5.0 g, 32 mmol) in pyridine (20 mL) was added acetic anhydride (10 mL) at 0 °C under argon, and the suspension was stirred for 12 h at room temperature. The reaction mixture was diluted with ethyl acetate, and successively washed with saturated $CuSO_4$ aqueous solution, water, and brine. The organic layer was dried over anhydrous $MgSO_4$ and concentrated in vacuo. The residue was purified by flash column chromatography on silica gel (hexane/ethyl acetate = 10/1) to afford **14a** (6.0 g, 95%) as a colorless oil: IR ($CHCl_3$): 2969, 2930, 1728, 1445, 1381, 1240, 1023 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 1.60 (3 H, s), 1.68 (3H, s), 1.70 (3H, s), 2.03–2.12 (4H, m), 2.06 (3H, s), 4.59 (2H, d, J = 7.0 Hz), 5.07–5.10 (1H, m), 5.33–5.36 (1H, m); ^{13}C NMR (125 MHz, $CDCl_3$): δ 16.4, 17.6, 21.0, 25.6, 26.2, 39.5, 61.4, 118.2, 123.7, 131.8, 142.2, 171.1; EI-LRMS MHz 196 (M^+), 136. EI-HRMS calcd for $C_{12}H_{20}O_2$ 196.1463. Found 196.1478.

3.2. (2*E*,6*E*)-3,7,11-Trimethyldodeca-2,6,10-trienyl acetate (Farnesyl acetate) (14b)

In a manner similar to that for the synthesis of **13a** from **12a**, a crude product, which was obtained from **13b** (5.0 g, 22 mmol) and acetic anhydride (10 mL) in pyridine (20 mL), was purified by flash column chromatography on silica gel (hexane/ethyl acetate = 10/1) to give **14b** (5.5 g, 93%) as a colorless oil: IR ($CHCl_3$): 2969, 2928, 1728, 1447, 1382, 1241, 1023 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 1.60 (6H, s), 1.68 (3H, s), 1.71 (3H, s), 1.96-2.13 (8H, m), 2.05 (3H, s), 4.59 (2H, d, J = 7.0 Hz), 5.08–5.11 (2H, m), 5.33–5.36 (1H, m); ^{13}C NMR (125 MHz, $CDCl_3$): δ 16.0, 16.4, 17.6, 21.0, 25.6, 26.1, 26.7, 39.5, 39.6, 61.4, 118.2, 123.6, 124.3, 131.3, 135.4, 142.2, 171.1; EI-LRMS MHz 264 (M^+), 288. EI-HRMS calcd for $C_{17}H_{28}O_2$ 264.2089. Found 264.2080.

3.3. (2*E*,6*E*,10*E*)-3,7,11,15-Tetramethylhexadeca-2,6,10,14-tetraenyl acetate (Geranylgeranyl acetate) (14c)

In a manner similar to that for the synthesis of **14a** from **13a**, a crude product, which was obtained from **13c** (5.0 g, 17 mmol) and acetic anhydride (10 mL) in pyridine (20 mL), was purified by flash column chromatography on silica gel (hexane/ethyl acetate = 10/1) to give **14c** (5.3 g, 92%) as a colorless oil: IR ($CHCl_3$): 2969, 2929, 1728, 1448, 1386, 1239, 1023 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 1.55 (3H, s), 1.60 (6H, s), 1.68 (3H, s), 1.71 (3H, s), 1.96–2.14 (12H, m), 2.05 (3H, s), 4.59 (2H, d, J = 7.0 Hz), 5.08–5.12 (3H, m), 5.33–5.36 (1H, m); ^{13}C NMR (125 MHz, $CDCl_3$): δ 15.90, 16.20, 16.5, 17.7, 21.0, 25.7, 26.2, 26.5, 26.8, 39.5, 39.6, 39.7, 61.4, 118.2, 123.6, 124.2, 124.4, 131.3, 135.0, 135.5, 142.3, 171.1; EI-LRMS MHz 332 (M^+), 272. EI-HRMS calcd for $C_{22}H_{36}O_2$ 332.2715. Found 332.2737.

3.4. (2*E*,6*E*)-8-Hydroxy-3,7-dimethylocta-2,6-dienyl acetate (15a)

To a suspension of selenium dioxide (283 mg, 2.5 mmol) and salicylic acid (353 mg, 2.5 mmol) in CH_2Cl_2 (125 mL) was added 70% *tert*-butyl hydroperoxide (10 mL) at room temperature, and the mixture was stirred at room temperature for 10 min. After the mixture was cooled to 0 °C, geranyl acetate (**14a**) (5.0 g, 25 mmol) in CH_2Cl_2 (5 mL) was added to it. The reaction mixture was stirred at the same temperature for 5 min and then continuously stirred at room temperature for 24 h. The suspension was diluted with ethyl acetate, and successively washed with 5% $NaHCO_3$ aqueous solution, saturated aqueous $CuSO_4$, saturated aqueous $Na_2S_2O_3$, water, and brine. The organic layer was dried over anhydrous $MgSO_4$ and concentrated in vacuo. The residue was chromatographed on silica gel (hexane/ethyl acetate = 10/1) to give **15a** (4.1 g, 75%) as a colorless oil: IR ($CHCl_3$): 3468, 2935, 1728, 1445, 1367, 1242, 1023 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 1.67 (3H, s), 1.71 (3H, s), 2.06 (3H, s), 2.08 (2H, dd, J = 7.0, 14.0 Hz), 2.18 (2H, dd, J = 7.0, 14.0 Hz), 3.99 (2H, s), 4.58 (2H, d, J = 7.5 Hz), 5.32–5.38 (2H, m); ^{13}C NMR (125 MHz, $CDCl_3$): δ 13.6, 16.4, 21.0, 25.6, 39.0, 61.4, 68.8, 118.6, 125.2, 135.2, 141.7, 171.2; EI-LRMS MHz 212 (M^+). EI-HRMS calcd for $C_{12}H_{20}O_3$ 212.1412. Found 212.1435.

