Bioorganic & Medicinal Chemistry 18 (2010) 5795–5806

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Efficient synthesis and biological evaluation of demethyl geranylgeranoic acid derivatives ☆

Akimori Wada [a,*], Fei Wang [a], Yoshitomo Suhara [b], Yumiko Yamano [a], Takashi Okitsu [a], Kimie Nakagawa [c], Toshio Okano [c]

[a] Department of Organic Chemistry for Life Science, Kobe Pharmaceutical University, 4-19-1 Motoyamakita-machi, Higashinada-ku, Kobe 658-8558, Japan
[b] Department of Environmental Sciences, Yokohama College of Pharmacy, Yokohama 245-0066, Japan
[c] Department of Hygienic Sciences, Kobe Pharmaceutical University, 4-19-1 Motoyamakita-machi, Higashinada-ku, Kobe 658-8558, Japan

ARTICLE INFO

Article history:
Received 28 May 2010
Revised 30 June 2010
Accepted 2 July 2010
Available online 8 July 2010

Keywords:
Retinoid receptors
Retinoic acid analog
Geranylgeranoic acid
Demethyl analog
Coupling reaction
Vinyl triflate

ABSTRACT

Synthetic retinoids have generated in the fields of dermatology and oncology due to their potent anti-proliferative and differentiation activities. We efficiently synthesized different demethyl geranylgeranoic acid (GGA) analogs, and evaluated their biological activities. Among the demethyl analogs synthesized, 3-demethyl derivative exhibited the highest anti-proliferative activity in HL-60 cells. In addition, a 3-demethyl derivative induced apoptosis more potently than 9*Z*-retinoic acid. These activities were due to the high binding affinity of 3-demethyl derivative for retinoid receptors. We found that, in a conjugated polyene system combined with a methyl substituent, the position of the methyl played an important role in the regulation of gene transcription and apoptosis-inducing activity. These results provided useful information on the structure–activity relationships of GGA derivatives that function as acyclic retinoic acid analogs. This information is likely to be useful in the development of new anti-cancer drugs.

1. Introduction

The two retinoids, all-*E*-retinoic acid (ATRA) **1** and 9*Z*-retinoic acid (9CRA) **2** (Fig. 1) are metabolites of vitamin A (retinol) and bind the retinoic acid receptors (RARα, β, and γ) and retinoid X receptors (RXRα, β, and γ), respectively.[2–4] These receptors are members of the nuclear receptor superfamily, and play important roles in cell differentiation, cell proliferation, and embryonic development through regulation of gene transcription. RXRs form heterodimers with other nuclear receptors, including RARs, the thyroid-hormone receptor (TR), the vitamin-D receptor (VDR), and the peroxisome-proliferator-activated receptors (PPARs). Retinoids and their synthetic analogs have been developed as therapeutic agents in the fields of dermatology and oncology due to their potent anti-proliferative and differentiation-inducing activities. In particular, ATRA potently induces differentiation of acute promyelocytic leukemia (APL) blasts and is widely used as a chemotherapeutic treatment for APL.[5,6] However, the clinical use of retinoid is very restricted due to its strong side effects, particularly-headaches and mucocutaneous toxicity.[7] To overcome these problems, various novel retinoid analogs have been synthesized and their biological activities have been evaluated.[8–15] Among these analogs, acyclic retinoid (AR) **3**, which has a didehydrated structure due to the 4–5 single bond in geranylgeranoic acid (GGA) **4**, is expected to be a potent antitumor agent[16,17] and is currently being tested in clinical trials.[18,19] GGA was considered to be a retinoic acid analog because it was derived from retinoic acid by cleaving the 1–6 single bond and subsequently hydrogenating the two double bonds at the 7–8 and 11–12 positions (numbering in retinoic acid). In addition, it is well known that GGA **4** and geranylgeraniol (GG-OH) **5** can strongly induce apoptosis in various cell lines.[20–23] These observations prompted us to investigate the structure–activity relationships of GGA analogs as the next step in our studies on the synthesis of retinoid analogs and their biological activities.[24–27] In this study, we synthesized 3-, 7-, 11-, and 15-demethyl geranylgeranoic acids **6a–d** and evaluated their biological activities.

2. Results and discussion

2.1. Chemistry

To obtain demethyl analogs, we followed the general synthetic strategy shown in Scheme 1. The key step was the introduction of the hydrogen atom **B** or methyl substituent **C** into enol triflate **A**, which was stereoselectively derived from β-keto ester[28]; these transformations were achieved with palladium-catalyzed coupling reactions.[29]

☆ See Ref. 1.

* Corresponding author. Tel.: +81 78 441 7561; fax: +81 78 441 7562.
E-mail address: a-wada@kobepharma-u.ac.jp (A. Wada).

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doi:10.1016/j.bmc.2010.07.003

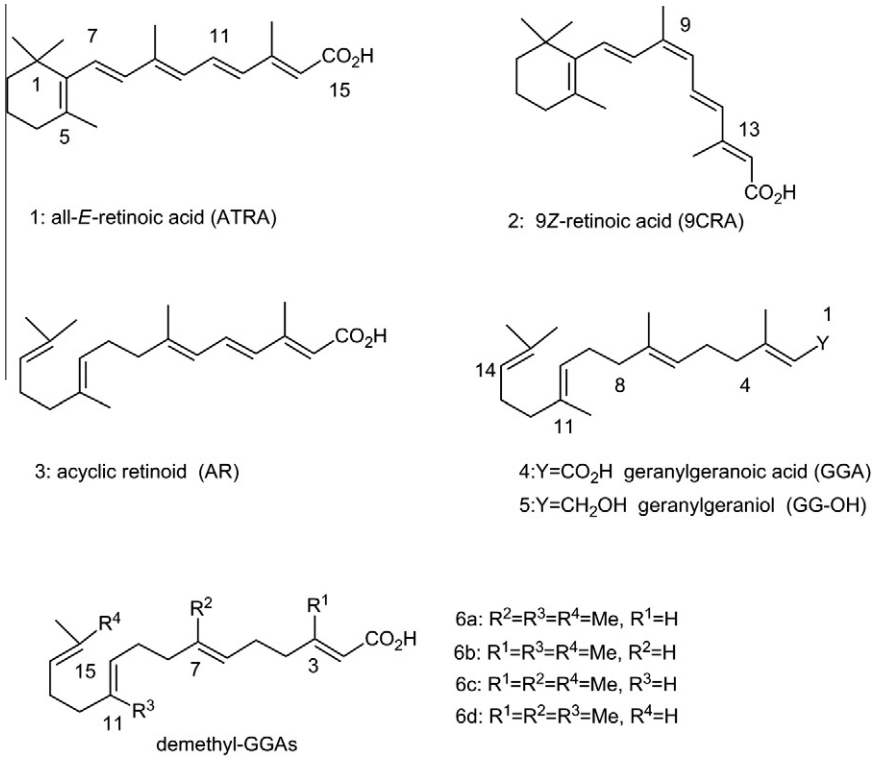
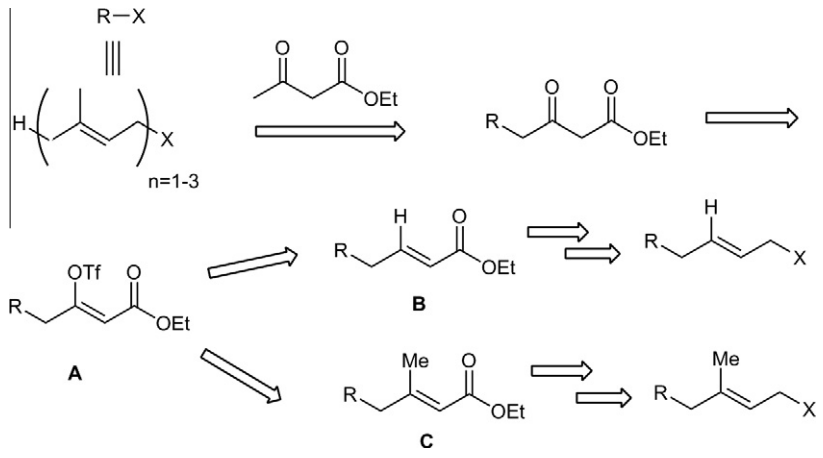


Figure 1. Structures of retinoic acids and related compounds.

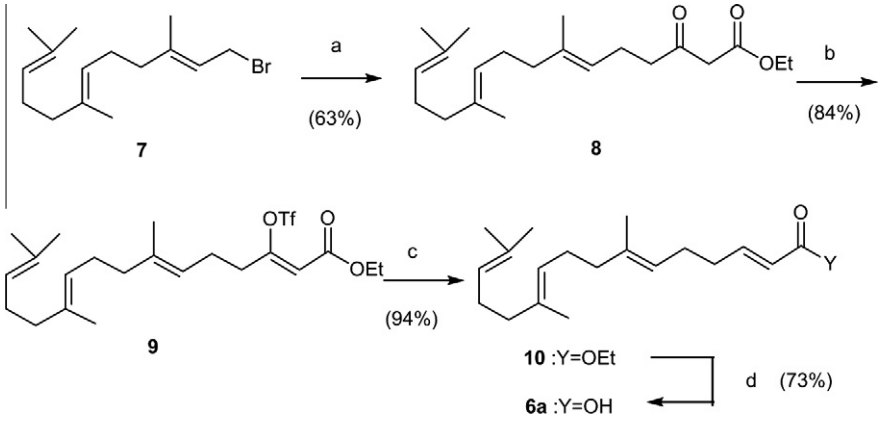


Scheme 1. Strategy for the synthesis of demethyl analogs.

