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Preparation and biological evaluation of 5-substituted retinoic acids ☆

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ABSTRACT

Various 5-substituted retinoic acids were prepared by a palladium-catalyzed cross coupling reactions of vinyl nonaflates and *E*- or *Z*-3-tributylstannyl-2-beten-1-ol as a key reaction. These coupling products were then converted to the corresponding all-*E*- and 9*Z*-retinoic acid analogs via Horner–Emmons reaction and subsequent basic hydrolysis, and their biological activities were evaluated. The all-*E*-derivatives, 5-butyl and isobutyl analogs exhibited stronger effects for anti-proliferative and differentiation-inducing activities in HL-60 cells. In contrast, in 9*Z*-derivatives, none of the analogs showed any activity.

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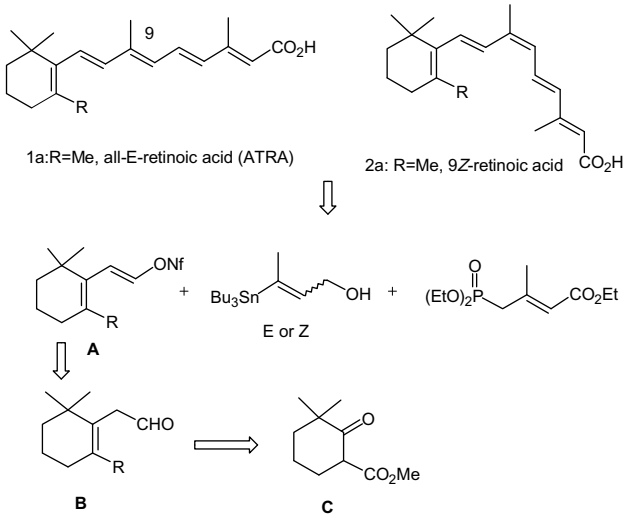
1. Introduction

The vitamin A metabolites all-*E*-retinoic acid (ATRA) **1** and 9*Z*-retinoic acid (9CRA) **2** are the ligand molecules for the retinoic acid receptors (RARα, β, γ) and retinoid X receptors (RXRα, β, γ, respectively.[2] These receptors are members of the nuclear receptor superfamily and are ligand-dependent factors that play important roles in a myriad of biological functions including cell differentiation, cell proliferation, and embryonic development, through regulation of gene transcription.[2,3] The RXRs form heterodimers with other nuclear receptors proteins such as the RARs, the thyroid hormone receptor (TR), the vitamin D receptor (VDR) and the peroxisome proliferator-activated receptors (PPARs). Currently, great efforts are underway for the preparation of receptor-selective retinoid complexes in order not only to define the functions of each receptor but also to develop therapeutic agents.[4] As part of our studies of the stereoselective synthesis of retinoids,[5–7] we wish to describe here a novel synthesis of 5-substituted all-*E*- and 9*Z*-retinoic acids and their biological evaluation.

2. Chemistry

We have developed a stereoselective synthesis of retinoid mono isomers and their related analogs by the Stille coupling reactions of vinyl triflate or nonaflate, which were derived from 2,6,6-trimethyl-1-cyclohexene-1-acetaldehyde, with 3-tributylstannyl-2-butenols.[7] To apply this methodology for preparation of 5-substituted retinoic acid analogs, we planned a synthetic strategy as shown in Scheme 1. We chose the vinylnonaflate as segment A because the reactivity of nonaflate for the coupling reaction is greater than that of the corresponding triflate.[8] The vinylnonaflates **A**, the key intermediates of our synthetic strategy, would be easily derived from the corresponding aldehydes **B**, and the aldehydes **B** could be obtained from the keto ester **C** (Scheme 1).



Scheme 1. Synthetic plan for 5-substituted retinoic acid.

☆ See Ref. 1.

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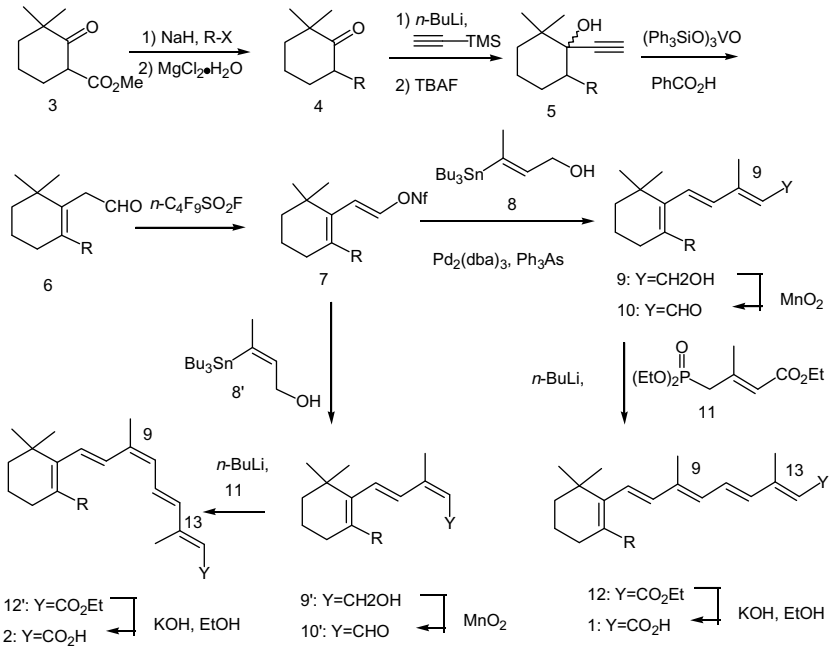

Scheme 2. Synthetic route of retinoic acid analogs.

6-Substituted 2,2-dimethylcyclohexanones **4** were prepared from the β-keto ester **3** by the introduction of alkyl chain according to the usual method and subsequent demethoxycarbonylation[9] in good yield. Treatment of **4** with lithium (trimethylsilyl)acetylene gave the adduct and, following treatment with tetrabutylammonium fluoride, afforded the hydroxyacetylene **5** in excellent yields. The methods for rearrangement of α-acetylenic alcohol into α,β-unsaturated compound using oxo-metallic catalysts have reported.[10] In the case of the application of this rearrangement to the cyclohexanol derivatives, our group has found good reaction conditions giving the β,γ-unsaturated carbonyl compounds in good yield using tris(triphenylsilyl) vanadate, triphenylsilanol and benzoic acid.[11] Adopting these reaction conditions, the acetylenic alcohols **5** were converted into the corresponding aldehydes **6** in 81–92% yields. Enol nonaflates **7** were prepared in moderate to good yields by treatment with nonafluorobutanesulfonyl fluoride (NfF) using potassium *tert*-butoxide as a base in THF.