3.5. (2*E*,6*E*,10*E*)-12-Hydroxy-3,7,11-trimethyldodeca-2,6,10-trienyl acetate (15b)

In a manner similar to that for the synthesis of **15a** from **14a**, a crude product, which was obtained from **14b** (1.0 g, 3.8 mmol), selenium dioxide (42 mg, 0.38 mmol), salicylic acid (52 mg, 0.38 mmol), and 70% *tert*-butyl hydroperoxide (3 mL) in CH_2Cl_2 (30 mL), was purified by flash column chromatography on silica gel (hexane/ethyl acetate = 10/1) to give **15b** (647 mg, 61%) as a colorless oil: IR ($CHCl_3$): 3504, 2932, 1725, 1448, 1367, 1238, 1023 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 1.56 (1H, s), 1.60 (3H, s), 1.67 (3H, s), 1.71 (3H, s), 2.00–2.07 (4H, m), 2.06 (3H, s), 2.11–2.13 (4H, m), 3.99 (2H, s), 4.58 (2H, d, J = 7.0 Hz), 5.10–5.12 (1H, m), 5.33–5.36 (1H, m), 5.37–5.41 (1H, m); ^{13}C NMR (125 MHz, $CDCl_3$): δ 13.6, 15.9, 16.4, 21.0, 26.08, 26.12, 39.2, 39.4, 61.3, 68.9, 118.3, 123.8, 125.8, 134.7, 135.0, 142.1, 171.1; EI-LRMS MHz 280 (M^+), 220. EI-HRMS calcd for $C_{17}H_{28}O_3$ 280.2038. Found 280.2045.

3.6. (2*E*,6*E*,10*E*,14*E*)-16-Hydroxy-3,7,11,15-tetramethylhexadeca-2,6,10,14-tetraenyl acetate (15c)

In a manner similar to that for the synthesis of **15a** from **14a**, a crude product, which was obtained from **14c** (1.4 g, 4.0 mmol), selenium dioxide (45 mg, 0.4 mmol), salicylic acid (55 mg, 0.4 mmol), and 70% *tert*-butyl hydroperoxide (3 mL) in CH_2Cl_2 (30 mL) was purified by flash column chromatography on silica gel (hexane/ethyl acetate = 10/1) to give **15c** (587 mg, 40%) as a colorless oil: IR ($CHCl_3$): 3504, 2927, 1727, 1448, 1383, 1240, 1022 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 1.60 (6H, s), 1.66 (3H, s), 1.71 (3H, s), 2.05 (3H, s), 1.99–2.13 (12H, m), 3.99 (2H, br s), 4.58 (2H, d, J = 7.5 Hz), 5.11 (2H, dd, J = 7.0, 12.5 Hz), 5.34 (1H, t, J = 7.0 Hz), 5.39 (1H, t, J = 7.0 Hz); ^{13}C NMR (125 MHz, $CDCl_3$): δ 13.6, 15.91, 15.94, 16.4, 21.0, 26.1, 26.2, 26.5, 39.2, 39.4, 39.6, 61.3, 68.9, 118.2, 123.6, 124.4, 125.9, 134.5, 134.7, 135.3, 142.2, 171.1; EI-LRMS MHz 348 (M^+), 288. EI-HRMS calcd for $C_{22}H_{36}O_3$ 348.2664. Found 348.2679.

3.7. (2*E*,6*E*)-3-Methyl-7-((tetrahydro-2H-pyran-2-yloxy)-methyl)octa-2,6-dienyl acetate (16a)

A catalytic amount of *p*-toluenesulfonic acid was added to a cooled solution of **15a** (500 mg, 2.4 mmol) and 3, 4-dihydro-2*H*-pyran (396 mg, 4.7 mmol) in CH_2Cl_2 (5 mL) at 0 °C under argon, and the mixture was stirred at room temperature for 2 h. The reaction mixture was poured into ice-water and extracted with ethyl acetate twice. The organic layer was combined and washed with brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residue was chromatographed on silica gel (hexane/ethyl acetate = 5/1) to give **16a** (566 mg, 81%) as a colorless oil: IR ($CHCl_3$): 3017, 2945, 2854, 1728, 1454, 1240, 1075 cm^{-1}; 1H NMR (500 MHz, $CDCl_3$): δ 1.51–162 (4 H, m), 166 (3H, s), 1.71 (3H, s), 1.81–1.86 (2H, m), 2.06 (3H, s), 2.09 (2H, t, J = 7.5 Hz), 2.17 (2H, t, J = 7.5 Hz), 3.49–3.53 (1H, m), 3.84 (1H, d, J = 11.5 Hz), 3.86–3.90 (2H, m), 4.10 (1H, d, J = 11.5 Hz), 4.58 (1H, d, J = 6.5 Hz), 4.60 (1H, d, J = 6.5 Hz), 5.33–5.37 (1H, m), 5.39–5.43 (1H, m); ^{13}C NMR (125 MHz, $CDCl_3$): δ 14.0, 16.4, 19.5, 21.0, 25.5, 25.9, 30.7, 39.1, 61.3, 62.2, 73.0, 97.4, 118.4, 127.0, 132.4, 141.9, 171.1; EI-LRMS MHz 296 (M^+). EI-HRMS calcd for $C_{17}H_{28}O_4$ 296.1988. Found 296.1958.