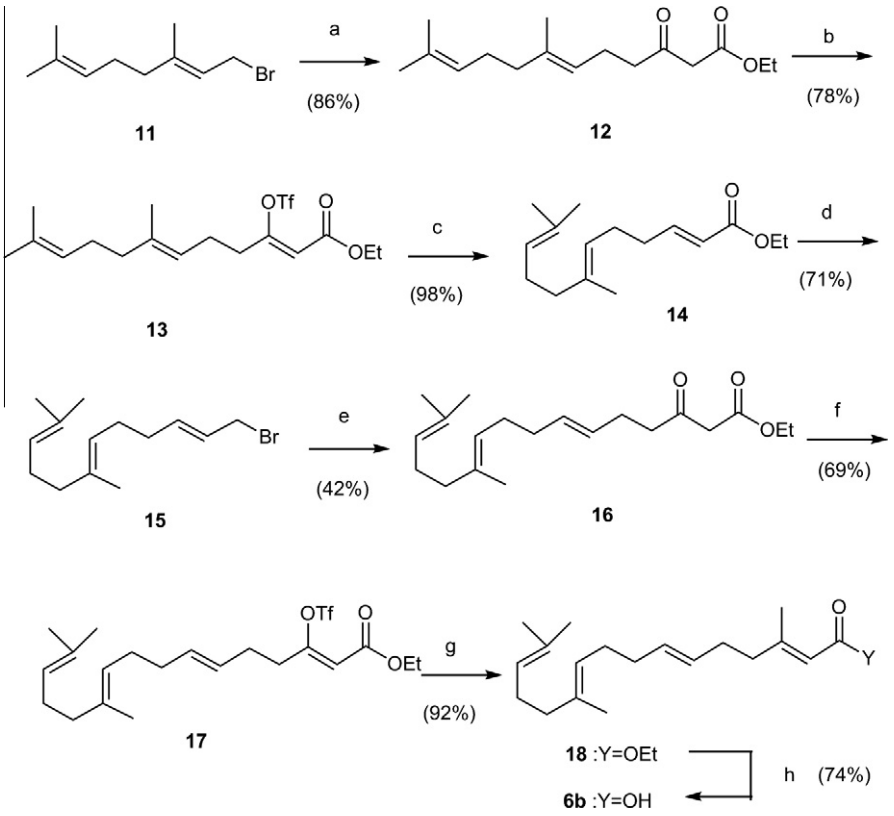
Scheme 2 shows the synthetic route for the 3-demethyl analog **6a**, starting from commercially available farnesyl bromide **7**. Compound **7** was converted into the (*Z*)-vinyl triflate **9** according to the previously reported method.[28] A coupling reaction between triflate **9** and tributyltin hydride proceeded smoothly to afford the ester **10** in 94% yield. A basic hydrolysis of the ester **10** generated the desired 3-demethyl acid **6a** in 73% yield.

The synthesis of the 7-demethyl analog **6b** started with geranyl bromide **11** (Scheme 3). In a manner similar to that for the synthesis of **10** from **7**, compound **11** was converted into the ester **14**. After reduction of the ester **14** with diisobutylaluminium hydride (DIBAL-H), the resulting alcohol was brominated with PPh_3 and CBr_4 to give **15**. Then, **15** was transformed into the triflate **17** in the same manner as described for the preparation of **13** from **12**. The coupling reaction between triflate **17** and tetramethyltin in the presence of a palladium catalyst achieved the introduction of a methyl substituent to give the ester **18**. Then, **18** was converted to the corresponding acid **6b** by basic hydrolysis.

For preparation of 11-demethyl and 15-demethyl analogs **6c** and **6d** (Scheme 4), we conducted the coupling reaction between allyl sulfones **23a** and **23b** and allyl bromides **25a** and **25b**. The sulfones **23a** and **23b** were readily obtained from the reaction of the corresponding allyl halides with the sodium salt of *p*-toluenesufinic acid; the vinyl bromides **25a** and **25b** were prepared from geranyl acetate **24a** and farnesyl acetate **24b**, respectively, according to a previously reported method.[30,31] The coupling reaction of **23** and **25** was accomplished with *n*-BuLi as a base and with a subsequent deacetylation in a DIBAL-H reduction. This gave the sulfonyl alcohol **26** in a mixture of isomers with double bonds at the carbon atom next to the connection position. The ratio of these



Scheme 2. Synthetic route for 3-demethyl GGA. Reagents: (a) ethyl acetoacetate, 60% NaH, *n*-BuLi; (b) $((CH_3)_3Si)_2NK$, Tf_2NPh; (c) Bu_3SnH, $Pd(PPh_3)_4$, LiCl; (d) 10% KOH, EtOH.



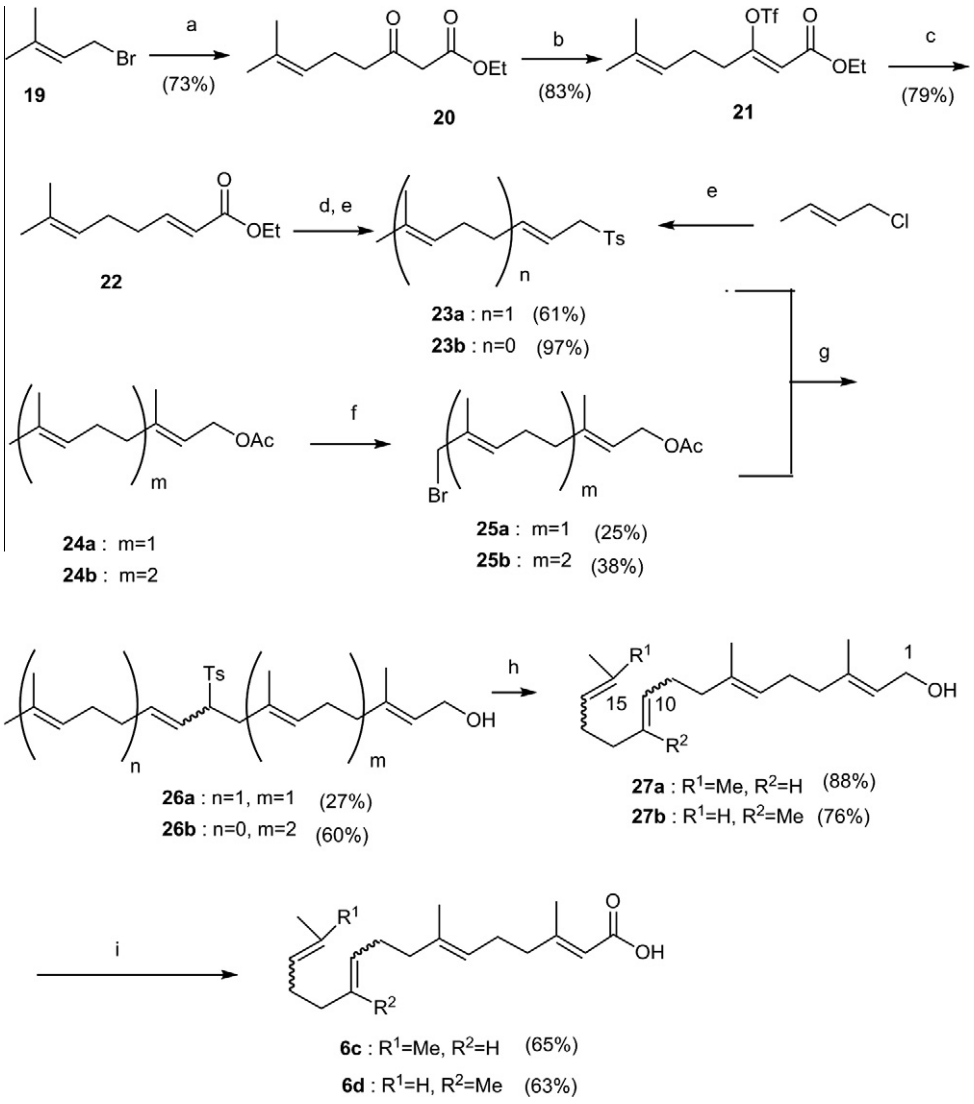
Scheme 3. Synthetic route for 7-demethyl GGA. Reagents: (a) ethyl acetoacetate, 60% NaH, *n*-BuLi; (b) $((CH_3)_3Si)_2NK$, Tf_2NPh; (c) Bu_3SnH, $Pd(PPh_3)_4$, LiCl; (d) i—DIBAL-H; ii—PPh_3, CBr_4; (e) ethyl acetoacetate, 60% NaH, *n*-BuLi; (f) $((CH_3)_3Si)_2NK$, Tf_2NPh; (g) CuI, Ph_3As, $Pd(PhCN)_2Cl_2$, Me_4Sn; (h) 10% KOH, EtOH.

isomers was determined by a ^1H NMR spectrum (10*E*:10*Z* = 2:1 for **26a**, 14*E*:14*Z* = 3:1 for **26b**). The separation of these isomers would have been difficult; therefore we used the mixture without further purification. Removal of the *p*-toluenesulfonyl group of **26** was achieved by treating with sodium amalgam and Na_2HPO_4 to give the alcohol **27**, which was finally converted into the corresponding acid **6** in two oxidation steps with aldehyde.

2.2. Biological evaluation

We evaluated our synthesized analogs for anti-proliferative activity, apoptosis-inducing activity, and receptor activation. To examine the anti-proliferative effect, we measured the cell cycle phase distribution of HL-60 cells treated with GGA and its analogs at 2.5×10^{-5} M. We found that, among the analogs, only the 3-demethyl analog **6a** could affect the G0/G1 cell cycle transition, and **6a** showed 1.7-fold higher potency than GGA **4** (Fig. 2).

We determined apoptosis-inducing activity of the analogs by cell cycle analysis in HL-60 cells treated with increasing concentrations of GGA and demethyl analogs. Dose–response relationships for GGA and its analogs (Fig. 3) indicated that the apoptosis-inducing activity of compound **6a** was 10-fold more potent than that of GGA **4**. The other demethyl analogs **6b**–**d** were less potent than **6a**, but at high concentrations, **6b** and **6c** exhibited slightly higher activities than GGA **4**.



Scheme 4. Synthetic route for 10-demethyl- and 14-demethyl GGAs. Reagents: (a) ethyl acetoacetate, 60% NaH, *n*-BuLi; (b) $((CH_3)_3Si)_2NK$, Tf_2NPh; (c) Bu_3SnH, $Pd(PPh_3)_4$, LiCl; (d) i—DIBAL-H; ii—PPh_3, NBS; (e) TsNa; (f) i—salicylic acid, SeO_2, *t*-BuOOH; ii—PPh_3, CBr_4; (g) i—*n*-BuLi; ii—DIBAL-H; (h) Na/Hg, Na_2HPO_4; (i) i—MnO_2; ii—$NaClO_2$, 2-methyl-2-butene, NaH_2PO_4.

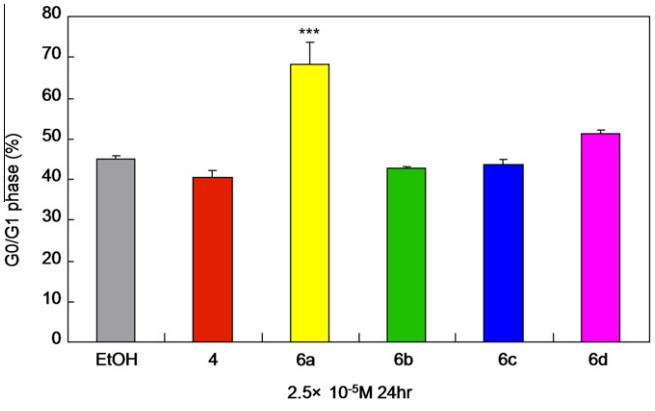


Figure 2. Cell cycle regulatory activity of GGA and its analogs in HL-60 cells. The percentage of cells in G0/G1 phase represents the number observed in the presence of the compounds (2.5×10^{-5} M for 24 h) compared to the number observed in the absence of 9CRA analogs. G0/G1-specific cell cycle arrest is evidenced by an increase in the relative number of cells with G0 compared to G1 DNA. The vertical bars indicate the SEM. *** $p < 0.001$.