The coupling reactions of enolnonaflates **7** with *E*- or *Z*-3-tributylstannyl-2-butenol **8** or **8′** in the presence of 5 mol% of tris(dibenzylideneacetone)dipalladium (0) ($Pd_2(dba)_3$) and triphenylarsin (Ph_3As) as a ligand at room temperature in DMF afforded the alcohols **9** and **9′**, respectively. These alcohols were oxidized by MnO_2 to give the aldehydes **10** and **10′** without isomerization of the double bonds. These aldehydes were easily converted into the esters **12** and **12′** by condensation with triethyl 3-phosphonocrotonate **11** accompanied by a small amount of the regioisomer at the 13 position. Basic hydrolysis and subsequent recrystallization afforded the all-*E*- and 9*Z*-5-substituted retinoic acids **1** and **2** in pure form (Scheme 2, Tables 1 and 2).

3. Biological evaluation

To examine the anti-proliferative effect of the novel compounds on HL-60 cell growth, we measured the cell cycle phase distribution of HL-60 cells treated with increasingly higher concentrations of ATRA and the retinoic acid analogs. Dose–response curves of ATRA (Fig. 1) and its analogs indicated that compound **1b** showed twofold stronger anti-proliferative activity than did ATRA **1a**, while the less potent branched analogs **1c** exhibited approximately one-third of the anti-proliferative activity of **1a** (Table 3). The analogs **1d** and **1e**, having more bulky subustituents, were markedly less active. With the exception of **2e**, which had almost no measurable activity, the 9CRA analogs exhibited approximately one-tenth of the activity of ATRA **1** (Table 4).

To evaluate the differentiation-inducing activity of analogs, the expression of cell surface CD11b antigen, a marker of granulocyte/monocyte/macrophage differentiation, was measured by flow cytometric analysis. The apoptosis-inducing activities in HL-60 cells were determined from the agarose gel electrophoresis of the DNA samples from HL-60 cells treated with increasing concentrations of ATRA, 9CRA, and the corresponding analogs. These results are summarized in Tables 3 and 4. In the differentiation-inducing activity, the analog **1b** showed almost the same activity of ATRA. It is noteworthy that analog **1c** showed threefold higher activity than that of ATRA. On the other hand, the 9*Z*-analogs exhibited very limited effects on the differentiation-inducing activity. This observation is consistent with earlier observations that in the structure–activity relationship of retinobenzoic acid, a medium-sized and hydrophobic alkyl group seems to be important for the differentiation-inducing acitivity.[12] With regard to apoptosis-inducing activity, none of the all-*E*- or the 9*Z*- analogs showed this ability toward HL-60 cells.

In order to determine whether the above biological activity was mediated by retinoidal receptors, the analogs were tested in two kinds of transcriptional assays. Initially, we assessed the transactivation activity towards a human RARβ gene promoter with three copies of retinoic acid responsive elements (RAREs) in transfected MG-63 cells. As shown in Figure 2, all-*E* analogs **1b** and **1c** at 10^{-6} M exhibited a comparable or even higher RARβ/RARE mediated gene expression than that of ATRA, the natural ligand of RAR.

However, none of the 9*Z*-analogs exhibited higher transactivation activity than that of 9CRA. In the transactivation activities of analogs toward a rat cellular retinoic acid-binding protein II (CRABPII) gene including RXREs in transfected MG-63 cells, none of the analogs exhibited higher transactivation activity than 9CRA, the natural ligand of RXR (Fig. 3). Although we tested the binding affinities of analogs for human RXRα-Gal4 expressed in

Table 1
Yields of reactions for the synthesis of enol nonaflates (%)

	Substituent R	**4** Yield[a]	**5** Yield	**6** Yield	**7** Yield	**10** Yield	**10'** Yield[a]
1	Bu	61	96	81	59	48	65
2	*i*-Bu	46	96	92	60	38	57
3	$PhCH_2$	67	97	88	75	49	58
4	2-Naphtyl-CH_2	77	Quant.	83	56	65	70

[a] Yield in 2 steps.

Table 2
Yields of reactions for the synthesis of 5-substituted retinoic acids

	Substituent R	Product	Yield (%)[a]	Product	Yield (%)
1	Bu	**12a**	94 (15:1)	**1b**	82
2	*i*-Bu	**12b**	92 (14:1)	**1c**	75
3	$PhCH_2$	**12c**	95 (41:1)	**1d**	69
4	2-Naphtyl-CH_2	**12d**	89 (50>:1)	**1e**	79
5	Bu	**12′a**	92 (13:1)	**2b**	70
6	*i*-Bu	**12′b**	93 (12:1)	**2c**	75
7	$PhCH_2$	**12′c**	89 (26:1)	**2d**	86
8	2-Naphtyl-CH_2	**12′d**	89 (50>:1)	**2e**	Quant.

[a] Ratio of all-*E*-: 13*Z*-isomers in the case of **12** or 9*Z*-: 9*Z*,13*Z*- isomers in the case of **12′**.

Table 3
Biological activity of the ATRA analogs[a]

Compound	Anti-proliferative activity	Differentiation-inducing activity	Apoptosis-inducing activity
1a (ATRA)	100	100	±
1b	193	129	–
1c	35	310	–
1d	2	2	–
1e	<0.81	<0.22	–

[a] Anti-proliferative activity was assessed by cell cycle evaluation, as described in Section 4. Differentiation inducing activity was measured by expression of cell surface CD11b antigen as determined by flow cytometry. The data were normalized with respect to the values obtained for ATRA. The apoptosis-inducing activities in HL-60 cells were determined from the agarose gel electrophoresis by judging the DNA fragmentation; +:positive, –:negative.

Table 4
Biological activity of the 9CRA analogs[a]

Compound	Anti-proliferative activity	Differentiation-inducing activity	Apoptosis- inducing activity
2a (9CRA)	184	110	±
2b	14	9	–
2c	16	10	–
2d	11	6	–
2e	<0.81	<0.22	–

[a] Anti-proliferative activity was assessed by cell cycle evaluation, as described in Section 4. Differentiation inducing activity was measured by expression of cell surface CD11b antigen as determined by flow cytometry. The data were normalized with respect to the values obtained for ATRA. The apoptosis-inducing activities in HL-60 cells were determined from the agarose gel electrophoresis by judging the DNA fragmentation; +:positive, –:negative.

transfected MG63 cells, the results were almost the same as those of RXRE assay (data not shown).