3.8. (2*E*,6*E*,10*E*)-3,7-Dimethyl-11-((tetrahydro-2H-pyran-2-yloxy)methyl)dodeca-2,6,10-trienyl acetate (16b)

In a manner similar to that for the synthesis **16a** from **15a**, a crude product, which was obtained from **15b** (1.4 g, 0.50 mmol), 3, 4-dihydro-2*H*-pyran (840 mg, 1.0 mmol), and catalytic amount of *p*-toluenesulfonic acid in CH_2Cl_2 (10 mL), was purified by flash column chromatography on silica gel (hexane/ethyl acetate = 5/1) to give **16b** (1.47 g, 81%) as a colorless oil: IR ($CHCl_3$): 3010, 2946, 2855, 1728, 1442, 1239, 1021 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 1.60 (3H, s), 1.66 (3H, s), 1.71 (3H, s), 1.51–1.63 (4H, m), 1.80–1.87 (2H, m), 2.05 (3H, m), 2.00–2.14 (8H, m), 3.49–3.54 (1H, m), 3.83–3.90 (2H, m), 4.09 (1H, d, J = 12.0 Hz), 4.59 (3H, d, J = 6.5 Hz), 5.09–5.12 (1H, m), 5.33–5.36 (1H, m), 5.40–5.43 (1H, m); ^{13}C NMR (125 MHz, $CDCl_3$): δ 14.1, 16.0, 16.5, 19.6, 21.1, 25.5, 26.2, 26.4, 30.7, 39.3, 39.5, 61.4, 62.2, 73.0, 97.4, 118.3, 123.9, 127.8, 131.9, 135.2, 142.3, 171.1; EI-LRMS MHz 364 (M^+), 304. EI-HRMS calcd for $C_{22}H_{36}O_4$ 364.2614. Found 364.2627.

3.9. (2*E*,6*E*,10*E*,14*E*)-3,7,11-Trimethyl-15-((tetrahydro-2H-pyran-2-yloxy)methyl)hexadeca-2,6,10,14-tetraenyl acetate (16c)

In a manner similar to that for the synthesis of **16a** from **15a**, a crude product, which was obtained from **15c** (730 mg, 2.1 mmol), 3, 4-dihydro-2*H*-pyran (353 mg, 4.2 mmol), and a catalytic amount of *p*-toluenesulfonic acid in CH_2Cl_2 (5 mL), was purified by flash column chromatography on silica gel (hexane/ethyl acetate = 5/1) to give **16c** (681 mg, 75%) as a colorless oil: IR ($CHCl_3$): 3008, 2946, 2855, 1728, 1440, 1239, 1018 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 1.60 (3H, s), 1.66 (3H, s), 1.69 (3H, s), 1.71 (3H, s), 1.73–1.79 (2H, m), 1.83–1.89 (2H, m), 2.05 (3H, m), 3.50–3.54 (1H, m), 3.86–3.90 (2H, m), 4.09 (1H, d, J = 11.5 Hz), 4.59 (2H, d, J = 7.0 Hz), 4.60 (1H, d, J = 3.0 Hz), 5.10 (2H, dd, J = 7.0, 13.5 Hz), 5.34 (1H, dt, J = 1.5, 7.0 Hz), 5.42 (1H, dt, J = 1.5, 7.0 Hz); ^{13}C NMR (125 MHz, $CDCl_3$): δ 14.0, 15.9, 16.0, 16.4, 19.5, 21.0, 25.5, 26.4, 26.6, 30.6, 39.3, 39.5, 39.6, 61.4, 62.1, 72.9, 97.4, 118.2, 123.6, 124.4, 127.9, 131.8, 134.7, 135.4, 142.2, 171.1; EI-LRMS MHz 432 (M^+). EI-HRMS calcd for $C_{27}H_{44}O_4$ 432.324. Found 432.3262.

3.10. (2*E*,6*E*)-3-Methyl-7-((tetrahydro-2H-pyran-2-yloxy)methyl)octa-2,6-dien-1-ol (17a)

To a solution of compound **16a** (525 mg, 1.8 mmol) in MeOH (10 mL) was added 1 N aqueous NaOH until the pH reached 11–12 at room temperature, and the reaction mixture was stirred at room temperature for 1 h. The solution was poured into ice-water and immediately extracted with ethyl acetate three times. The combined extracts were successively washed with water and brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residue was chromatographed on silica gel (hexane/ethyl acetate = 5/1) to afford **17a** (369 mg, 82%) as a colorless oil: IR ($CHCl_3$): 3448, 3009, 2945, 2856, 1667, 1454, 1231, 1118 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 1.38 (1H, t, J = 5.5 Hz), 1.51–1.63 (4H, m), 1.66 (3H, s), 1.67 (3H, s), 1.69–1.75 (1H, m), 1.78–1.88 (1H, m), 2.08 (2H, t, J = 7.5 Hz), 2.13–2.23 (2H, m), 3.49–3.53 (1H, m), 3.85 (1H, d, J = 11.0 Hz), 3.84–3.89 (1H, m), 4.09 (1H, d, J = 11.0 Hz), 4.11–4.15 (2H, m), 4.61 (1H, t, J = 3.5 Hz), 5.39–5.42 (2H, m); ^{13}C NMR (125 MHz, $CDCl_3$): δ 14.0, 16.1, 19.4, 25.5, 25.8, 30.6, 39.0, 59.4, 62.0, 72.9, 97.0, 123.9, 127.7, 132.1, 139.1; EI-LRMS MHz 254 (M^+). EI-HRMS calcd for $C_{15}H_{26}O_3$ 254.1882. Found 254.1862.