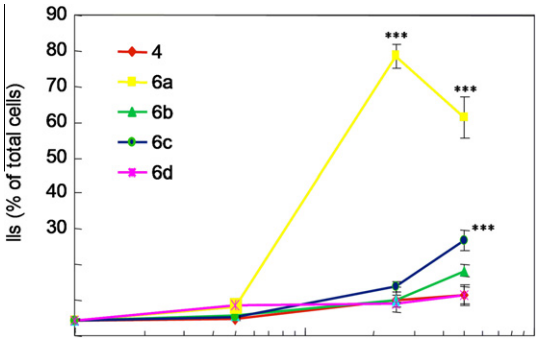


Figure 3. Dose–response relationships indicate the apoptosis-inducing activity of GGA and its analogs. Different concentrations of GGA analogs (10^{-6}–10^{-4} M) were added to HL-60 cells. Apoptosis is expressed as the percent of dead cells compared to the total number of untreated cells. The vertical bars indicate the SEM. *** $p < 0.001$; ** $p < 0.01$.

In order to determine whether the biological activity of the analogs was mediated by retinoid receptors, the analogs were tested in a transcriptional assay, where the transcriptional activation of RXRα was indicated by a luciferase reporter. The results are summarized in Figure 4. GGA did not show any transcriptional activity compared to the EtOH negative control. Among the demethyl analogs, the 3-demethyl derivative **6a** exhibited the highest activity; it activated RXRα with similar or higher potency than that 9CRA or ATRA.

It is well documented that ATRA can induce the differentiation of HL-60 cells into granulocytes and subsequently cause apoptosis by binding to RARs.[32,33] In contrast, 9CRA controls cell's biological activities, including apoptosis, by binding with almost equal affinity to RARs and RXRs.[34–37] Recently, an RAR-independent RXR signaling pathway was demonstrated with exposure to high concentrations of 9CRA in HL-60 cell lines. In that study, high 9CRA concentrations caused apoptosis through the activated RXR homodimer.[38,39] The high apoptosis-inducing activity of **6a** (Fig. 3) was observed at concentrations on the order of a micromolar or more; this concentration was three orders of magnitude higher than the doses of ATRA and 9CRA (nM) required to induce apoptosis. Therefore, the activity of **6a** would be attributed to RXR homodimer.

The high potency of apoptosis-inducing activity of **6a** was further supported by the following fact. Thus, in the transcriptional activity of analogs toward a rat cellular retinoic acid-binding protein II (CRABPII) gene including RXREs in transfected MG-63 cells, although none of the analogs **6a**–**d** at 10^{-6} M exhibited higher transcriptional activity than 9CRA, 3-demethyl derivative **6a** exhibited the highest activity among the analogs and its potency was about two-thirds of 9CRA (Fig. 5).

Finally, we assessed the transcriptional activity toward a human RARβ gene promoter with three copies of retinoic acid responsive elements (RAREs) in transfected MG-63 cells. None of the analogs **6a**–**d** exhibited a comparable or even higher RARβ/RARE mediated gene expression than did ATRA (Fig. 6). This fact strongly indicated that the apoptosis-inducing activity of **6a** was mediated through RXR homodimer not RAR.

In this study, we examined the anti-proliferative and apoptosis-inducing activities of demethyl GGAs in HL-60 cells. We found that the activities of GGA analogs were dramatically affected by the position of the demethylated in GGA. Thus, the 3-demethyl GGA **6a** showed the highest activities in both assays. This result contrasted markedly with our previous findings that 13-demethyl analogs of ATRA and 9CRA exhibited approximately half the potency of ATRA and 9CRA in transcriptional activation of RXRα.[27] Taken together, our results strongly suggested that the combination of methyl substituents and conjugated configurations might play an important role in the potency of retinoid biological activities. Further work is needed to define a more precise role for demethylated position in GGA analogs; these studies are currently ongoing.

3. Conclusion

In summary, we discovered a novel method for the synthesis of demethyl GGA derivatives that function as acyclic retinoid mimetics. The biological evaluation of the demethyl GGA analogs synthesized in this study indicated that **6a** represented a potent GGA analog. Our findings will provide useful information for further analog research and for biological studies in the mechanism of action of retinoids.

4. Experimental

4.1. General

IR spectra were recorded on a Perkin-Elmer FT-IR Paragon 1000 spectrometer in $CHCl_3$. ^1H NMR and ^{13}C NMR spectra were obtained on a Varian Gemini-300 NMR or a Varian VXR-500 spectrometer with tetramethylsilane as an internal standard in $CDCl_3$. Mass spectra were determined on a Hitachi M-4100 instrument. Column chromatography was performed with a Merck silica gel 60. Commercially available chemicals were used without further purification except when otherwise noted. Diisopropylamine was purified by distillation from CaH_2. A standard workup indicated that the organic layers were washed with brine, dried over anhydrous sodium sulfate, filtered, and concentrated in vacuo with a rotary evaporator.

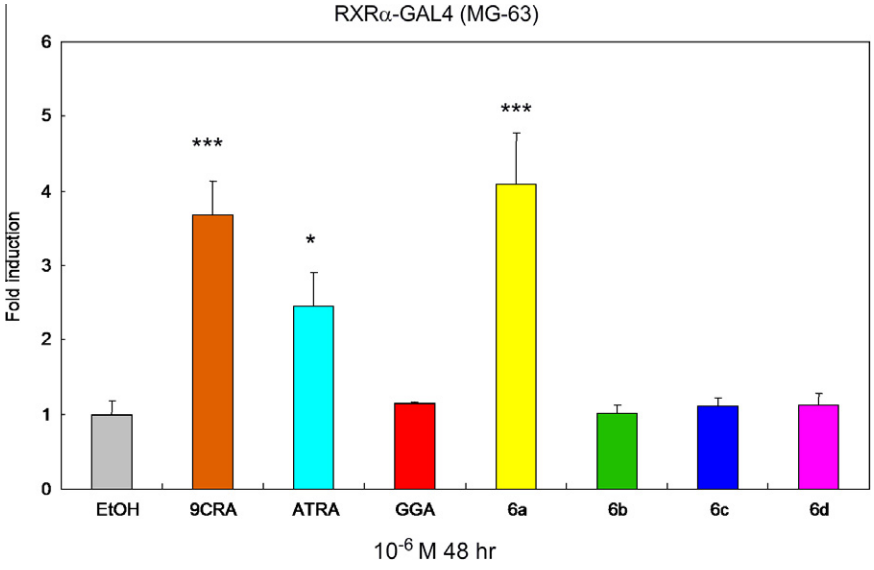


Figure 4. Transcriptional potency of ATRA, 9CRA, and their analogs on a human RXRα-GAL4 transgene expressed in MG-63 cells. The fold induction represents the increase in the expression of a RXR-regulated reporter gene relative to that observed for the ethanol treated control, which is defined as 1.0. Results represent the mean of three experiments where cells were exposed to analog concentrations at 10^{-6} M for 48 h; vertical bars show the SEM. $^{***}p < 0.001$; $^{*}p < 0.05$.

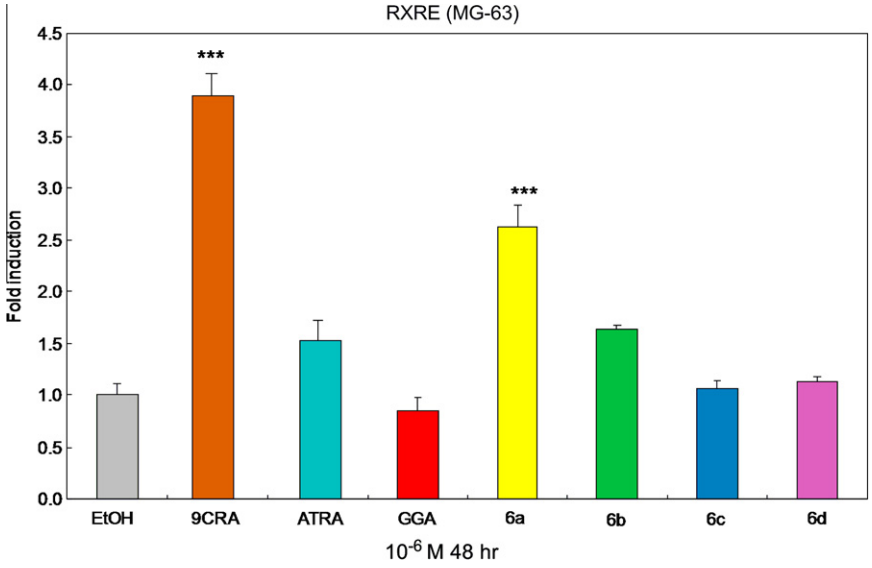


Figure 5. Transcriptional activity of ATRA, 9CRA, and their analogs toward a rat CRBPII-RXRE expression gene in transfected MG-63 cells. The cells were cotransfected with a luciferase reporter plasmid (pGVB2 vector) containing a rat CRABPII gene promoter including a RXRE and a pRL-CMV vector as an internal control. Luciferase activities induced by ATRA, 9CRA and the analogs in MG-63 cells were quantified and are shown as the fold increase in induction as compared with luciferase activity observed in the ethanol treated control cells (defined as 1.0). Results represent the mean of three experiments (values in column) at 10^{-6} M for 48 h; vertical bars indicate the SEM. *** $p < 0.001$.

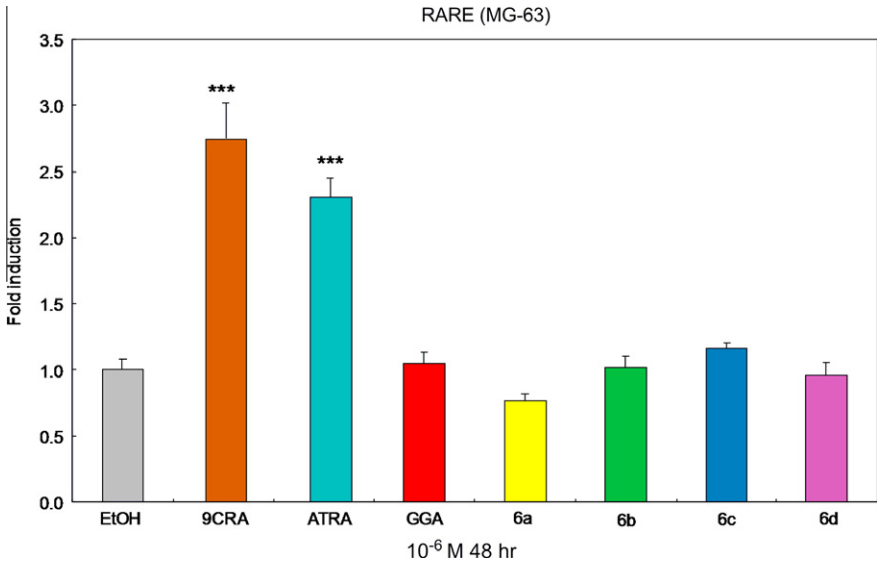


Figure 6. Transcriptional activity of ATRA, 9CRA, and their analogs toward a human RARα-RARE expression gene in transfected MG-63 cells. The cells were cotransfected with a luciferase reporter plasmid (pGVB2 vector) containing a human RARα gene promoter including a RARE and a pRL-CMV vector as an internal control. Luciferase activities induced by ATRA, 9CRA and the analogs in MG-63 cells were quantified and are presented as fold increase in induction as compared to the luciferase activity observed in the ethanol treated control cells, which is defined as 1.0. Each result represents the mean of three experiments (values in column) at 10^{-6} M for 48 h; vertical bars indicate the SEM. *** $p < 0.001$.