It is well documented that ATRA has the ability to induce differentiation of HL-60 cells into granulocytes/macrophages via binding to RAR but lacks the ability to induce apoptosis.[13] In contrast, 9CRA has the ability to induce both HL-60 cell differentiation and apoptosis *via* binding to RXR.[14] Our results obtained above were consistent with these observations. Thus, the lack of biological activity of the 9*Z*-analogs in all assays was probably due to the lack of the binding affinity for RAR and RXR, hence, stronger gene expressions through both signaling pathways of RAR/RARE and RXR/RXRE were not found. The higher activities of **1b** and **1c** toward the anti-proliferative, differentiation activities of HL-60 cells are attributed to their binding affinity for RAR. The reason of weak anti-proliferative activity of **1c** compared to that of ATRA is not clear at the present time. These results indicate that the all-*E*-analogs having a medium-alkyl substituent are potent candidates for development of an RAR agonist.

In this study, we examined the influence of regulatory activity of 5-substituted analogs of ATRA and 9CRA toward the anti-proliferative, differentiation, and apoptosis activities of HL-60 cells. The influence of such modification on the 5 position was more prominent in the ATRA analogs than the 9CRA variants. However, it is important to note that an excessively bulky substituent greatly reduces the potency to regulate cell proliferation and differentiation.

In summary, we have developed a novel method for the stereoselective synthesis of 5-substituted retinoic acid using a Stille coupling reaction between a vinyl nonaflate with *E*- or *Z*-alkenylstannane. The all-*E*-analogs having butyl and isobutyl substituents exhibited stronger anit-prolifelative and differentiation-inducing activities than did ATRA due to their binding affinity to RARs.

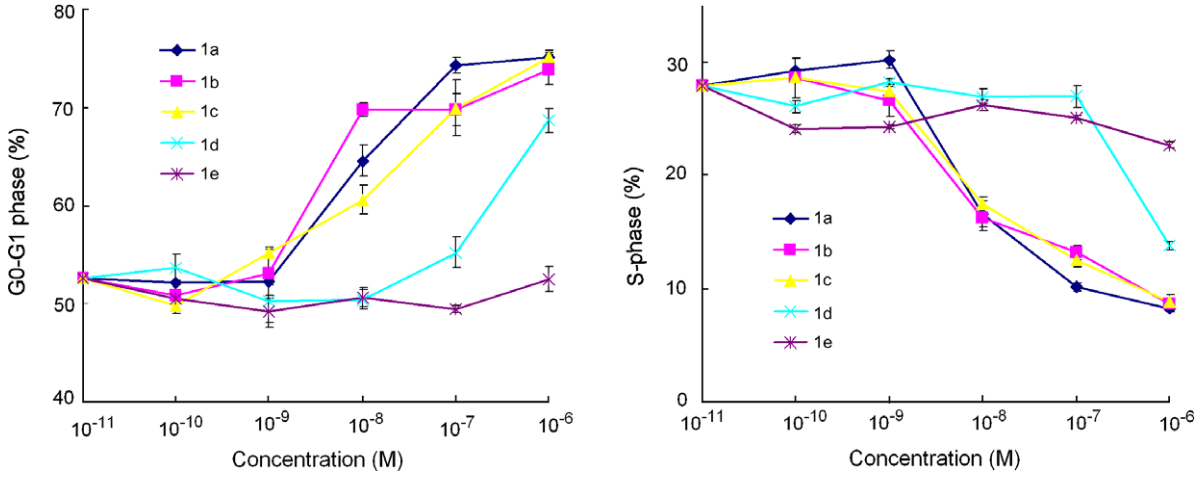

Figure 1. Cell cycle regulatory activity of ATRA and its analogs in HL-60 cells. Percentage of cells in G0/G1 phase (A) and S phase (B) as a function of concentration in culture in the absence of 9CRA analogs (10^{-11}–10^{-6} M). G0/G1-specific cell cycle arrest is evidenced by an increase in the relative number of G0/G1 DNA cells.

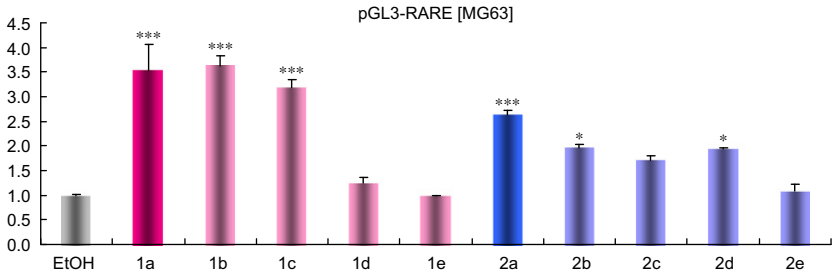

Figure 2. Transcriptional activity of ATRA, 9CRA and their analogs toward a human RARβ-RARE expression gene in transfected MG-63 cell. The cells were cotransfected with a luciferase reporter plasmid (pGVB2 vector) containing a human RARβ gene promoter including a RARE and a pRL-CMV vector as an internal control. Luciferase activities induced by ATRA, 9CRA and its analogs in MG-63 cells were quantified and represented as fold-induction as compared with luciferase activity observed in the control cells. Results represent the mean of three experiments (values in column) at 10^{-6} M, and standard errors (vertical bars).

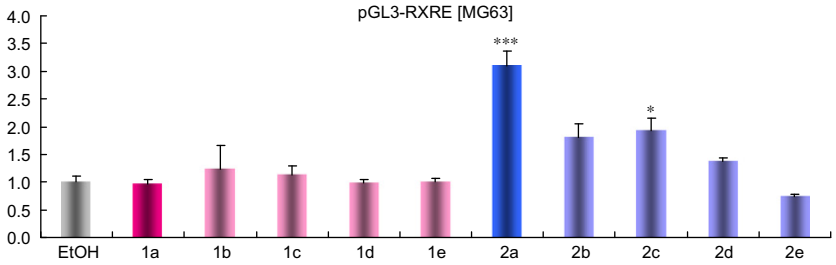

Figure 3. Transcriptional activity of ATRA, 9CRA and their analogs toward a rat CRBPII-RXRE expression gene in transfected MG-63 cell. The cells were cotransfected with a luciferase reporter plasmid (pGVB2 vector) containing a rat CRABPII gene promoter including a RXRE and a pRL-CMV vector as aninternal control. Luciferase activities induced by ATRA, 9CRA and its analogs in MG-63 cells were quantified and represented as fold-induction as compared with luciferase activity observed in the control cells. Results represent the mean of three experiments (values in column) at 10^{-6} M, and standard errors (vertical bars).