3.11. (2*E*,6*E*,10*E*)-3,7-Dimethyl-11-((tetrahydro-2H-pyran-2-yloxy)methyl)dodeca-2,6,10-trien-1-ol (17b)

In a manner similar to that for the synthesis of **17a** from **16a**, a crude product, which was obtained from **16b**

(1.1 g, 3.0 mmol), 1 N aqueous NaOH in MeOH (20 mL), was purified by flash column chromatography on silica gel (hexane/ethyl acetate = 5/1) to give **17b** (777 mg, 80%) as a colorless oil: IR ($CHCl_3$): 3455, 3010, 2944, 2855, 1667, 1454, 1231, 1118 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 1.38 (1H, br s), 1.51–1.63 (4H, m), 1.60 (3H, s), 1.66 (3H, s), 1.68 (3H, s), 1.69–1.75 (1H, m), 1.81–1.88 (1H, m), 2.00–2.06 (4H, m), 2.08–2.16 (4H, m), 3.48–3.53 (1H, m), 3.85 (1H, d, J = 12.0 Hz), 3.85–3.91 (1H, m), 4.09 (1H, d, J = 12.0 Hz), 4.15–4.17 (2H, m), 4.59–4.61 (1H, m), 5.10–5.13 (1H, m), 5.40–5.43 (2H, m); ^{13}C NMR (125 MHz, $CDCl_3$): δ 14.1, 16.0, 16.3, 19.6, 25.5, 26.26, 26.29, 30.7, 39.2, 39.5, 59.4, 62.2, 73.0, 97.4, 123.5, 124.1, 127.8, 131.9, 135.0, 139.6; EI-LRMS MHz 322 (M^+). EI-HRMS calcd for $C_{20}H_{34}O_3$ 322.2508. Found 322.2509.

3.12. (2*E*,6*E*,10*E*,14*E*)-3,7,11-Trimethyl-15-((tetrahydro-2H-pyran-2-yloxy)methyl)hexadeca-2,6,10,14-tetraen-1-ol (17c)

In a manner similar to that for the synthesis of **17a** from **16a**, a crude product, which was obtained from **16c** (550 mg, 1.3 mmol), 1 N aqueous NaOH in MeOH (5 mL), was purified by flash column chromatography on silica gel (hexane/ethyl acetate = 5/1) to give **17c** (387 mg, 78%) as a colorless oil: IR ($CHCl_3$): 3450, 3010, 2940, 2855, 1667, 1454, 1231, 1118 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 1.27 (4H, m), 1.60 (3H, s), 1.61 (3H, s), 1.66 (3H, s), 1.68 (3H, s), 1.97–2.14 (12H, m), 3.48–3.53 (1H, m), 3.83–3.91 (2H, m), 4.09 (1H, d, J = 12.0 Hz), 4.15 (2H, d, J = 7.0 Hz), 4.61 (1H, dd, J = 2.5, 7.0 Hz), 5.10–5.13 (2H, m), 5.40–5.43 (2H, m); ^{13}C NMR (125 MHz, $CDCl_3$): δ 14.0, 16.0, 16.3, 19.5, 22.6, 25.5, 26.3, 26.6, 30.7, 31.6, 39.3, 39.5, 39.6, 59.4, 62.1, 73.0, 97.4, 123.4, 123.8, 124.4, 127.9, 131.8, 134.7, 135.3, 139.7; EI-LRMS MHz 390 (M^+). EI-HRMS calcd for $C_{25}H_{42}O_3$ 390.3134. Found 390.3152.

3.13. 1-Hydroxy-3-methyl-2-((2*E*,6*E*)-3-methyl-7-((tetrahydro-2H-pyran-2-yloxy)methyl)octa-2,6-dienyl)naphthalen-4-yl acetate (19a)

Compound **18** (205 mg, 0.95 mmol) was dissolved in ethyl acetate/dioxane 1:1 (1 mL), and a catalytic amount of boron trifluoride ether complex was added to the solution. After the resulting mixture was stirred at 45 °C for 5 min, compound **17a** (330 mg, 1.0 mmol) in dioxane (0.2 mL) was added to the mixture and continuously stirred at the same temperature for 3 h. The reaction mixture was cooled to room temperature, poured into ice-water, and extracted with ethyl acetate three times. The combined organic layer was washed with water and brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residue was chromatographed on silica gel using hexane/ ethyl acetate 5:1 to give **19a** (193 mg, 45%) as a yellow oil: IR ($CHCl_3$): 3690, 3006, 2937, 2854, 1758, 1601, 1456, 1232, 1020 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 1.53–1.73 (6H, m), 1.65 (3H, s), 1.86 (3H, s), 2.12–2.18 (4H, m), 2.25 (3H, s), 2.47 (3H, s), 3.50–3.53 (3H, m), 3.85–3.96 (2H, m), 4.09 (1H, d, J = 12.0 Hz), 4.61 (1H, t, J = 3.5 Hz), 5.21–5.24 (1H, m), 5.36–5.39 (1H, m), 5.89 (1H, br s), 7.41–7.47 (2H, m), 7.63–7.64 (1H, m), 8.12–8.14 (1H, m); ^{13}C NMR (125 MHz, $CDCl_3$): δ 13.5, 14.0, 16.3, 19.3, 20.7, 25.5, 25.9, 26.4, 30.6, 39.2, 62.0, 72.8, 96.9, 119.9, 120.6, 121.7, 121.8, 124.1, 125.0, 126.1, 126.3, 127.5, 132.4, 138.4, 147.8, 169.7; EI-LRMS MHz 452 (M^+). EI-HRMS calcd for $C_{28}H_{36}O_5$ 452.2563. Found 452.2560.

3.14. 1-Hydroxy-3-methyl-2-((2*E*,6*E*,10*E*)-3,7-dimethyl-11-((tetrahydro-2H-pyran-2-yloxy)methyl)dodeca-2,6,10-trienyl)naphthalen-4-yl acetate (19b)