4.2. Synthesis of 3-demethyl analog (6a)

4.2.1. Ethyl (6*E*,10*E*)-7,11,15-trimethyl-3-oxo-6,10,14-hexadecatrienoate (8)

To a suspension of NaH (60% dispersion in oil, 440 mg, 11.0 mmol) in oil in THF (10 mL) was added ethyl acetoacetate (1.27 mL, 10.0 mmol), and the mixture was stirred at 0 °C for 10 min. Then *n*-BuLi (1.59 M hexane solution, 6.60 mL, 10.5 mmol) was added to the suspension, followed by stirring at 0 °C for additional 10 min. After addition of farnesyl bromide **7** (3.3 g, 11.0 mmol), the solution was stirred at room temperature for 1 h, quenched with saturated NH_4Cl aqueous solution. The mixture was extracted with ether (3× 50 mL), followed by standard work-up. The residue was purified by flash column chromatography on silica gel (hexane/AcOEt = 10:1) to give **8** (2.1 g, 63% yield) as a pale yellow oil. This compound is known, but there is no spectral data.[40]

IR ν_{max} cm^{-1}: 3029, 2927, 1741, 1715, 1650; ^1H NMR (300 MHz) δ: 1.26 (3H, t, J = 7.2 Hz, CH_2CH_3), 1.57 (6H, s, $CH_3 \times 2$), 1.60 (3H, s, CH_3), 1.66 (3H, s, CH_3), 1.96–2.05 (8H, m, $CH_2 \times 4$), 2.26–2.28 (2H, m, CH_2), 2.53–2.58 (2H, m, CH_2), 3.41 (2H, s, CH_2), 4.18 (2H, q, J = 7.2 Hz, OCH_2), 5.05–5.08 (3H, br s, =CH × 3); ^{13}C NMR (75 MHz) δ: 14.0, 15.9 (2C), 17.6, 22.1, 25.6, 26.5, 26.7, 39.5, 39.6,

43.0, 49.3, 61.3, 122.0, 124.0, 124.3, 131.2, 135.0, 136.7, 167.1, 202.5; EI-LRMS *m*/*z* 334 (M^+), 135, 109; EI-HRMS calcd for $C_{21}H_{34}O_3$ 334.2506; found: 334.2527.

4.2.2. Ethyl (2*Z*,6*E*,10*E*)-7,11,15-trimethyl-3-(trifluoromethylsulfonyloxy)-2,6,10,14-hexadecatetraenoate (9)

To a solution of 0.5 M potassium bis(trimethylsilyl)amide solution in toluene (14.0 mL, 7.0 mmol) were added compound **8** (1.94 g, 5.84 mmol) dissolved in THF (3 mL) and *N*-phenyl-bis(trifluoromethanesulfonimide) (2.53 g, 7.1 mmol) at −78 °C under Ar. After the mixture was warmed to room temperature, then stirred for 12 h. The resulting solution was diluted with ether and quenched with saturated NH_4Cl aqueous solution. The mixture was extracted with ether (3× 50 mL), followed by standard workup. The residue was purified by flash column chromatography on silica gel (hexane/AcOEt = 80:1) to afford **9** (2.32 g, 84% yield) as a pale yellow oil. This compound is known, but there is no spectral data.[40]

IR ν_{max} cm^{-1}; 3033, 2928, 1727, 1678, 1428; ^1H NMR (300 MHz) δ: 1.30 (3H, t, *J* = 7.2 Hz, CH_2CH_3), 1.59 (6H, s, $CH_3 \times 2$), 1.62 (3H, d, *J* = 0.9 Hz, CH_3), 1.67 (3H, d, *J* = 0.6 Hz, CH_3), 1.97–2.06 (8H, m, $CH_2 \times 4$), 2.26–2.31 (2H, m, CH_2), 2.38–2.43 (2H, m, CH_2), 4.24 (2H, q, *J* = 7.2 Hz, OCH_2), 5.06–5.11 (3H, m, =CH × 3), 5.74 (1H, s, =CH); ^{13}C NMR (75 MHz) δ: 14.0, 15.9, 16.0, 17.6, 24.4, 25.7, 26.5, 26.7, 34.6, 39.6, 39.7, 61.2, 112.0, 120.6, 123.8, 124.3, 131.3, 135.3, 138.2, 158.4, 162.5; EI-LRMS *m*/*z* 466 (M^+), 136; EI-HRMS calcd for $C_{22}H_{33}F_3O_5S$ 466.1999; found: 466.2001.

4.2.3. Ethyl (2*E*,6*E*,10*E*)-7,11,15-trimethyl-2,6,10,14-hexadecatetraenoate (10)

To a solution of compound **9** (50 mg, 0.107 mmol) in THF (5 mL) was added LiCl (13.6 mg, 0.321 mmol), $Pd(PPh_3)_4$ (6.2 mg, 5.35 μmol), and Bu_3SnH (62.3 mg, 0.214 mmol) and stirred at room temperature for 10 min. The reaction mixture was extracted with ether (3× 10 mL), followed by standard workup. The residue was purified by flash column chromatography on silica gel (hexane/AcOEt = 10:1) to give **10** (32 mg, 94% yield) as a pale yellow oil.

IR ν_{max} cm^{-1}; 3027, 2928, 1709, 1654, 1447; ^1H NMR (300 MHz) δ: 1.28 (3H, t, *J* = 7.2 Hz, CH_2CH_3), 1.59 (9H, br s, $CH_3 \times 3$) 1.67 (3H, s, CH_3), 1.98–2.09 (8H, m, $CH_2 \times 4$), 2.13–2.24 (4H, m, $CH_2 \times 2$), 4.18 (2H, q, *J* = 7.2 Hz, OCH_2), 5.07–5.12 (3H, br s, =CH × 3), 5.82 (1H, dt, *J* = 15.6, 1.5 Hz, =CH), 6.96 (1H, dt, *J* = 15.6, 6.9 Hz, =CH); ^{13}C NMR (75 MHz) δ: 14.3, 16.0, 16.1, 17.7, 25.7, 26.4, 26.5, 26.7, 32.4, 39.7 (2C), 60.1, 121.4, 122.7, 124.1, 124.4, 131.2, 135.0, 136.4, 148.9, 166.7; EI-LRMS *m*/*z* 318 (M^+), 136, 114; EI-HRMS calcd for $C_{21}H_{34}O_2$ 318.2557; found: 318.2538.

4.2.4. (2*E*,6*E*,10*E*)-7,11,15-Trimethyl-2,6,10,14-hexadecatetraenoic acid (6a)

To a solution of compound **10** (180 mg, 0.565 mmol) in ethanol (3 mL) was added 10% KOH aqueous solution (3 mL), and then heated at 70 °C for 2 h. After cooled to room temperature, the reaction mixture was neutralized with 10% HCl aqueous solution and the resulting solution was extracted with AcOEt (3× 10 mL), followed by standard workup. The residue was purified by flash column chromatography on silica gel (hexane/AcOEt = 1:1) to give **6a** (119 mg, 73% yield) as a pale yellow oil.

IR ν_{max} cm^{-1}; 3521, 2926, 1697, 1651, 1421; ^1H NMR (300 MHz) δ: 1.60 (9H, s, $CH_3 \times 3$), 1.68 (3H, s, CH_3), 1.95–2.13 (8H, m, $CH_2 \times 4$), 2.16–2.20 (2H, m, CH_2), 2.24–2.29 (2H, m, CH_2), 5.08–5.14 (3H, m, =CH × 3), 5.83 (1H, d, *J* = 15.6 Hz, =CH), 7.09 (1H, dt, *J* = 15.6, 6.6 Hz, =CH); ^{13}C NMR (75 MHz) δ: 16.0, 16.1, 17.6, 25.7, 26.3, 26.5, 26.7, 32.5, 39.6, 39.7, 120.8, 122.5, 124.0, 124.4, 131.2, 135.1, 136.6, 151.9, 172.1; EI-LRMS *m*/*z* 290 (M^+), 136; EI-HRMS calcd for $C_{19}H_{30}O_2$ 290.2244; found: 290.2239.

4.3. Synthesis of 7-demethylgeranylgeranoic acid (6b)

4.3.1. Ethyl (6*E*)-7,11-dimethyl-3-oxo-6,10-dodecadienoate (12)

In a manner similar to that for the synthesis of **8** from **7**, a crude product, which was obtained from **11** (2.39 g, 11.0 mmol), NaH (60% dispersion in oil, 440 mg, 11.0 mmol), *n*-BuLi (1.59 M hexane solution, 6.60 mL, 0.5 mmol), and ethyl acetoacetate (1.27 mL, 10.0 mmol), was purified by flash column chromatography on silica gel (hexane/AcOEt = 10:1) to give **12** (2.3 g, 86% yield) as a pale yellow oil. The spectral data of this compound were identical with those of the literature.[41]

^1H NMR (300 MHz) δ: 1.27 (3H, t, *J* = 7.2 Hz, CH_2CH_3), 1.59 (3H, s, CH_3), 1.60 (3H, s, CH_3), 1.67 (3H, s, CH_3), 1.94–2.06 (4H, m, $CH_2 \times 2$), 2.22–2.32 (2H, m, CH_2), 2.56 (2H, t, *J* = 7.5 Hz, CH_2CH_2CO), 3.42 (2H, s, $COCH_2CO$), 4.18 (2H, q, *J* = 7.2 Hz, CH_2O), 5.04–5.09 (2H, m, =CH × 2).