4. Experimental

4.1. General methods

Melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. UV spectra were recorded on a JASCO Ubest-55 instrument in EtOH. IR spectra were recorded on a Perkin-Elmer FT-IR Paragon 1000 spectrometer in $CHCl_3$. ^1H NMR spectra were obtained on a Varian Gemini-300 NMR or a Varian VXR-500 spectrometer with tetramethylsilane as an internal standard in $CDCl_3$. ^{13}C NMR and ^{19}F NMR were obtained on a Varian VXR-500 (125 and 470 MHz) NMR spectrometer in $CDCl_3$, respectively. Mass spectra were determined on a Hitachi M-4100 instrument. Column chromatography (CC) under reduced pressure (ca. 30 mmHg) was performed using Merck Silica gel 60. All reactions were carried out under a nitrogen atmosphere. THF and ether were purified by distillation from benzophenone sodium ketyl under nitrogen. Materials obtained from commercial suppliers were used without further purification. Standard workup means that the organic layers were finally washed with brine, dried over anhydrous sodium sulfate (Na_2SO_4), filtered, and concentrated in vacuo below 30 C using a rotary evaporator.

4.2. General procedure for the preparation of 6-substituted 2,2-dimethylcyclohexanone (4a–d)

To a suspension of NaH (60% dispersion in oil, 3.4 g, 0.085 mol) in THF (65 mL) and DMF (7 mL) was added a solution of β-keto ester **3**[15] (12.2 g, 0.066 mol) in THF (70 mL) at 0 °C. After stirring for 15 min at room temperature, a solution of alkyl halide (0.13 mmol) in THF (70 mL) was added dropwise and the resulting mixture was heated under reflux for 18 h. After cooling, the mixture was poured into 10% HCl (100 mL), and the organics were extracted with ether (2 × 100 mL), followed by standard workup. The residue was purified by CC (Et_2O–hexane, 1:4) to give 3-alkylated keto ester (14.5 g, 70%) as a colorless oil.

A mixture of this keto ester (17 g, 54.5 mmol) and $MgCl_26H_2O$ (5 equiv) in DMSO (50 mL) was heated under reflux for 18 h. After cooling, water (150 mL) was added, and the organics were extracted with ether (3 × 80 mL), followed by standard workup to give the crude ketone. The residue was purified by CC (Et_2O−hexane, 1:6) to afford the ketone **4** (9.7 g, 70%) as a pale yellow oil.

4.2.1. 6-Butyl-2,2-dimethylcyclohexanone (4a)

The 3-butyl keto ester was prepared from keto ester (**3**, 5.0 g, 27.17 mmol) and butyl iodide (7.0 g, 38.04 mmol) in 85% yield (5.56 g) as a colorless liquid, and this keto ester (5.04 g, 21.00 mmol) was converted to the ketone (**4a**) in 72% yield (2.75 g) as a colorless oil.

IR ν_{max} cm^{-1}: 1699; ^1H NMR (300 MHz) δ: 0.86 (3H, t, J = 6.5 Hz), 1.01 (3H, s) 1.15 (3H, s), 1.17–2.52 (12H, m), 2.46 (1H); ^{13}C NMR (75 MHz) δ: 13.98, 21.55, 22.87, 25.12, 25.57, 29.08, 29.53, 34.51, 41.86, 45.37, 46.02, 217.08; HRMS (EI) m/z: Calcd $C_{12}H_{22}O$ (M^+) 182.1670. Found: 182.1671.

4.2.2. 2,2-Dimethyl-6-(2-methylpropyl)cyclohexanone (4b)

The 3-isobutyl keto ester was prepared from keto ester (**3**, 5.0 g, 27.17 mmol) and isobutyl iodide (7.0 g, 38.04 mmol) in 68% yield (4.43 g) as a colorless liquid, and the keto ester (4.16 g, 17.33 mmol) was converted to the ketone (**4b**) in 68% yield (2.15 g) as a colorless oil.

IR ν_{max} cm^{-1}: 1701; ^1H NMR (300 MHz) δ: 0.81 (3H, d, J = 6.3 Hz), 0.83 (3H, d, J = 6.3 Hz), 1.01 (3H, s), 1.16 (3H, s), 1.21 (2H, dd, J = 3.9, 12.9 Hz), 1.40–1.94 (6H, m), 1.99–2.09 (1H, m), 2.51–2.62 (1H, m); ^{13}C NMR (75 MHz) δ: 21.60, 22.20, 23.04, 25.06, 25.36, 25.60, 34.95, 38.40, 41.92, 43.71, 45.47, 217.17; HRMS (EI) m/z: Calcd $C_{12}H_{22}O$ (M^+) 182.1670. Found: 182.1663.

4.2.3. 6-Benzyl-2,2-dimethylcyclohexanone (4c)

The 3-benzyl keto ester was prepared from keto ester (**3**, 2.0 g, 5.43 mmol) and benzyl bromide (1.3 g, 7.60 mmol) in 81% yield (2.40 g) as a colorless liquid, and this keto ester (1.55 g, 5.66 mmol) was converted to the ketone (**4c**) in 83% yield (1.00 g) as a colorless oil.

IR ν_{max} cm^{-1}: 1496, 1604, 3028, 1702; ^1H NMR (300 MHz) δ: 1.07 (3H, s), 1.19 (3H, s), 1.58–1.67 (2H, m, 3-CH_2), 1.69–1.82 (2H, m), 1.22–3.25 (5H, m), 7.12–7.29 (5H, m); ^{13}C NMR (75 MHz) δ: 21.38, 25.10, 25.60, 33.91, 35.71, 41.83, 45.49, 47.81, 125.80, 128.20 (2C), 129.05 (2C), 140.78, 216.25; HRMS (EI) m/z: Calcd $C_{15}H_{20}O$ (M^+) 216.1513. Found: 216.1516.

4.2.4. 2,2-Dimethyl-6-(2-naphthylmethyl)cyclohexanone (4d)

The 3-(2-naphtylmethyl) keto ester was prepared from keto ester (**3**, 6.3 g, 34.24 mmol) and 2-naphtylmethyl bromide (10.6 g, 47.94 mmol) in 93% yield (10.27 g) as a colorless liquid, and this keto ester (8.91 g, 27.50 mmol) was converted to the ketone (**4d**) in 83% yield (6.01 g) as a colorless oil. IR ν_{max} cm^{-1}: 1601, 1702; ^1H NMR (300 MHz) δ: 1.09 (3H, s), 1.21 (3H, s), 1.52–1.66 (2H, m), 1.69–1.81 (2H, m), 1.26–3.40 (5H, m), 7.29 (1H, d, J = 8.4 Hz), 7.37–7.48 (2H, m), 7.59 (1H, s), 7.74–7.81 (3H, m); ^{13}C NMR (75 MHz) δ: 21.38, 25.13, 25.62, 34.00, 35.89, 41.83, 45.55, 47.82, 125.11, 125.84, 127.31, 127.36, 127.54, 127.69, 127.75, 131.96, 133.48, 138.32, 216.23; HRMS (EI) m/z: Calcd $C_{19}H_{22}O$ (M^+) 266.1670. Found: 266.1674.