In a manner similar to that for the synthesis of **19a** from **17a**, a crude product, which was obtained from **17b** (328 mg, 1.0 mmol), a catalytic amount of boron trifluoride ether complex, and compound **18** (200 mg, 0.93 mmol) in acetate/dioxane 1:1 (1 mL), was purified by flash column chromatography on silica gel (hexane/ethyl acetate = 5/1) to give **19b** (246 mg, 51%) as a yellow oil: IR ($CHCl_3$): 3691, 3008, 2929, 2856, 1762, 1669, 1456, 1233, 1021 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 1.59 (3H, s), 1.63 (3H, s), 1.85 (3H, s), 1.99–2.13 (8H, m), 2.24 (3H, s), 2.47 (3H, s), 3.49 (2H, d, J = 7.0 Hz), 3.51 (1H, m), 3.82 (1H, d, J = 11.5 Hz), 3.85–3.90 (1H, m), 4.07 (1H, d, J = 11.5 Hz), 4.59 (1H, m), 5.07 (1H, t, J = 7.5 Hz), 5.23 (1H, t, J = 7.5 Hz), 5.38 (1H, t, J = 7.0 Hz), 5.86 (1H, s), 7.38–7.45 (2H, m), 7.62 (1H, d, J = 8.5 Hz), 8.10 (1H, d, J = 8.5 Hz); ^{13}C NMR (125 MHz, $CDCl_3$): δ 13.5, 14.0, 14.1, 16.1, 16.4, 19.5, 20.6, 22.7, 25.5, 26.3, 26.4, 30.7, 31.6, 39.2, 39.6, 62.1, 73.0, 97.4, 120.0, 120.6, 121.3, 121.8, 123.8, 124.9, 126.1, 126.3, 127.9, 131.8, 135.4, 137.9, 138.8, 147.8, 169.7; EI-LRMS MHz 520 (M^+). EI-HRMS calcd for $C_{33}H_{44}O_5$ 520.3189. Found 520.3192.

3.15. 1-Hydroxy-3-methyl-2-((2*E*,6*E*,10*E*,14*E*)-3,7,11-trimethyl-15-((tetrahydro-2H-pyran-2-yloxy)methyl)-hexadeca-2,6,10,14-tetraenyl)naphthalen-4-yl acetate (19c)

In a manner similar to that for the synthesis of **19a** from **17a**, a crude product, which was obtained from **17c** (258 mg, 0.66 mmol), a catalytic amount of boron trifluoride ether complex, and compound **18** (130 mg, 0.6 mmol) in acetate/dioxane 1:1 (0.8 mL), was purified by flash column chromatography on silica gel (hexane/ethyl acetate = 5/1) to give **19c** (170 mg, 48%) as a yellow oil: IR ($CHCl_3$): 3690, 3503, 2987, 2942, 2856, 1732, 1601, 1445, 1250, 1046 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 1.57 (3H, s), 1.51–1.71 (6H, m), 1.60 (3H, s), 1.65 (3H, s), 1.87 (3H, s), 1.98–2.14 (12H, m), 2.26 (3H, s), 2.47 (3H, s), 3.41–3.51 (1H, m), 3.53 (2H, d, J = 6.5 Hz), 3.83 (1H, d, J = 11.5 Hz), 3.85–3.90 (1H, m), 4.08 (1H, d, J = 11.5 Hz), 4.59 (1H, t, J = 4.0 Hz), 5.06–5.13 (2H, m), 5.24–5.27 (1H, m), 5.39–5.42 (1H, m), 5.76 (1H, s), 7.40–7.46 (2H, m), 7.62–7.64 (1H, m), 8.11–8.13 (1H, m); ^{13}C NMR (125 MHz, $CDCl_3$): δ 13.7, 14.1, 16.0, 16.1, 16.4, 19.5, 20.6, 22.7, 25.5, 26.3, 26.41, 26.46, 26.59, 30.7, 39.3, 39.7, 62.2, 73.0, 97.4, 120.6, 121.2, 121.8, 123.5, 124.0, 124.4, 124.9, 126.06, 126.13, 126.3, 128.0, 131.8, 134.7, 135.8, 138.0, 139.3,

148.0, 169.7; EI-LRMS MHz 588 (M^+). EI-HRMS calcd for $C_{38}H_{52}O_5$ 588.3815. Found 588.3831.

3.16. 2-((2*E*,6*E*)-8-(1,4-dimethoxy-2-methylnaphthalen-3-yl)-2,6-dimethylocta-2,6-dienyloxy)-tetrahydro-2H-pyran (20a)

To a stirred suspension of 30% potassium hydride (dispersion in mineral oil) (142 mg, 1.1 mmol) in THF (8 mL) was added compound **19a** (120 mg, 0.27 mmol) in THF (2 mL) at 0 °C under argon. The mixture was warmed to room temperature and then stirred for 20 min. After the color of the suspension changed to dark green, methyl iodide (1 mL, 1.6 mmol) was added, and the reaction mixture was stirred at room temperature further for 12 h. Addition of saturated aqueous NH_4Cl to the mixture at 0 °C was followed by extraction with ether three times. The organic layer was dried over anhydrous $MgSO_4$ and concentrated dry in vacuo. The residue was chromatographed on silica gel using hexane/ethyl acetate 10:1 to 5:1 to give **20a** (76 mg, 65%) as a pale yellow oil: IR ($CHCl_3$): 3691, 3009, 2944, 2853, 1594, 1456, 1353, 1206, 1020 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 1.48-1.60 (4H, m), 1.63 (3H, s), 1.65–1.73 (2H, m), 1.83 (3H, s), 2.05 (2H, t, J = 8.0 Hz), 2.14 (2H, t, J = 8.0 Hz), 2.37 (3H, s), 3.45–3.49 (1H, m), 3.56 (2H, d, J = 5.5 Hz), 3.79 (1H, d, J = 11.5 Hz), 3.82–3.86 (1H, m), 3.87 (3H, s), 3.88 (3H, s), 4.05 (1H, d, J = 11.5 Hz), 4.56 (1H, t, J = 3.0 Hz), 5.11–5.13 (1H, m), 5.37–5.40 (1H, m), 7.44–7.46 (2H, m), 8.03–8.06 (2H, m); ^{13}C NMR (125 MHz, $CDCl_3$): δ 12.4, 14.0, 16.4, 19.5, 25.5, 26.29, 26.32, 30.6, 39.3, 61.3, 62.1, 62.2, 72.9, 97.4, 122.1, 122.3, 125.3, 125.4, 126.9, 127.2, 127.5, 127.6, 130.8, 132.0, 135.5, 149.7, 150.1; EI-LRMS MHz 438 (M^+). EI-HRMS calcd for $C_{28}H_{38}O_4$ 438.2770. Found 438.2781.