4.3.2. Ethyl (2*Z*,6*E*)-7,11-dimethyl-3-(trifluoromethylsulfonyloxy)-2,6,10-dodecatrienoate (13)

In a manner similar to that for the synthesis of **9** from **8**, a crude product, which was obtained from **12** (1.71 g, 6.42 mmol), 0.5 M potassium bis(trimethylsilyl)amide solution in toluene (15.4 mL, 7.70 mmol), and *N*-phenyl-bis(trifluoromethanesulfonimide) (2.75 g, 7.70 mmol), was purified by flash column chromatography on silica gel (hexane/AcOEt = 10:1) to afford **13** (2.0 g, 78% yield) as a pale yellow oil. The spectral data of this compound were identical with those of the literature.[41]

^1H NMR (300 MHz) δ: 1.29 (3H, t, *J* = 7.2 Hz, CH_2CH_3), 1.58 (3H, s, CH_3), 1.60 (3H, s, CH_3), 1.66 (3H, s, CH_3), 1.99–2.06 (4H, m, $CH_2 \times 2$), 2.25–2.30 (2H, m, CH_2), 2.37–2.42 (2H, m, CH_2), 4.23 (2H, q, *J* = 7.2 Hz, OCH_2), 5.03–5.07 (2H, m, =CH × 2), 5.73 (1H, s, =CH).

4.3.3. Ethyl (2*E*,6*E*)-7,11-dimethyl-2,6,10-dodecatrienoate (14)

In a manner similar to that for the synthesis of **10** from **9**, a crude product, which was obtained from **13** (1.44 g, 3.62 mmol), LiCl (460 mg, 10.9 mmol), $Pd(PPh_3)_4$ (25 mg, 21.6 μmol), and Bu_3SnH (2.1 g, 7.25 mmol), was purified by flash column chromatography on silica gel (hexane/AcOEt = 10:1) to give **14** (886 mg, 98% yield) as a pale yellow oil.

IR ν_{max} cm^{-1}; 3030, 2929, 1712, 1643; ^1H NMR (300 MHz) δ: 1.26 (3H, t, *J* = 7.2 Hz, CH_2CH_3), 1.58 (6H, s, $CH_3 \times 3$), 1.66 (3H, s, CH_3), 1.97–2.06 (4H, m, $CH_2 \times 2$), 2.12–2.23 (4H, m, $CH_2 \times 2$), 4.16 (2H, q, *J* = 7.2 Hz, CH_2O), 5.06–5.10 (2H, m, =CH × 2), 5.80 (1H, d, *J* = 15.6 Hz, =CH), 6.95 (1H, dt, *J* = 15.6, 6.6 Hz, =CH); ^{13}C NMR (75 MHz) δ: 14.2, 16.0, 17.6, 25.6, 26.4, 26.6, 32.4, 39.6, 60.0, 121.4, 122.7, 124.1, 131.3, 136.3, 148.8, 166.6; EI-LRMS *m*/*z* 250 (M^+), 235, 207, 135; EI-HRMS calcd for $C_{16}H_{26}O_2$ 250.1932; found: 250.1956.

4.3.4. (2*E*,6*E*)-1-Bromo-7,11-dimethyl-2,6,10-dodecatriene (15)

Compound **14** (585 mg, 2.34 mmol) was dissolved in CH_2Cl_2 (3 mL) and cooled to −78 °C. After DIBAL-H (1 M solution in toluene, 4.9 mL, 4.9 mmol) was added to the solution, the mixture was stirred at room temperature for 1 h. The resulting solution was quenched with saturated NH_4Cl aqueous solution, then filtered through Celite. The filtrate was extracted with ether (3× 50 mL), followed by standard workup. The residue was purified by flash column chromatography on silica gel (hexane/AcOEt = 10:1) to give (2*E*,6*E*)-7,11-dimethyl-2,6,10-dodecatrien-1-ol (413 mg, 85% yield) as a pale yellow oil.

IR ν_{max} cm^{-1}; 3430, 3010, 2978, 2874, 1618, 1456, 1378; ^1H NMR (300 MHz) δ: 1.45–1.60 (1H, br s, OH), 1.59 (6H, br s, $CH_3 \times 2$), 1.68 (3H, s, CH_3), 1.80–2.20 (8H, m, $CH_2 \times 4$), 4.03–4.12 (2H, m, OCH_2), 5.09 (2H, br s, =CH), 5.59–5.78 (2H, m, =CH × 2);

^{13}C NMR (75 MHz) δ: 16.0, 17.7, 25.7, 26.7, 27.6, 32.4, 39.7, 63.8, 123.6, 124.3, 129.0, 131.3, 133.0, 135.6.

To a solution of alcohol (0.407 g, 1.95 mmol) in CH_2Cl_2 was added PPh_3 (666 mg, 2.54 mmol) and CBr_4 (972 mg, 2.93 mmol) and stirred at room temperature for 1 h. After the solution was concentrated, the residue was dissolved in hexane (20 mL), then filtered through Celite. The filtrate was concentrated. The residue was purified by flash column chromatography on silica gel (hexane/AcOEt = 10:1) to give **15** (445 mg, 84% yield) as a pale yellow oil.

IR ν_{max} cm^{-1}; 3020, 2928, 1660, 1439, 1377; ^{1}H NMR (300 MHz) δ: 1.59 (6H, br s, $CH_3 \times 2$), 1.68 (3H, s, CH_3), 1.98–2.11 (8H, m, $CH_2 \times 4$), 3.94 (2H, d, J = 6.6 Hz, CH_2Br), 5.09–5.11 (2H, br s, =CH × 2), 5.71–5.76 (2H, m, =CH × 2); ^{13}C NMR (75 MHz) δ: 16.0, 17.7, 25.7, 26.6, 27.2, 32.2, 33.4, 39.6, 123.2, 124.3, 126.4, 131.2, 135.8, 136.2.

4.3.5. Ethyl (6*E*,10*E*)-11,15-dimethyl-3-oxo-6,10,14-hexadecatrienoate (16)

In a manner similar to that for the synthesis of **8** from **7**, a crude product, which was obtained from **15** (245 mg, 1.64 mmol), NaH (60% dispersion in oil, 72 mg, 1.80 mmol), 1.59 M (*n*-BuLi hexane solution, 1.08 mL, 1.72 mmol), and ethyl acetoacetate (209 μL, 1.64 mmol), was purified by flash column chromatography on silica gel (hexane/AcOEt = 10:1) to give **16** (220 mg, 42% yield) as a pale yellow oil.

IR ν_{max} cm^{-1}; 3027, 2929, 1741, 1715, 1648; ^{1}H NMR (300 MHz) δ: 1.27 (3H, t, J = 7.2 Hz, CH_2CH_3), 1.57 (3H, d, J = 0.9 Hz, CH_3), 1.59 (3H, s, CH_3), 1.67 (3H, d, J = 0.9 Hz, CH_3), 1.94–2.06 (8H, m, $CH_2 \times 4$), 2.24–2.29 (2H, m, CH_2), 2.59 (2H, t, J = 7.5 Hz, CH_2), 3.42 (2H, s, CH_2), 4.18 (2H, q, J = 7.2 Hz, CH_2O), 5.06–5.10 (2H, m, =CH × 2), 5.38–5.45 (2H, m, =CH × 2); ^{13}C NMR (75 MHz) δ: 14.0, 16.0, 17.6, 25.6, 26.4, 26.7, 27.8, 32.7, 39.6, 42.8, 49.3, 61.3, 123.8, 124.3, 127.9, 131.2, 131.5, 135.3, 167.1, 202.3; EI-LRMS *m*/*z* 320 (M$^+$), 279, 259, 147; EI-HRMS calcd for $C_{20}H_{32}O_3$ 320.2350; found: 320.2360.

4.3.6. Ethyl (2*Z*,6*E*,10*E*)-11,15-dimethyl-3-(trifluoromethylsulfonyloxy)-2,6,10,14-hexadecatetraenoate (17)

In a manner similar to that for the synthesis of **9** from **8**, a crude product, which was obtained from **16** (821 mg, 2.56 mmol), bis(trimethylsilyl)amide solution in toluene (6.14 mL, 3.07 mmol), and *N*-phenyl-bis(trifluoromethanesulfonimide) (1.10 g, 3.07 mmol), was purified by flash column chromatography on silica gel (hexane/AcOEt = 10:1) to afford **17** (800 mg, 69% yield) as a pale yellow oil.

IR ν_{max} cm^{-1}; 3023, 2930, 1728, 1679, 1428; ^{1}H NMR (300 MHz) δ: 1.30 (3H, t, J = 7.2 Hz, CH_2CH_3), 1.59 (3H, s, CH_3), 1.60 (3H, s, CH_3), 1.68 (3H, d, J = 1.2 Hz, CH_3), 1.97–2.04 (8H, m, $CH_2 \times 4$), 2.29 (2H, q, J = 6.8 Hz, CH_2), 2.42 (2H, t, J = 6.8 Hz, CH_2), 4.25 (2H, q, J = 7.2 Hz, OCH_2), 5.07–5.11 (2H, br s, =CH × 2), 5.30–5.60 (2H, m, =CH × 2), 5.74 (1H, s, =CH); ^{13}C NMR (75 MHz) δ: 14.0, 16.0, 17.7, 25.7, 26.7, 27.8, 28.8, 32.7, 34.5, 39.7, 61.2, 112.1, 123.6, 124.3, 126.4, 131.3, 133.1, 135.5, 158.2, 162.4; EI-LRMS *m*/*z* 452 (M$^+$), 411, 321, 275; EI-HRMS calcd for $C_{21}H_{31}F_3O_5S$ 452.1843; found: 452.1869.

4.3.7. Ethyl (2*E*,6*E*,10*E*)-3,11,15-trimethyl-2,6,10,14-hexadecatetraenoate (18)

To a solution of compound **17** (262 mg, 0.58 mmol) in 1-methyl-2-pyrrolidinone (NMP, 10 mL) was added CuI (11 mg, 0.058 mmol), Ph_3As (17.8 mg, 0.058 mmol), $Pd(PhCN)_2Cl_2$ (11.1 mg, 0.029 mmol), and $SnMe_4$ (169 μL, 1.16 mmol), and heated at 100 °C for 10 h. The mixture was diluted with AcOEt (30 mL) and the resulting mixture was washed with saturated KF aqueous solution (2× 20 mL) and water (2× 20 mL), dried over $MgSO_4$, and concentrated. The residue was purified by flash column chromatography on silica gel (hexane/AcOEt = 10:1) to give **18** (169 mg, 92% yield) as a pale yellow oil.