4.3. General procedure for the preparation of 6-substituted acetylenic alcohol (5a–d)

To a stirred suspension of the 6-substituted ketone (**4**, 0.8 g, 3.3 mmol) in THF (13 mL) was added dropwise at −70 °C a solution of lithium trimethylsillylacetylene, prepared from trimethylsilylacethylene (0.45 mL, 3.3 mmol) and *n*-BuLi (1.7 M hexane solution, 1.9 mL, 3.3 mmol) in THF (6 mL). After stirring for 1 h at −70 °C, the reaction mixture was quenched with a saturated NH_4Cl (20 mL) and extracted with ether (3 × 20 mL), followed by standard workup to give the crude trimethylsilylated acetylene. To a silylated acetylene in THF (6 mL) was added tetrabutyl ammonium fluoride (1 mol solution in THF, 3.3 mL) at 0 °C. After stirring for an additional 1 h, the reaction mixture was quenched with a saturated NH_4Cl (20 mL) and extracted with ether (3 × 20 mL), followed by standard workup. The residue was purified by CC (ether–hexane, 1:19) to afford the acetylenic alcohol **5** as a colorless oil.

4.3.1. 6-Butyl-1-ethynyl-2,2-dimethylcyclohexanol (5a)

This was prepared from ketone (**4a**, 2.41 g, 13.22 mmol), trimethylsilylacetylene (2.05 g, 20.89 mmol) and 1 M TBAF in THF (13.22 mL, 13.22 mmol) in 96% yield (2.65 g) as a colorless liquid. IR ν_{max} cm^{-1}: 2358, 3305, 3608; ^1H NMR (300 MHz) δ: 0.88 (3H, t, J = 7.2 Hz), 0.97 (3H, s), 1.09 (3H, s), 1.04–1.83 (13H, m), 1.88 (1H, s), 2.44 (1H, s); ^{13}C NMR (75 MHz) δ: 14.11, 19.70, 21.02, 23.10, 26.54, 29.35, 29.99, 30.08, 37.70, 38.79, 42.04, 74.93, 78.08, 84.60; HRMS (EI) m/z: Calcd $C_{14}H_{24}O$ (M^+) 208.1825. Found: 208.1825.

4.3.2. 1-Ethynyl-2,2-dimethyl-6-(2-methylpropyl)cyclohexanol (5b)

This was prepared from ketone (**4b**, 3.86 g, 21.21 mmol), trimethylsilylacetylene (3.28 g, 33.51 mmol) and 1 M TBAF in THF (21.21 mL, 21.21 mmol) in 96% yield (4.22 g) as a colorless liquid. IR ν_{max} cm^{-1}: 2361, 3306, 3609; ^1H NMR (300 MHz) δ: 0.84 (3H, d, J = 6.5 Hz), 0.91 (3H, d, J = 6.5 Hz), 0.98 (3H, s), 1.09 (3H, s), 1.14–1.77 (10H, m), 1.85 (1H, s), 2.44 (1H, s); ^{13}C NMR (75 MHz) δ: 19.74, 21.06, 21.64, 24.22, 25.44, 26.62, 29.61, 37.65, 38.84, 39.52, 39.69, 74.95, 77.96, 84.68; HRMS (EI) m/z: Calcd $C_{14}H_{24}O$ (M^+) 208.1826. Found: 208.1822.

4.3.3. 6-Benzyl-1-ethynyl-2,2-dimethylcyclohexanol (5c)

This was prepared from ketone (**4c**, 1.70 g, 7.87 mmol), trimethylsilylacetylene (1.22 g, 12.43 mmol) and 1 M TBAF in THF (7.87 mL, 7.87 mmol) in 97% yield (1.84 g) as a colorless liquid. IR ν_{max} cm^{-1}: 1495, 1603, 2360, 3012, 3305, 3606; ^1H NMR (300 MHz) δ: 1.00 (3H, s), 1.14 (3H, s), 1.15–3.26 (10H, m), 2.58 (1H, s), 7.18–7.28 (5H, m); ^{13}C NMR (75 MHz) δ: 19.73, 20.84, 26.56, 29.17, 37.37, 37.68, 39.02, 44.27, 75.51, 78.03, 84.33, 125.74, 128.23 (2C), 129.39 (2C), 141.40; HRMS (EI) m/z: Calcd $C_{17}H_{22}O$ (M^+) 242.1670. Found: 242.1675.

4.3.4. 1-Ethynyl-6-(2-naphthylmethyl)-2,2-dimethylcyclohexanol (5d)

This was prepared from ketone (**4d**, 6.0 g, 22.64 mmol), trimethylsilylacetylene (3.51 g, 35.77 mmol) and 1 M TBAF in THF (22.64 mL, 22.64 mmol) in quantitative yield (6.58 g) as a pale yellow solid. IR ν_{max} cm^{-1}: 1508, 1601, 2107, 3057, 3305, 3608; ^1H NMR (300 MHz) δ: 1.03 (3H, s), 1.16 (3H, s), 1.25–1.47 (2H, m), 1.57–1.71 (3H, m), 1.99–2.12 (1H, m), 2.55–2.63 (2H, m), 2.62 (1H, s), 3.40 (1H, dd, J = 2.9, 13.2 Hz), 3.51 (1H, dd, J = 2.9, 13.2 Hz), 7.36 (1H, d, J = 8.4 Hz), 7.38–7.48 (2H, m), 7.64 (1H, s), 7.75–7.82 (3H, m); ^{13}C NMR (75 MHz) δ: 19.76, 20.84, 26.57, 29.25, 37.55, 37.68, 39.07, 44.24, 75.5, 78.09, 84.34, 125.08, 125.83, 127.35, 127.51, 127.56, 127.75, 128.04, 131.94, 133.51, 138.97; HRMS (EI) m/z: Calcd $C_{21}H_{24}O$ (M^+) 292.1826. Found: 292.1830.

4.4. General procedure for the preparation of aldehyde (6a–d)

A solution of acetylenic alcohol (**5**, 1.0 equiv), $(Ph_3SiO)_3VO$ (0.02 equiv), and benzoic acid (0.02 equiv) in xylene (50 mL) was heated under reflux for 2 days. After cooling, the solvent was removed in vacuo and the residue was purified by CC [ether-hexane (1:19)] to afford the aldehyde (**6**).