3.17. 2-((2*E*,6*E*,10*E*)-12-(1,4-dimethoxy-2-methylnaphthalen-3-yl)-2,6,10-trimethyldodeca-2,6,10-trienyloxy)-tetrahydro-2H-pyran (20b)

In a manner similar to that for the synthesis of **20a** from **19a**, a crude product, which was obtained from **19b** (110 mg, 0.21 mmol), 30% potassium hydride (dispersion in mineral oil) (113 mg, 0.85 mmol), and methyl iodide (0.78 mL, 1.3 mmol), was purified by flash column chromatography on silica gel (hexane/ethyl acetate = 10/1 to 5/1) to give **20b** (66 mg, 62%) as a pale yellow oil: IR ($CHCl_3$): 3690, 2939, 2854, 1732, 1602, 1456, 1376, 1227, 1017 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 1.49–1.71 (6H, m), 1.57 (3H, s), 1.62 (3H, s), 1.83 (3H, s), 1.95–2.09 (8H, m), 2.37 (3H, s), 3.47–3.50 (1H, m), 3.56 (2H, d, J = 6.5 Hz), 3.78–3.85 (1H, m), 3.82 (1H, d, J = 11.5 Hz), 3.87 (3H, s), 3.88 (3H, s), 4.07 (1H, d, J = 11.5 Hz), 4.58 (1H, t, J = 3.5 Hz), 5.07–5.11 (2H, m), 5.36–5.39 (1H, m), 7.44–7.47 (2H, m), 8.03–8.06 (2H, m); ^{13}C NMR (125 MHz, $CDCl_3$): δ 12.4, 14.0, 16.0, 16.4, 19.5, 25.5, 26.34, 26.37, 26.6, 30.7, 39.3, 39.7, 61.3, 62.16, 62.19, 73.0, 97.4, 122.1, 122.2, 122.8, 124.3, 125.3, 125.4, 126.9, 127.3, 127.5, 127.8, 130.9, 131.8, 134.8, 135.7, 149.7, 150.1; EI-LRMS MHz 506 (M^+). EI-HRMS calcd for $C_{33}H_{46}O_4$ 506.3396. Found 506.3401.

3.18. 2-((2*E*,6*E*,10*E*,14*E*)-16-(1,4-dimethoxy-2-methylnaphthalen-3-yl)-2,6,10,14-tetramethylhexadeca-2,6,10,14-tetraenyloxy)-tetrahydro-2H-pyran (20c)

In a manner similar to that for the synthesis of **20a** from **19a**, a crude product, which was obtained from **19c** (100 mg, 0.17 mmol), 30% potassium hydride (dispersion in mineral oil) (91 mg, 0.68 mmol), and methyl iodide (0.63 mL, 1.0 mmol), was purified by flash column chromatography on silica gel (hexane/ethyl acetate = 10/1 to 5/1) to give **20c** (59 mg, 60%) as a pale yellow oil: IR ($CHCl_3$): 3690, 2989, 2940, 2854, 1732, 1602, 1456, 1353, 1250, 1046 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 1.51–1.71 (6H, m), 1.56 (3H, s), 1.56 (3H, s), 1.65 (3H, s), 1.83 (3H, s), 1.92–2.12 (12H, m), 2.37 (3H, s), 3.48–3.51 (1H, m), 3.56 (2H, d, J = 6.5 Hz), 3.83 (1H, d, J = 11.5 Hz), 3.84–3.92 (1H, m), 3.87 (3H, s), 3.88 (3H, s), 4.09 (1H, d, J = 11.5 Hz), 4.59 (1H, t, J = 4.0 Hz), 5.06–5.12 (3H, m), 5.39–5.41 (1H, m), 7.44–7.48 (2H, m), 8.03–8.07 (2H, m); ^{13}C NMR (125 MHz, $CDCl_3$): δ 12.3, 14.0, 15.95, 16.03, 16.4, 19.6, 25.5, 26.3, 26.4, 26.6, 26.7, 30.7, 39.3, 39.7, 61.3, 62.17, 62.19, 73.0, 97.4, 122.1, 122.3, 122.8, 124.1, 124.4, 125.3, 125.4, 126.9, 127.3, 127.5, 127.9, 130.9, 131.8, 134.7, 135.1, 135.8, 149.7, 150.1; EI-LRMS MHz 574 (M^+). EI-HRMS calcd for $C_{38}H_{54}O_4$ 574.4022. Found 574.4042.

3.19. 2-((2*E*,6*E*)-8-hydroxy-3,7-dimethylocta-2,6-dienyl)-3-methylnaphthalene-1,4-dione (MK-2 ω-OH) (7)

To a stirred suspension of di-ammonium cerium (IV) nitrate (158 mg, 0.29 mmol) in water (0.1 mL) was added **20a** (42 mg, 96 μmol) in CH_3CN/ ether 5:1 (0.6 mL). After addition of extra CH_3CN (0.3 mL), the mixture was stirred for 20 min. The reaction mixture was poured into water and extracted with ether three times. The combined ether layer was washed with water and brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residue was purified by preparative TLC (hexane/ethyl acetate = 3/1) to give **7** (22 mg, 72%) as a yellow oil: IR ($CHCl_3$): 3690, 3602, 2997, 2928, 1660, 1597, 1458, 1378, 1332, 1297, 973 cm^{-1};^1H NMR (500 MHz, $CDCl_3$): δ 1.63 (3H, s), 1.79 (3H, s), 2.03 (2H, t, J = 7.5 Hz), 2.12 (2H, t, J = 7.5 Hz), 2.19 (3H, s), 3.36 (2H, d, J = 6.5 Hz), 3.94 (2H, br s), 5.01 (1H, t, J = 7.0 Hz), 5.32 (1H, t, J = 7.0 Hz), 7.68–7.70 (2H, m), 8.07–8.09 (2H, m); ^{13}C NMR (125 MHz, $CDCl_3$): δ 12.7, 13.7, 16.3, 25.9, 26.1, 39.2, 68.9, 119.6, 125.6, 126.2, 126.3, 132.1, 133.3, 133.4, 135.0, 137.0, 143.4, 146.1, 184.6, 185.4; EI-LRMS MHz 324 (M^+). EI-HRMS calcd for $C_{21}H_{24}O_3$ 324.1725. Found 324.1748.