IR ν_{max} cm^{-1}; 3018, 2929, 1706, 1646; ^{1}H NMR (300 MHz) δ: 1.27 (3H, t, J = 7.2 Hz, CH_2CH_3), 1.59 (3H, s, CH_3), 1.60 (3H, s, CH_3), 1.68 (3H, s, CH_3), 1.90–2.10 (8H, m, $CH_2 \times 4$), 2.14–2.18 (4H, m, $CH_2 \times 2$), 2.15 (3H, s, CH_3), 4.14 (2H, q, J = 7.2 Hz, CH_2O), 5.09–5.12 (2H, m, =CH × 2), 5.40–5.43 (2H, m, =CH × 2), 5.65 (1H, s, =CH); ^{13}C NMR (75 MHz) δ: 14.3, 16.0, 17.7, 18.8, 25.7, 26.7, 28.0, 30.5, 32.7, 39.7, 40.9, 59.4, 115.7, 123.9, 124.3, 128.7, 131.1, 131.2, 135.3, 159.4, 166.8; EI-LRMS *m*/*z* 318 (M$^+$), 303, 168; EI-HRMS calcd for $C_{21}H_{34}O_2$ 318.2557; found: 318.2549.

4.3.8. (2*E*,6*E*,10*E*)-3,11,15-Trimethyl-2,6,10,14-hexadecatetraenoic acid (6b)

In a manner similar to that for the synthesis of **6a** from **10**, a crude product, which was obtained from **18** (140 mg, 0.314 mmol) in ethanol (5 mL) and 10% KOH (3 mL), was purified by flash column chromatography on silica gel (hexane/AcOEt = 1:1) to afford **6b** (94 mg, 74% yield) as a pale yellow oil.

IR ν_{max} cm^{-1}; 3520, 3030, 2927, 1691, 1640, 1438; ^{1}H NMR (300 MHz) δ: 1.59 (3H, s, CH_3), 1.60 (3H, s, CH_3), 1.68 (3H, s, CH_3), 1.98–2.08 (8H, m, $CH_2 \times 4$), 2.10–2.30 (4H, m, $CH_2 \times 2$) 2.17 (3H, s, CH_3), 5.10–5.12 (2H, m, =CH × 2), 5.40–5.44 (2H, m, =CH × 2), 5.69 (1H, s, =CH–CO); ^{13}C NMR (75 MHz) δ: 16.0, 17.7, 19.1, 25.7, 26.7, 28.0, 30.4, 32.7, 39.7, 41.2, 115.4, 123.9, 124.4, 128.5, 131.2, 131.3, 135.3, 162.7, 172.4; EI-LRMS *m*/*z* 290 (M$^+$), 275, 249, 123; EI-HRMS calcd for $C_{19}H_{30}O_2$ 290.2244; found: 290.2247.

4.4. Synthesis of 11-demethylgeranylgeranoic acid (6c)

4.4.1. Ethyl 7-methyl-3-oxo-6-octenoate (20)

In a manner similar to that for the synthesis of **8** from **7**, a crude product, which was obtained from **19** (4.5 g, 30.2 mmol), NaH (60% dispersion in oil, 1.45 g, 36.2 mmol), 1.59 M (*n*-BuLi hexane solution, 23 mL, 36.0 mmol), and ethyl acetoacetate (4.3 g, 33.0 mmol) was purified by flash column chromatography on silica gel (hexane/AcOEt = 10:1) to give **20** (4.19 g, 73% yield) as a pale yellow oil.

IR ν_{max} cm^{-1}; 3027, 2984, 1741, 1716, 1645; ^{1}H NMR (300 MHz) δ: 1.22 (3H, t, J = 7.2 Hz, CH_2CH_3), 1.56 (3H, s, CH_3), 1.61 (3H, s, CH_3), 2.22 (2H, q, J = 7.2 Hz, CH_2), 2.51 (2H, t, J = 7.2 Hz, CH_2), 3.37 (2H, s, CH_2), 4.13 (2H, q, J = 7.2 Hz, CH_2O), 5.00 (1H, tq, J = 7.2, 1.2 Hz, =CH); ^{13}C NMR (75 MHz) δ: 14.0, 17.5, 22.1, 25.5, 42.9, 49.3, 61.2, 122.2, 132.9, 167.1, 202.5; EI-LRMS *m*/*z* 198 (M$^+$), 180, 130, 111; EI-HRMS calcd for $C_{11}H_{18}O_3$ 198.1255; found: 198.1274.

4.4.2. Ethyl (2*Z*)-7-methyl-3-(trifluoromethylsulfonyloxy)-2,6-octadienoate (21)

In a manner similar to that for the synthesis of **9** from **8**, a crude product, which was obtained from **20** (4.62 g, 23.3 mmol), 0.5 M potassium bis(trimethylsilyl)amide solution in toluene (55.9 mL, 27.9 mmol), and *N*-phenyl-bis(trifluoromethanesulfonimide) (9.98 g, 27.9 mmol), was purified by flash column chromatography on silica gel (hexane/AcOEt = 10:1) to afford **21** (12.1 g, 83% yield) as a pale yellow oil.

IR ν_{max} cm^{-1}; 3032, 2983, 1727, 1678, 1428; ^{1}H NMR (300 MHz) δ: 1.29 (3H, t, J = 7.2 Hz, CH_2CH_3), 1.60 (3H, s, CH_3), 1.69 (3H, d, J = 0.9 Hz, CH_3), 2.21–2.28 (2H, m, CH_2), 2.37–2.41 (2H, m, CH_2), 4.24 (2H, q, J = 7.2 Hz, CH_2O), 5.01–5.07 (1H, m, =CH), 5.73 (1H, s, =CH); ^{13}C NMR (75 MHz) δ: 13.9, 17.6, 24.4, 25.6, 34.5, 61.2, 112.0, 120.7, 134.4, 158.4, 162.4; EI-LRMS *m*/*z* 331 (M+H$^+$), 262, 197, 123; EI-HRMS calcd for $C_{12}H_{17}F_3O_5S$ 331.0826; found: 331.0839.

4.4.3. Ethyl (2*E*)-7-methyl-2,6-octadienoate (22)

In a manner similar to that for the synthesis of **10** from **9**, a crude product, which was obtained from **21** (6.5 g, 19.7 mmol), LiCl

(2.5 g, 59 mmol), $Pd(PPh_3)_4$ (455 mg, 0.39 mmol), and Bu_3SnH (10.6 mL, 39.3 mmol) was purified by flash column chromatography on silica gel (hexane/AcOEt = 10:1) to give **22** (2.84 g, 79% yield) as a pale yellow oil. This compound is known, but there is no spectral data.[42]

IR ν_{max} cm^{-1}; 3025, 2931, 1707, 1654, 1447; ^1H NMR (300 MHz) δ: 1.27 (3H, t, J = 7.2 Hz, CH_2CH_3), 1.59 (3H, s, CH_3), 1.68 (3H, d, J = 0.6 Hz, CH_3), 2.11–2.25 (4H, m, $CH_2 \times 2$), 4.17 (2H, q, J = 7.2 Hz, CH_2O), 5.05 (1H, t, J = 6.5 Hz, =CH), 5.81 (1H, dt, J = 15.6, 1.8 Hz, =CH), 6.95 (1H, dt, J = 15.6, 6.6 Hz, =CH); ^{13}C NMR (75 MHz) δ: 14.2, 17.6, 25.6, 26.5, 32.4, 60.1, 121.4, 122.8, 132.7, 149.9, 166.7; EI-LRMS m/z 182 (M^+), 137, 114; EI-HRMS calcd for $C_{11}H_{18}O_2$ 182.1306; found: 182.1329.

4.4.4. (2*E*)-1-Methyl-4-(7-methyl-2,6-octadienylsulfonyl)-benzene (23a)

Compound **22** (2.84 g, 15.6 mmol) was dissolved in CH_2Cl_2 (30 mL) and cooled to −78 °C under Ar. After DIBAL-H (1 M solution in toluene, 32 mL, 32 mmol) was added to the solution, the mixture was stirred at room temperature for 1 h. The resulting solution was quenched with saturated NH_4Cl aqueous solution, then filtered through Celite. The filtrate was extracted with ether (3× 50 mL), followed by standard workup.

The residue was purified by flash column chromatography on silica gel (hexane/AcOEt = 10:1) to give (2*E*,6*E*)-7-methyl-2,6-octadien-1-ol as a pale yellow oil.

^1H NMR (300 MHz, $CDCl_3$) δ: 1.55 (3H, s, CH_3), 1.67 (3H, s, CH_3), 1.77 (1H, s, OH), 1.91–2.20 (4H, m, $CH_2 \times 2$), 4.06 (2H, br s, OCH_2), 5.12 (1H, br s, =CH), 5.56–5.80 (2H, m, =CH × 2); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 17.7, 25.6, 27.6, 32.3, 63.6, 123.7, 129.0, 131.9, 132.9.

The alcohol was dissolved in THF (30 mL) and cooled to −20 °C under Ar. To a solution of this alcohol was added PPh_3 (5.5 g, 21.0 mmol) and NBS (3.74 g, 21.0 mmol), and the mixture was stirred at −10 °C to 0 °C for 30 min. Further addition of sodium salt of *p*-toluenesulfinic acid (7.0 g, 28.0 mmol) and NaI (210 mg, 1.4 mmol) followed by stirring at room temperature for 5 h, and then AcOEt and saturated $Na_2S_2O_3$ aqueous solution was added. After separation of AcOEt layer, the aqueous layer was extracted with AcOEt (3× 30 mL), and combined organic layers were followed by standard workup. The residue was purified by flash column chromatography on silica gel (hexane/AcOEt = 3:1) to give **23a** (2.65 g, 61% yield in two steps) as a pale yellow oil.

IR ν_{max} cm^{-1}; 3027, 2927, 1598, 1318; ^1H NMR (300 MHz) δ: 1.53 (3H, s, CH_3), 1.64 (3H, d, J = 1.6 Hz, CH_3), 1.92–2.01 (4H, m, $CH_2 \times 2$), 2.41 (3H, s, $PhCH_3$), 3.69 (2H, d, J = 7.2 Hz, CH_2SO_2Ph), 4.98 (1H, t, J = 6.5 Hz, =CH), 5.37–5.48 (2H, m, =CH × 2), 7.30 (2H, d, J = 8.1 Hz, Ar-H), 7.70 (2H, d, J = 8.1 Hz, Ar-H); ^{13}C NMR (75 MHz) δ: 17.6, 21.5, 25.5, 27.1, 32.6, 60.1, 116.1, 123.2, 128.4, 129.5, 132.1, 135.4, 141.1, 144.4.