4.4.1. 2-(2-Butyl-6,6-dimethylcyclohexen-1-yl)ethanal (6a)

This was prepared from the alcohol (**5a**, 2.65 g, 12.75 mmol), $(Ph_3SiO)_3VO$ (239 mg, 0.26 mmol), and benzoic acid (31.7 mg, 0.26 mmol) in 81% yield (2.14 g) as a pale yellow oil. IR ν_{max} cm^{-1}: 1716; ^1H NMR (300 MHz) δ: 0.86 (3H, t, J = 6.9 Hz), 0.94 (6H, s), 1.22–1.32 (4H, m), 1.43–1.49 (2H, m), 1.53–1.62 (2H, m), 1.87 (2H, t, J = 7.5 Hz), 1.99 (2H, t, J = 6.0 Hz), 3.06 (2H, d, J = 2.4 Hz), 9.50 (1H, t, J = 2.4 Hz); ^{13}C NMR (75 MHz) δ: 13.97, 19.36, 22.81, 27.48, 28.17 (2C), 30.28, 33.71, 34.69, 39.34, 43.37, 128.31, 137.00, 201.92; HRMS (EI) m/z: Calcd $C_{14}H_{24}O$ (M^+) 208.1826. Found: 208.1840.

4.4.2. 2-[6,6-Dimethyl-2-(2-methylpropyl)cyclohexen-1-yl]ethanal (6b)

This was prepared from the alcohol (**5b**, 4.22 g, 20.27 mmol), $(Ph_3SiO)_3VO$ (365.7 mg, 0.41 mmol), and benzoic acid (50.0 mg, 0.41 mmol) in 92% yield (3.87 g) as a pale yellow oil. IR ν_{max} cm^{-1}: 1715; ^1H NMR (300 MHz) δ: 0.84 (6H, d, J = 6.6 Hz), 0.97 (6H, s), 1.46–1.52 (2H, m), 1.56–1.66 (2H, m), 1.77–1.84 (1H, m), 1.81 (2H, d, J = 8.0 Hz), 1.99 (2H, t, J = 6.3 Hz), 3.10 (2H, d, J = 2.3 Hz), 9.51 (1H, t, J = 2.3 Hz,); ^{13}C NMR (75 MHz) δ: 19.33, 22.29 (2C), 26.56, 28.26 (2C), 30.46, 34.92, 39.40, 42.66, 43.47, 129.58, 135.83, 202.07; HRMS (EI) m/z: Calcd $C_{14}H_{24}O$ (M^+) 208.1826. Found: 208.1846.

4.4.3. 2-(2-Benzyl-6,6-dimethylcyclohexen-1-yl)ethanal (6c)

This was prepared from the alcohol (**5c**, 1.20 g, 4.96 mmol), $(Ph_3SiO)_3VO$ (89.2 mg, 0.10 mmol), and benzoic acid (12.2 mg, 0.10 mmol) in 88% yield (1.05 g) as a pale yellow oil. IR ν_{max} cm^{-1}: 1494, 1602, 1716, 3012; ^1H NMR (300 MHz) δ: 1.04 (6H, s), 1.50–1.56 (2H, m), 1.56–1.67 (2H, m), 1.96 (2H, t, J = 6.0 Hz), 3.19 (2H, d, J = 2.1 Hz), 3.31 (2H, s), 7.08–7.30 (5H, m), 9.59 (1H,

t, J = 2.1 Hz); ^{13}C NMR (75 MHz) δ: 19.26, 28.18 (2C), 30.69, 34.92, 39.16, 39.26, 43.76, 125.98, 128.16 (2C), 128.39 (2C), 131.06, 134.90, 139.47, 201.07; HRMS (EI) m/z: Calcd $C_{17}H_{22}O$ (M^+) 242.1669. Found: 242.1668.

4.4.4. 2-[6,6-Dimethyl-2-(2-naphthylmethyl)cyclohexen-1-yl]ethanal (6d)

This was prepared from the alcohol (**5d**, 6.62 g, 22.74 mmol), $(Ph_3SiO)_3VO$ (401.4 mg, 0.45 mmol), and benzoic acid (54.9 mg, 0.45 mmol) in 83% yield (5.52 g) as a pale yellow oil. IR ν_{max} cm^{-1}: 1508, 1602, 1717, 3011; ^{1}H NMR (300 MHz) δ: 1.07 (6H, s), 1.51–1.59 (2H, m), 1.59–1.67 (2H, m), 1.99 (2H, t, J = 5.9 Hz), 3.25 (2H, d, J = 2.0 Hz), 3.46 (2H, s), 7.24 (1H, d, J = 8.6 Hz), 7.39–7.47 (2H, m), 7.52 (1H, s), 7.74–7.81 (3H, m), 9.63 (1H, t, J = 2.0 Hz); ^{13}C NMR (75 MHz) δ: 19.29, 28.21 (2C), 30.75, 35.00, 39.17, 39.48, 43.83, 125.24, 125.92, 126.30, 126.98, 127.42, 127.56, 128.01, 131.25, 132.10, 133.52, 134.86, 137.04, 200.98; HRMS (EI) m/z: Calcd $C_{21}H_{24}O$ (M^+) 292.1826. Found: 292.1821.

4.5. General procedure for the preparation enol nonaflate (7a–d)

A mixture of the aldehyde (1.0 equiv), t-BuOK (1.5 equiv) and n-$C_4F_9SO_2F$ (1.5 equiv) in THF (40 mL) was heated under reflux for 3–4 h. After cooling, the reaction mixture was quenched with saturated aqueous NH_4Cl (30 mL), the organics were extracted with ether (3 × 70 mL), followed by standard workup. The residue was purified by CC (ether–hexane, 1:9) to give the nonaflate **7**.

4.5.1. (*E*)-2-(2-Butyl-6,6-dimethylcyclohexen-1-yl)ethenyl Nonafluorobutanesulfonate (7a)

This was prepared from the aldehyde (**6a**, 1.58 g, 12.75 mmol), n-$C_4F_9SO_2F$ (3.44 g, 11.4 mmol) in 59% yield (2.14 g) as a pale yellow oil. IR ν_{max} cm^{-1}: 1210, 1424, 1649; ^{1}H NMR (300 MHz) δ: 0.86 (3H, t, J = 7.2 Hz), 0.95 (6H, s), 1.15–1.32 (4H, m), 1.40–1.64 (6H, m), 1.98 (2H, t, J = 6.5 Hz), 6.20 (1H, d, J = 11.4 Hz), 6.43 (1H, d, J = 11.4 Hz); ^{13}C NMR (125 MHz) δ: 13.90, 19.07, 22.68, 28.40 (2C), 30.13, 31.24, 34.04, 34.65, 39.05, 108.42 (J = 40.2, 271.2 Hz), 109.77 (J = 31.3, 270.6 Hz), 114.64 (J = 35.8, 301.6 Hz), 117.06 (J = 3.0, 288.6 Hz), 120.02, 130.04, 137.07, 137.69; ^{19}F NMR (470 MHz) δ: −127.39, −122.74, −111.22, −82.20; HRMS (EI) m/z: Calcd $C_{18}H_{23}F_9O_3S$ (M^+) 490.1223. Found: 490.1239.