3.20. 2-((2*E*,6*E*,10*E*)-12-hydroxy-3,7,11-trimethyldodeca-2,6,10-trienyl)-3-methylnaphthalene-1,4-dione (MK-3 ω-OH) (8)

In a manner similar to that for the synthesis of **7** from **20a**, a crude product, which was obtained from **20b** (45 mg, 88 μmol), di-ammonium cerium (IV) nitrate (146 mg, 0.27 mmol) in water, and CH_3CN/ ether 5:1 (0.6 mL), was purified by preparative TLC (hexane/ethyl acetate = 3/1) to give **8** (26 mg, 76%) as a yellow oil: IR

($CHCl_3$): 3677, 3620, 2979, 2926, 1657, 1430, 1332, 1229, 929 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 1.57 (3H, s), 1.63 (3H, s), 1.79 (3H, s), 1.93–2.09 (8H, m), 2.17 (3H, s), 3.67 (2H, d, J = 7.5 Hz), 3.97 (2H, br s), 5.00–5.07 (2H, m), 5.32–5.35 (1H, m), 7.68–7.70 (2H, m), 8.07–8.09 (2H, m); ^{13}C NMR (125 MHz, $CDCl_3$): δ 12.7, 13.6, 16.0, 16.4, 26.0, 26.2, 26.3, 39.2, 39.6, 69.0, 119.2, 124.1, 126.0, 126.2, 126.3, 132.1, 132.2, 133.28, 133.34, 134.7, 134.8, 137.4, 143.3, 146.1, 184.5, 185.5; EI-LRMS MHz 392 (M^+). EI-HRMS calcd for $C_{26}H_{32}O_3$ 392.2351. Found 392.2376.

3.21. 2-((2*E*,6*E*,10*E*,14*E*)-16-hydroxy-3,7,11,15-tetramethylhexadeca-2,6,10,14-tetraenyl)-3-methylnaphthalene-1,4-dione (MK-4 ω-OH) (9)

In a manner similar to that for the synthesis of **7** from **20a**, a crude product, which was obtained from **20c** (41 mg, 71 μmol), di-ammonium cerium (IV) nitrate (117 mg, 0.21 mmol) in water, and CH_3CN/ether 5:1 (0.6 mL), was purified by preparative TLC (hexane/ethyl acetate = 3/1) to give **9** (23 mg, 71%) as a yellow oil: IR ($CHCl_3$): 3607, 3605, 2979, 2926, 1658, 1430, 1332, 1229, 973 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 1.53 (3H, s), 1.56 (3H, s), 1.66 (3H, s), 1.79 (3H, s), 1.91–2.13 (12H, m), 2.19 (3H, s), 3.37 (2H, d, J = 7.5 Hz), 3.99 (2H, br s), 5.00–5.08 (3H, m), 5.38 (1H, dt, J = 1.5, 7.5 Hz), 7.68–7.70 (2H, m), 8.07–8.09 (2H, m); ^{13}C NMR (125 MHz, $CDCl_3$): δ 12.7, 13.7, 15.96, 16.0, 16.4, 26.0, 26.2, 26.5, 26.6, 39.3, 39.6, 39.7, 69.0, 119.1, 123.9, 124.5, 126.16, 126.22, 126.3, 132.2, 133.3, 133.4, 134.5, 135.1, 137.5, 143.4, 146.2, 184.5, 185.4; EI-LRMS MHz 460 (M^+). EI-HRMS calcd for $C_{31}H_{40}O_3$ 460.2977. Found 460.2965.

3.22. (2*E*,6*E*,10*E*,14*E*)-16-(1,4-dihydro-2-methyl-1,4-dioxonaphthalen-3-yl)-2,6,10,14-tetramethylhexadeca-2,6,10,14-tetraenal (MK-2 ω-CHO) (10)

To a solution of **7** (15 mg, 46 μmol) in CH_2Cl_2 (3 mL) was added pyridinium dichromate (35 mg, 92 μmol) at room temperature, and the mixture was stirred for 12 h. After the solution was concentrated, the residue was purified by preparative TLC (hexane/ethyl acetate 5/1) to give **10** (5.2 mg, 35%) as a yellow oil: IR ($CHCl_3$): 2929, 2855, 1682, 1660, 1618, 1597, 1258 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 1.72 (3H, s), 1.83 (3H, s), 2.18 (2H, dd, J = 7.5, 15.0 Hz), 2.19 (3H, s), 2.45 (2H, dd, J = 7.5, 15.0 Hz), 3.39 (2H, d, J = 7.0 Hz), 5.09 (1H, dt, J = 1.5, 7.5 Hz), 6.41 (1H, dt, J = 1.5, 7.5 Hz), 7.69–7.71 (2H, m), 8.07–8.10 (2H, m), 9.33 (1H, s); ^{13}C NMR (125 MHz, $CDCl_3$): δ 9.2, 12.7, 16.3, 26.1, 27.3, 38.0, 120.5, 121.3, 126.27, 126.31, 132.1, 133.41, 133.44, 136.0, 143.5, 145.7, 153.8, 184.5, 185.4, 195.1; EI-LRMS MHz 322 (M^+). EI-HRMS calcd for $C_{21}H_{22}O_3$ 322.1569. Found 322.1554.