4.4.5. (2*E*,6*E*)-8-Bromo-3,7-dimethyl-2,6-octadienyl acetate (25a)

To a solution of *t*-BuOOH (10.7 g) in CH_2Cl_2 (100 mL) was added SeO_2 (181 mg, 1.6 mmol) and salicylic acid (5.06 g, 36.6 mmol), and the mixture was stirred at room temperature for 1 h. Geranyl acetate **24a** (6.46 g, 24.4 mmol) in CH_2Cl_2 (50 mL) was added by dropwise at 0 °C, and stirred for 12 h. After the reaction mixture was concentrated, the residue was dissolved in ether (150 mL), washed with 25% $Na_2S_2O_4$ aqueous solution (2× 100 mL), and water (100 mL), and the organic layer was concentrated. The resulting residue was dissolved in ethanol (100 mL) and cooled to 0 °C. $NaBH_4$ (1.84 g, 48.8 mmol) was added to the solution over 20 min, then quenched with saturated NH_4Cl aqueous solution (50 mL). The reaction mixture was extracted with ether (3× 100 mL), followed by standard workup. The residue was purified by flash column chromatography on silica gel (hexane/AcOEt = 3:1) to give (2*E*,6*E*)-8-hydroxy-3,7-dimethyl-2,6-octadienyl acetate. The spectral data of this compound were identical with those of the literature.[31]

^1H NMR (300 MHz, $CDCl_3$) δ: 1.59 (3H, s, CH_3), 1.64 (3H, s, CH_3), 1.99 (3H, s, CH_3), 2.00–2.18 (4H, m, $CH_2 \times 2$), 2.21 (1H, br s, OH), 3.91 (2H, s, OCH_2), 4.52 (2H, d, J = 7.0 Hz, OCH_2), 5.28 (1H, t, J = 7.0 Hz, =CH)), 5.30 (1H, t, J = 6.5 Hz, =CH).

To a solution of this alcohol in CH_2Cl_2 (100 mL) was continuously added PPh_3 (7.37 g, 28.1 mmol) and CBr_4 (10.7 g, 32.4 mmol) at 0 °C under Ar, and stirred at room temperature for 12 h. The mixture was washed with saturated NH_4Cl aqueous solution and brine, dried over $MgSO_4$, and concentrated. The residue was purified by flash column chromatography on silica gel (hexane/AcOEt = 3:1) to give **25a** (3.16 g, 25% yield in two steps) as a pale yellow oil. This compound is known, but there is no spectral data.[31]

^1H NMR (300 MHz) δ: 1.62 (3H, s, CH_3), 1.69 (3H, s, CH_3), 1.97 (3H, s, CH_3), 1.96–2.18 (4H, m, $CH_2 \times 2$), 3.87 (2H, s, CH_2Br), 4.49 (2H, d, J = 7.0 Hz, OCH_2), 5.26 (1H, t, J = 7.0 Hz, =CH), 5.48 (1H, t, J = 7.0 Hz, =CH).

4.4.6. (2*E*,6*E*,10*E*)- and (2*E*,6*E*,10*Z*)-3,7,15-Trimethyl-9-(4-methylphenylesulfony)l-2,6,10,14-hexadecatetraen-1-ol (26a)

To a solution of **23a** (546 mg, 1.96 mmol) in THF/HMPA (10:1, 10 mL) was added *n*-BuLi (1.59 M hexane solution, 1.84 mL, 2.94 mmol) at 0 °C under Ar, and the resulting mixture was stirred for an additional 1 h at 0 °C. Then **25a** (593 mg, 2.16 mmol) in THF (5 mL) was added to the solution and was stirred at 0 °C for 12 h. After warmed to room temperature, the mixture was quenched with 1 M HCl aqueous solution, then extracted with ether (3× 30 mL), followed by standard workup. Continuously, the residue was dissolved in ether (20 mL) at 0 °C and DIBAL-H (1 M in toluene solution, 2.94 mL, 2.94 mmol) was added to the solution. The mixture was stirred at 0 °C to room temperature for 2 h, quenched with saturated NH_4Cl aqueous solution at 0 °C, extracted with ether (3× 50 mL), followed by standard workup. The residue was purified by flash column chromatography on silica gel (hexane/AcOEt = 3:1) to give **26a** (228 mg, 27% yield) as a mixture of stereoisomer (10*E*:10*Z* = 3:1, a pale yellow oil).

(2*E*,6*E*,10*E*)-isomer: IR ν_{max} cm^{-1}: 3608, 3533, 2968, 2927, 1664, 1597, 1447; ^1H NMR (300 MHz, $CDCl_3$) δ: 1.45 (3H, s, CH_3), 1.48 (3H. s, CH_3), 1.57 (3H, s, CH_3), 1.60 (3H, s, CH_3), 1.62–2.10 (9H, m, $CH_2 \times 4$, OH), 2.23 (1H, dd, J = 13.5, 11.0 Hz, CH_2), 2.40 (3H, s, CH_3), 2.81 (1H, br d, J = 13.5 Hz, CH_2), 3.92 (1H, td, J = 11.0, 3.5 Hz, CH), 4.05 (2H, d, J = 7.0 Hz, OCH_2), 4.92 (1H, t, J = 7.0 Hz, =CH), 5.02–5.38 (4H, m, =CH × 4), 7.24 (2H, d, J = 8.5 Hz, Ar-H), 7.63 (2H, d, J = 8.5 Hz, Ar-H); ^{13}C NMR (75 MHz) δ: 15.6, 15.9, 17.4, 21.4, 25.5, 26.1, 27.1, 32.5, 37.1, 39.0, 58.9, 67.6, 121.7, 123.2, 123.5, 127.9, 129.0, 129.2, 129.8, 131.8, 134.4, 138.5, 139.5, 144.2.

4.4.7. (2*E*,6*E*,10*E*)- and (2*E*,6*E*,10*Z*)-3,7,15-Trimethyl-2,6,10,14-hexadecatetraen-1-ol (27a)

To a solution of compound **26a** (127 mg, 0.295 mmol) in anhydrous ethanol (10 mL) was added Na_2HPO_4 (168 mg, 1.18 mmol) and sodium amalgam 5.0% (543 mg, 1.18 mmol), and the reaction mixture was heated to reflux for 10 h. After the solution was cooled to room temperature, the reaction was quenched with 1 M HCl aqueous solution, and extracted with ether (3× 50 mL), followed by standard workup. The residue was purified by flash column chromatography on silica gel (hexane/AcOEt = 3:1) to give **27a** (72 mg, 88% yield) as a mixture of stereoisomer (10*E*:10*Z* = 3:1, a pale yellow oil).[43]

(2*E*,6*E*,10*E*)-isomer: IR ν_{max} cm^{-1}; 3611, 3010, 2928, 1667, 1450, 1383; ^1H NMR (300 MHz) δ: 1.36 (1H, br s, OH), 1.58 (6H, s, $CH_3 \times 2$), 1.64 (3H, s, CH_3), 1.67 (3H, s, CH_3), 1.90–2.20 (12H, m, $CH_2 \times 6$), 4.12 (2H, d, J = 7.0 Hz, OCH_2), 5.01 (2H, t, J = 6.0 Hz,

=CH × 2), 5.28–5.50 (3H, m, =CH × 3); EI-LRMS *m*/*z* 276 (M^+), 189, 135; EI-HRMS calcd for $C_{19}H_{32}O$ 276.2452; found: 276.2469.

4.4.8. (2*E*,6*E*,10*E*)- and (2*E*,6*E*,10*Z*)-3,7,15-Trimethyl-2,6,10,14-hexadecatetraenoic acid (6c)

To a solution of **27a** (140 mg, 0.506 mmol) in ether (20 mL) was added MnO_2 (1.4 g, 16.1 mmol), and stirred at room temperature for 2 h. Then the mixture was filtered through Celite and concentrated to afford the crude aldehyde. The aldehyde was dissolved in *t*-BuOH (30 mL), then $NaClO_2$ (203 mg, 2.24 mmol), 2-methyl-2-butene (15 mL, 134 mmol), water (10 mL), and NaH_2PO_4 (215 mg, 1.79 mmol) were added. After stirring for 12 h, AcOEt (30 mL) and water (50 mL) was added, and organic layer was separated, dried over $MgSO_4$, and concentrated. The residue was purified by flash column chromatography on silica gel (hexane/AcOEt = 3:1) to give **6c** (96 mg, 65% yield in two steps) as a mixture of stereoisomer (*10E*:*10Z* = 3:1, a pale yellow oil).

(2*E*,6*E*,10*E*)-isomer: IR ν_{max} cm^{-1}; 3611, 3010, 2928, 1667, 1450, 1383; ^1H NMR (300 MHz) δ: 1.42 (3H, s, CH_3), 1.68 (6H, br s, CH_3 × 2), 1.96–2.10 (8H, m, CH_2 × 4), 2.10–2.40 (4H, m, CH_2 × 2), 2.12 (3H, s, CH_3), 5.10 (2H, br s, =CH × 2), 5.20–5.50 (2H, m, =CH × 2), 5.69 (1H, s, =CH); EI-LRMS *m*/*z* 290 (M^+), 191, 135; EI-HRMS calcd for $C_{19}H_{30}O_2$ 290.2244; found: 290.2269.

4.5. Synthesis of 15-demethylgeranylgeranoic acid (6d)

4.5.1. (2*E*)-1-(2-Butenylsulfonyl)-4-methylbenzene (23b)

To a solution of crotyl chloride (4.4 mL, 0.44 mmol) in methanol (40 mL) was added sodium salt of *p*-toluenesulfinic acid (22 g, 88 mmol), and the solution was refluxed for 3 h. After removal of methanol, the residue was poured into water, and extracted with ether (3× 100 mL), followed by standard workup. The residue was purified by flash column chromatography on silica gel (hexane/AcOEt = 5:1 to 3:1) to give **23b** (4.51 g, 97% yield). The spectral data of this compound were identical with those of the literature.[44]

IR ν_{max} cm^{-1}; 3027, 2921, 1598, 1319, 1142; ^1H NMR (300 MHz) δ: 1.65 (3H, d, *J* = 5.5 Hz, CH_3), 2.42 (3H, s, $PhCH_3$), 3.68 (2H, d, *J* = 7.2 Hz, SO_2CH_2), 5.34–5.44 (1H, m, =CH), 5.48–5.60 (1H, m, =CH), 7.31 (2H, d, *J* = 8.4 Hz, Ar-H), 7.70 (2H, d, *J* = 8.4 Hz, Ar-H); ^{13}C NMR (75 MHz) δ: 18.0, 21.5, 60.1, 117.0, 128.3, 129.5, 135.5, 136.2, 144.5.

4.5.2. (2*E*,6*E*,10*E*)-12-Bromo-3,7,11-trimethyl-2,6,10-dodecatrienyl acetate (25b)

In a manner similar to that for the synthesis of **25a** from **24a**, farnesyl acetate **24b** (5.0 g, 18.9 mmol) was converted into the bromoacetate **25b** (1.1 g, 38% yield) as a pale yellow oil. The spectral data of this compound were identical with those of the literature.[45]

IR ν_{max} cm^{-1}: 3075, 2936, 1666, 1647, 1449, 1384; ^1H NMR (300 MHz) δ: 1.59 (3H, s, CH_3), 1.70 (3H, s, CH_3), 1.74 (3H, s, CH_3), 1.98–2.15 (8H, m, CH_2 × 4), 2.05 (3H, s, CH_3), 3.96 (2H, s, CH_2Br), 4.58 (2H, d, *J* = 6.9 Hz, $-CH_2OAc$), 5.09 (1H, t, *J* = 6.0 Hz, =CH), 5.33 (1H, t, *J* = 6.9 Hz, =CH), 5.56 (1H, t, *J* = 6.6 Hz, =CH); ^{13}C NMR (75 MHz) δ: 14.6, 15.9, 16.4, 21.0, 26.1, 26.8, 38.7, 39.4, 41.8, 61.3, 118.3, 124.2, 131.1, 131.9, 134.6, 142.1, 171.0.