4.5.2. (*E*)-2-[6,6-Dimethyl-2-(2-methylpropyl)cyclohexen-1-yl]ethenyl nonafluorobutanesulfonate (7b)

This was prepared from the aldehyde (**6b**, 1.43 g, 6.88 mmol), n-$C_4F_9SO_2F$ (3.12 g, 10.32 mmol) in 60% yield (2.02 g) as a pale yellow oil. IR ν_{max} cm^{-1}: 1208, 1424, 1649; ^{1}H NMR (300 MHz) δ: 0.81 (6H, d, J = 6.3 Hz), 0.98 (6H, s), 1.43–1.51 (2H, m), 1.53–1.66 (2H, m), 1.67–1.89 (1H, m), 1.93 (2H, d, J = 7.8 Hz), 1.97 (2H, d, J = 3.0 Hz), 6.19 (1H, d, J = 12.0 Hz), 6.42 (1H, d, J = 12.0 Hz); ^{13}C NMR (125 MHz) δ: 19.05, 22.16 (2C), 26.79, 28.43 (2C), 30.32, 34.26, 39.12, 43.39, 108.42 (J = 39.7, 270.7 Hz), 109.78 (J = 32.3, 271.5 Hz), 114.61 (J = 36.3, 301.0 Hz), 117.06 (J = 34.6, 288.4 Hz), 120.34, 131.19, 136.48, 137.50; ^{19}F NMR (470 MHz) δ: −127.37, −122.73, −111.17, −82.19; HRMS (EI) m/z: Calcd $C_{18}H_{23}F_9O_3S$ (M^+) 490.1223. Found: 490.1217.

4.5.3. (*E*)-2-(2-Benzyl-6,6-dimethylcyclohexen-1-yl)ethenyl nonafluorobutanesulfonate (7c)

This was prepared from the aldehyde (**6c**, 200 mg, 0.83 mmol), n-$C_4F_9SO_2F$ (377.6 mg, 1.25 mmol) in 75% yield (265.0 mg) as a pale yellow oil. IR ν_{max} cm^{-1}: 1206, 1425, 1494, 1602, 1647, 3064; ^{1}H NMR (300 MHz) δ: 1.05 (6H, s), 1.48–1.55 (2H, m), 1.58–1.67 (2H, m), 1.96 (2H, t, J = 6.0 Hz), 3.42 (2H, s), 6.29 (1H, d, J = 12.2 Hz), 6.47 (1H, d, J = 12.2 Hz), 7.05 (2H, d, J = 7.5 Hz), 7.14–7.31 (3H, m); ^{13}C NMR (125 MHz) δ: 18.94, 28.43 (2C), 30.33, 34.37, 38.88, 40.33, 108.20 (J = 37.5, 290.0 Hz), 109.72 (J = 33.8, 304.8 Hz), 114.57 (J = 34.3, 295.9 Hz), 117.02 (J = 32.8,287.0 Hz), 119.88, 126.00, 128.07 (2C), 128.46 (2C), 132.58, 135.28, 137.34, 140.10; ^{19}F NMR (470 MHz) δ: −127.40, −122.75, −111.19, −82.21.; HRMS (EI) m/z: Calcd $C_{21}H_{21}F_9O_3S$ (M^+) 524.1066. Found: 524.1082.

4.5.4. (*E*)-2-[6,6-Dimethyl-2-(2-naphthylmethyl)cyclohexen-1-yl]ethenyl Nonafluorobutanesulfonate (7d)

This was prepared from the aldehyde (**6d**, 4.57 g, 15.72 mmol), n-$C_4F_9SO_2F$ (7.12 g, 23.58 mmol) in 56% yield (5.03 g) as a pale yellow oil. IR ν_{max} cm^{-1}: 1202, 1426, 1508, 1601, 1643, 3065; ^{1}H NMR (300 MHz) δ: 1.08 (6H, s), 1.501.57 (2H, m), 1.57–1.66 (2H, m), 2.00 (2H, t, J = 3.7 Hz), 3.59 (2H, s), 6.36 (1H, d, J = 12.0 Hz), 6.54 (1H, d, J = 12.0 Hz), 7.21 (1H, d, J = 8.6 Hz), 7.42–7.48 (2H, m), 7.50 (1H, s), 7.75–7.82 (3H, m); ^{13}C NMR (125 MHz) δ: 18.95, 28.46 (2C), 29.23, 34.45, 38.88, 40.56, 108.40 (J = 34.0, 314.1 Hz), 109.69 (J = 31.0, 269.1 Hz), 114.58 (J = 35.6, 263.7 Hz), 116.99 (J = 33.9, 286.4 Hz), 120.00, 125.31, 126.00, 126.35, 126.82, 127.43, 127.59, 128.09, 132.09, 132.66, 133.55, 135.33, 137.47, 137.64; ^{19}F NMR (470 MHz) δ: −127.38, −122.72, −111.16, −82.18; HRMS (EI) m/z: Calcd $C_{25}H_{23}F_9O_3S$ (M^+) 574.1222. Found: 574.1225.

4.6. General procedure for the preparation of β-Ionylideneacetoaldehyde analogs (10a–d and 10′a–d)

To a stirred solution of nonaflate (**7,** 200 mg, 0.67 mmol) in DMF (2 mL) was added $Pd(PPh_3)_4$ (40 mg, 0.034 mmol, 0.05 equiv) at room temperature under nitrogen. After 10 min, a solution of *E*- or *Z*-3-tributylstannylbutenol (**8 or 8′**, 300–400 mg, ca. 1 mmol, 1.5 equiv) in DMF (2 mL) was added and the resulting mixture was stirred for 2 h. The reaction mixture was quenched with saturated aqueous NaCl (5 mL) and extracted with Et_2O (10 mL × 3), followed by the standard work up. The residue was purified by CC (ether:hexane = 1:4–3:7 as an eluent) to give the coupled alcohol (**9 or 9′**). The alcohol (**9**, 0.5 mmol) was dissolved in Et_2O (20 mL), and MnO_2 (10 equiv) was added. After stirring the mixture for 4–5 h min, MnO_2 was filtered with Celite. The filtrate was concentrated in vacuo and the residue was purified by CC (ether:hexane = 3:7 as eluent) to give the aldehyde (**10 or 10′**) as a pale yellow oil.