3.23. (2*E*,6*E*,10*E*)-12-(1,4-dihydro-2-methyl-1,4-dioxonaphthalen-3-yl)-2,6,10-trimethyldodeca-2,6,10-trienal (MK-3 ω-CHO) (11)

In a manner similar to that for the synthesis of **10** from **7**, a crude product, which was obtained from **8** (14 mg, 36 μmol) and pyridinium dichromate (27 mg, 71 μmol) in CH_2Cl_2, was purified by preparative TLC (hexane/ethyl acetate = 5/1) to give **11** (7.7 mg, 55%) as a yellow oil: IR ($CHCl_3$): 3016, 1682, 1660, 1598, 1435, 1332, 1227 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 1.59 (3H, s), 1.70 (3H, s), 1.79 (3H, s), 2.00 (2H, t, J = 7.5 Hz), 2.06–2.11 (4H, m), 2.19 (3H, s), 2.39 (2H, dd, J = 7.5, 15.0 Hz), 3.37 (2H, d, J = 7.5 Hz), 5.02 (1H, dt, J = 1.5, 7.5 Hz), 5.09 (1H, dt, J = 1.5, 7.5 Hz), 6.40 (1H, dt, J = 1.5, 7.5 Hz), 7.68–7.70 (2H, m), 8.07–8.09 (2H, m), 9.35 (1H, s); ^{13}C NMR (125 MHz, $CDCl_3$): δ 9.19, 12.7, 15.9, 16.4, 26.1, 26.4, 27.4, 37.9, 39.5, 119.4, 125.2, 126.3, 126.3, 132.17, 132.21, 133.37, 133.41, 133.6, 137.3, 139.4, 143.4, 146.2, 154.4, 184.6, 185.5, 195.3; EI-LRMS MHz 390 (M^+). EI-HRMS calcd for $C_{26}H_{30}O_3$ 390.2195. Found 390.2201.

3.24. (2*E*,6*E*)-8-(1,4-dihydro-2-methyl-1,4-dioxonaphthalen-3-yl)-2,6-dimethylocta-2,6-dienal (MK-4 ω-CHO) (12)

In a manner similar to that for the synthesis of **10** from **7**, a crude product, which was obtained from **9** (10 mg, 22 μmol) and pyridinium dichromate (16 mg, 43 μmol) in CH_2Cl_2, was purified by preparative TLC (hexane/ethyl acetate = 5/1) to give **12** (5.1 mg, 51%) as a yellow oil: IR ($CHCl_3$): 2978, 2929, 2855, 1682, 1660, 1618, 1597, 1440, 1331, 1227 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 1.56 (3H, s), 1.59 (3H, s), 1.74 (3H, s), 1.79 (3H, s), 1.92 (2H, t, J = 7.0 Hz), 1.98–2.07 (6H, m), 2.14 (2H, t, J = 7.5 Hz), 2.19 (3H, s), 2.43 (2H, dd, J = 7.0, 14.5 Hz), 3.37 (2H, d, J = 7.5 Hz), 5.00-5.12 (3H, m), 6.45 (1H, dt, J = 1.5, 7.5 Hz), 7.68–7.70 (2H, m), 8.07–8.09 (2H, m), 9.37 (1H, s); ^{13}C NMR (125 MHz, $CDCl_3$): δ 9.23, 12.7, 15.9, 16.4, 26.0, 26.5, 26.6, 27.4, 38.0, 39.5, 39.7, 119.2, 124.1, 125.6, 126.2, 126.3, 132.2, 133.3, 133.4, 135.0, 137.5, 139.4, 143.4, 146.2, 154.5, 184.6, 185.5, 195.3; EI-LRMS MHz 458 (M^+). EI-HRMS calcd for $C_{31}H_{38}O_3$ 458.2821. Found 458.2812.

3.24.1. Carboxylase activity assays. Carboxylase activity was assayed by previously described methods.[26–29] The amount of $^{14}CO_2$ incorporated into exogenous substrates was measured in reaction mixtures of 125 μL containing substrate at the indicated concentration, 222 μM of reduced MK-4, 16 μM of the propeptide ProFIX19 which contains the sequence AVFLDHENANKILNRPKRY, 1.4 mM $NaH^{14}CO_3$ (5 μCi), 25 mM MOPS (pH 7.0), 500 mM NaCl, 0.16% (w/v) phosphatidylcholine, 0.16% (w/v) CHAPS, 8 mM DTT, and 0.8 M ammonium sulfate, unless stated otherwise. All of the assay components except for the microsomal fraction were prepared as a master mixture. The incorporation of $^{14}CO_2$ into peptide substrates for over 30 min was assayed in a scintillation counter. Stimulation experiments with reduced MK-4 were performed at a constant concentration of the enzyme sample and substrate (3.6 mM FLEEL) with increasing concentrations of reduced MK-4, as indicated. All assays were performed in quadruplicate.

3.24.2. Evaluation of apoptosis-inducing activity of vitamin K analogues. MG-63 cells were maintained in continuous culture in DMEM supplemented with 10%

dextran-coated charcoal-treated fetal calf serum and 1% penicillin–streptomycin-mixed solution at 37 °C in a humidified atmosphere of 5% CO_2 in air. For synchronization at S phase, cells (4×10^5 cells/mL) were cultured in 30 mL of DMEM supplemented with 2.5 mM thymidine for 12 h. After two washes with Ca, Mg-free phosphate-buffered saline (PBS), the synchronization of the cell cycle was repeated in the same manner. Cells were placed in 6-well tissue culture plates and cultured for 3 days with vitamin K analogues (10^{-5} and 10^{-6} M) in DMEM. To reduce the effects of contact inhibition, control cells were adjusted to 60% to 70% confluency at the time of the FACS analysis. After each group of cells was collected in PBS, they were resuspended in PBS containing 0.2% Triton X and 1 μL RNase and then incubated at 37 °C for 1 h. Cells were washed with PBS and incubated with 0.5 mL of DNA-staining solution containing propidium iodide (50 μg/mL) at 4 °C for 20 min. The cells were analyzed with a flow cytometer equipped with an argon laser (488 nm) and the cell cycle distribution was analyzed.

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Supplementary data

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