4.5.3. (2*E*,6*E*,10*E*,14*E*)- and (2*E*,6*E*,10*E*,14*Z*)-3,7,11-Trimethyl-13-(4-methylphenylsulfonyl)-2,6,10,14-hexadecatetraen-1-ol (26b)

In a manner similar to that for the synthesis of **26a** from **23a**, a crude product, which was obtained from **23b** (1.68 g, 8 mmol) and **25b** (2.49 g, 7.25 mmol), was purified by flash column chromatography on silica gel (hexane/AcOEt = 1:1) to give **26b** (3.34 g, 97% yield) as a mixture of stereoisomer (14*E*:14*Z* = 2:1, a pale yellow oil).

(2*E*,6*E*,10*E*,14*E*)-isomer: IR ν_{max} cm^{-1}; 3610, 3530, 3026, 2921, 1666, 1598, 1446; ^1H NMR (300 MHz) δ: 1.45 (3H, s, CH_3), 1.52 (3H, s, CH_3), 1.55 (3H, d, *J* = 5.5 Hz, CH_3), 1.63 (3H, s, CH_3), 1.70–2.10 (9H, m, CH_2 × 4, OH), 2.21 (1H, dd, *J* = 14.0, 12.0 Hz, CH_2), 2.39 (3H, s, CH_3), 2.68 (1H, br d, *J* = 14 Hz), 3.51 (1H, td, *J* = 12.0, 3.5 Hz, SO_2CH), 4.10 (2H, d, *J* = 6.5 Hz, CH_2O), 5.00–5.40 (5H, m, =CH × 5), 7.27 (2H, d, *J* = 8.5 Hz, Ar-H), 7.66 (2H, d, *J* = 8.5 Hz, Ar-H); ^{13}C NMR (75 MHz) δ: 15.7, 15.8, 16.1, 17.9, 21.5, 26.1, 26.4, 37.2, 39.2, 39.4, 59.1, 67.6, 122.7, 123.4, 123.9, 128.4, 129.1, 129.3, 129.4, 134.5, 134.6, 134.8, 139.2, 144.2.

4.5.4. (2*E*,6*E*,10*E*,14*E*)- and (2*E*,6*E*,10*E*,14*Z*)-3,7,11-Trimethyl-2,6,10,14-hexadecatetraen-1-ol (27b)

In a manner similar to that for the synthesis of **27a** from **26a**, a crude product, which was obtained from **26b** (106 mg, 0.247 mmol), Na_2HPO_4 (140 mg, 0.986 mmol), sodium amalgam 5.0% (378 mg, 378 mg), was purified by flash column chromatography on silica gel (hexane/AcOEt = 3:1 to 2:1) to give **27b** (51.8 mg, 76% yield) as a mixture of stereoisomer (14*E*:14*Z* = 2:1, a pale yellow oil).[46]

(2*E*,6*E*,10*E*,14*E*)-isomer: IR ν_{max} cm^{-1}; 3611, 3444, 3010, 2935, 1666, 1649, 1450, 1383; ^1H NMR (300 MHz) δ: 1.49 (1H, br s, OH), 1.55 (3H, d, *J* = 4.0 Hz, CH_3), 1.59 (3H, s, CH_3), 1.67 (6H, s, CH_3 × 2), 1.90–2.20 (12H, m, CH_2 × 6), 4.12 (2H, d, *J* = 6.5 Hz, CH_2O), 5.09 (2H, t, *J* = 5.5 Hz, =CH × 2), 5.25–5.50 (3H, m, =CH × 3); EI-LRMS *m*/*z* 276 (M^+), 259, 205, 191; EI-HRMS calcd for $C_{19}H_{32}O$ 276.2452; found: 276.2426.

4.5.5. (2*E*,6*E*,10*E*,14*E*)- and (2*E*,6*E*,10*E*,14*Z*)-3,7,11-Trimethyl-2,6,10,14-hexadecatetraenoic acid (6d)

In a manner similar to that for the synthesis of **6c** from **27a**, a crude product, which was obtained from **27b** (253 mg, 0.918 mmol), MnO_2 (2.53 g, 29.1 mmol), *t*-BuOH (10 mL), $NaClO_2$ (386 mg, 4.26 mmol), NaH_2PO_4 (409 mg, 3.41 mmol), 2-methyl-2-butene (15 mL, 134 mmol), water (10 mL), was purified by flash column chromatography on silica gel (hexane/AcOEt = 3:1) to give **6d** (168 mg, 63% yield) as a mixture of stereoisomer (14*E*:14*Z* = 2:1, a pale yellow oil).

(2*E*,6*E*,10*E*,14*E*)-isomer: IR ν_{max} cm^{-1}; 3528, 3029, 2931, 1691, 1640, 1438; ^1H NMR (300 MHz) δ: 1.57 (3H, s, CH_3), 1.58 (3H, d, *J* = 6.5 Hz, CH_3), 1.61 (3H, s, CH_3), 1.90–2.20 (8H, m, CH_2 × 4), 2.15–2.20 (4H, m, CH_2 × 2), 2.18 (3H, s, CH_3), 5.00–5.50 (4H, m, =CH × 4), 5.70 (1H, s, =CH); EI-LRMS *m*/*z* 290 (M^+), 209, 15; EI-HRMS calcd for $C_{19}H_{30}O_2$ 290.2245; found: 290.2258.

4.6. Biological assay

4.6.1. HL-60 cells and cell cycle synchronization

HL-60 cells were maintained in continuous culture in RPMI-1640 medium (Nissui Seiyaku Co., Ltd, Tokyo, Japan), supplemented with 10% dextran-coated, charcoal-treated fetal calf serum (FCS) (Gibco BRL, Grand Island, NY, USA) and kanamycin (0.06 mg/mL) (Sigma, St. Lois, MO, USA), at 37 °C in a humidified atmosphere of 5% CO_2 in air. The doubling time of HL-60 cells was approximately 24 h. For synchronization at S phase, cells (4×10^5 cells/mL) were cultured in 30 mL of RPMI-1640 medium, supplemented with 2.5 mM thymidine. After two washes with Ca-, Mg-free phosphate-buffered saline [PBS(−)], the synchronization of the cell cycle was repeated. The cells thus obtained were used in biological assays.

4.6.2. Flow cytometry

Cells (10^5 cells/well) were placed in 24-well tissue culture plates, and cultured for 3d with retinoids (10^{-6}–10^{-4} M) in RPMI-1640 medium at 37 °C in a humidified atmosphere of 5% CO_2 in air. To reduce the effects of contact inhibition, control cells were adjusted to 60–70% confluency at the time of the fluorescent activated cell sorting (FACS) analysis. Each group of cells was collected in PBS(−). Then, cells were resuspended in PBS(−) that

contained 0.2% Triton X and 1 μL RNase, and incubated at 37 °C for 1 h. Cells were washed with PBS(−) and incubated with 0.5 mL of DNA-staining solution that contained 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, Becton–Dickinson FACScan™), and the cell cycle distribution was analyzed with ModiFiT LT software (Verity Software House; Topsham, ME).

4.6.3. Transcriptional activity assay of RXRα-Gal4

Human osteosarcoma MG-63 cells, which are positive for RXR gene expression, were maintained in Dulbecco's modified Eagle medium (Gibco BRL) supplemented with 1% penicillin, 1% streptomycin, and 10% dextran-coated, charcoal-treated, FCS (Gibco BRL). The day before transfection, cells were seeded on six-well culture plates at a density of 2×10^5 cells per well in order to ensure that they were confluent on the day of transfection. The RXRα-Gal4 transgene comprised a one-hybrid plasmid (pM vector) that contained a human RXR sequence connected to a yeast GAL4-DNA binding domain sequence. The cells were cotransfected with 1.0 μg of RXR-Gal4 and either 0.5 mg of a luciferase reporter plasmid (pGVP2 vector) that also contained a GAL4-DNA binding site or 0.5 mg of a pRL-CMV vector as an internal control. Each set of experiments was repeated at least three times with 10^{-6} M of the test compound. The results are presented as means ± SEM of the fold increase in induction.

4.6.4. Transcriptional activity assay of RARE or RXRE

Human osteosarcoma MG-63 cells, which are positive for RXR gene expression, were maintained in Dulbecco's modified Eagle's medium (Gibco BRL) supplemented with 1% penicillin, 1% streptomycin, and 10% dextran-coated charcoal-treated FCS (Gibco BRL). The day before transfection, cells were seeded on six-well culture plates at a density of 2×10^5 cells per well so that they were confluent on day of transfection. The retinoid-responsive luciferase reporter constructs human RARβ-RARE3-SV40-Luc and rat CRBPII-RXRE-SV40-Luc were generated by cloning three copies of the retinoic acid response element (RARE) from the RARβ promoter (59/33: GGGTAAAGTTCACCGAAAGTTCACTCG) or the RXRE from the rat CRBPII promoter (639/605: GCTGTCACAGGTCACAGGTCACAGGTCACAGTTCA) in the pGL3 vector. The pRL-CMV vector was an internal control using the Tfx-50 reagent. After transfection, the cells were incubated with retinoids (10^{-6} M) for 2 days. Luciferase activity of the cell lysates was measured with a luciferase assay system (Toyo Ink Co., Ltd), according to the manufacture's instructions. Transactivation determined from the luciferase activity was standardized with respect to the luciferase activity of the same cells measured with the Sea Pansy luciferase assay system as a control (Toyo Ink Co., Ltd). Each set of experiments was repeated at least three times, and the results (means ± SEM) are presented in terms of folds in increase induction compared to the vehicle (ethanol)-treated control, which is expressed as 1.0.

4.7. Statistical analysis

Statistical significance was determined with Dunnett's test. The data were compared to EtOH-treated control cells, and levels of significance were evaluated as $^{***}p < 0.001$, $^{**}p < 0.01$, and $^{*}p < 0.05$.

Acknowledgments

This work was supported in part by a Grant-in-Aid for Scientific Research (C) (A.W.) from the Ministry of Education, Culture, Sports, Science, and Technology of Japan, and by grants from the Kobe Pharmaceutical University Collaboration Fund and the Science Research Promotion Fund of the Japanese Private School Promotion Foundation.

A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2010.07.003. These data include MOL files and InChiKeys of the most important compounds described in this article.

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