4.6.1. (2*E*,4*E*)-5-(2-Butyl-6,6-dimethylcyclohexen-1-yl)-3-methyl-2,4-pentadienal (10a)

This was prepared from the nonaflate (**7a**, 915 mg, 1.87 mmol) and *E*-stannyl olefin (**8**, 1.02 g, 2.81 mmol) in 48% yield (231 mg, 2 steps) as a pale yellow oil. UV–vis λ_{max} nm: 277.8, 328.6; IR ν_{max} cm^{-1}: 1657; ^{1}H NMR (300 MHz) δ: 0.87 (3 H, t, J = 7.2 Hz), 1.03 (6H, s), 1.20–1.40 (6H, m), 1.40–1.70 (4H, m), 2.04 (2H, t, J = 7.4 Hz), 2.30 (3H, s), 5.92 (1H, d, J = 8.1 Hz), 6.20 (1H, d, J = 16.2 Hz), 6.74 (1H, d, J = 16.2 Hz), 10.12 (1H, d, J = 8.1 Hz); ^{13}C NMR (75 MHz) δ: 12.88, 13.96, 19.07, 22.69, 28.94 (2C), 30.66, 31.19, 34.27, 34.65, 39.72, 128.73, 134.69, 135.75, 137.04, 137.19, 155.03, 191.29; HRMS (EI) m/z: Calcd C18H28O (M+) 260.2139. Found: 260.2147.

4.6.2. (2*E*,4*E*)-3-Methyl-5-[6,6-dimethyl-2-(2-methylpropyl)cyclohexen-1-yl]-2,4-pentadienal (10b)

This was prepared from the nonaflate (**7b**, 1.0 g, 2.04 mmol) and *E*-stannyl olefin (**8**, 1.11 g, 3.06 mmol) in 38% yield (203 mg, 2 steps) as a pale yellow oil. UV–vis λ_{max} nm: 278.4, 326.2; IR ν_{max} cm^{-1}: 1657; ^{1}H NMR (300 MHz) δ: 0.82 (6H, d, J = 6.6 Hz), 1.05 (6H, s), 1.45–1.52 (2H, m, 2-CH2), 1.56–1.67 (2H, m), 1.70–1.84 (1H, m), 1.96 (2H, d, J = 7.5 Hz), 2.02 (2H, t, J = 5.4 Hz), 2.30 (3H, s), 5.93 (1H, d, J = 8.4 Hz), 6.19 (1H, d, J = 16.2 Hz), 6.74 (1H, d, J = 16.2 Hz), 10.12 (1H, d, J = 8.4 Hz); ^{13}C NMR (75 MHz) δ: 12.91, 19.06, 22.49 (2C), 27.27, 28.94 (2C), 30.94, 34.48, 39.86, 43.60, 128.70, 134.95, 135.98, 136.22, 138.17, 155.02, 191.29; HRMS (EI) m/z: Calcd C18H28O (M+) 260.2139. Found: 260.2142.

4.6.3. (2*E*,4*E*)-5-(2-Benzyl-6,6-dimethylcyclohexen-1-yl)-3-methyl-2,4-pentadienal (10c)

This was prepared from the nonaflate (**7c**, 390 mg, 0.91 mmol) and *E*-stannyl olefin (**8**, 495 mg, 1.37 mmol) in 49% yield (131 mg, 2 steps) as a pale yellow oil. UV–vis λ_{max} nm: 317.8; IR ν_{max} cm^{-1}: 1493, 1610, 1657; ^1H NMR (300 MHz) δ: 1.10 (6H, s), 1.48–1.57 (2H, m), 1.591.65 (2H, m), 1.96 (2H, t, J = 5.5 Hz), 2.27 (3H, s), 3.45 (2H, s), 5.87 (1H, d, J = 8.1 Hz), 6.25 (1H, d, J = 16.0 Hz), 6.78 (1H, d, J = 16.0 Hz), 7.09 (2H, J = 7.5 Hz), 7.13–7.21 (1H, m), 7.23–7.31 (2H, m), 10.09 (1H, d, J = 8.1 Hz,); ^{13}C NMR (75 MHz) δ: 12.88, 18.98, 28.96 (2C), 30.64, 34.60, 39.42, 40.54, 100.22, 125.84, 128.20 (2C), 128.35 (2C), 129.11, 134.02, 135.31, 139.46, 140.58, 154.51, 191.26; HRMS (EI) *m*/*z*: Calcd C21H26O (M+) 294.1983. Found: 294.1979.

4.6.4. (2*E*,4*E*)-3-Methyl-5-[6,6-dimethyl-2-(2-naphthylmethyl)cyclohexen-1-yl]-2,4-pentadienal (10d)

This was prepared from the nonaflate (**7d**, 1.70 g, 2.96 mmol) and *E*-stannyl olefin (**8**, 1.60 g, 4.44 mmol) in 65% yield (664 mg, 2 steps) as a pale yellow solid. UV–vis λ_{max} nm: 224.0, 275.4; IR ν_{max} cm^{-1}: 1508, 1602, 1661, 3013; ^1H NMR (300 MHz) δ: 1.14 (6H, s), 1.51–1.57 (2H, m), 1.58–1.67 (2H, m), 2.03 (2H, t, J = 5.4 Hz), 2.27 (3H, s), 3.62 (2H, s), 5.83 (1H, d, J = 8.3 Hz), 6.31 (1H, d, J = 16.2 Hz), 6.83 (1H, d, J = 16.2 Hz), 7.23 (1H, d, J = 8.4 Hz), 7.39–7.49 (2H, m), 7.51 (1H, s), 7.75–7.82 (3H, m), 10.09 (1H, d, J = 8.3 Hz); ^{13}C NMR (75 MHz) δ: 12.93, 19.02, 29.00 (2C), 30.72, 34.69, 39.45, 40.75, 125.20, 125.93, 126.42, 126.95, 127.39, 127.59, 127.97, 129.14, 132.00, 133.52, 133.96, 135.33, 135.50, 138.12, 139.64, 154.47, 191.23; HRMS (EI) *m*/*z*: Calcd C25H28O (M+) 344.2139. Found: 344.2143.

4.6.5. (2*Z*,4*E*)-5-(2-Butyl-6,6-dimethylcyclohexen-1-yl)-3-methyl-2,4-pentadienal (10′a)

This was prepared from the nonaflate (**7a**, 800 mg, 1.63 mmol) and *Z*-stannyl olefin (**8′**, 885 mg, 2.45 mmol) in 65% yield (276 mg, 2 steps) as a pale yellow oil. UV–vis λ_{max} nm: 276.4, 321.0; IR ν_{max} cm^{-1}: 1615, 1660; ^1H NMR (300 MHz) δ: 0.85 (3H, t, J = 7.1 Hz), 1.03 (6H, s), 1.18–2.08 (12H, m), 2.10 (3H, s), 5.84 (1H, d, J = 8.3 Hz), 6.62 (1H, d, J = 15.9 Hz), 7.50 (1H, d, J = 15.9 Hz), 10.14 (1H, d, J = 8.3 Hz); ^{13}C NMR (75 MHz) δ: 13.96, 19.08, 21.12, 22.76, 28.97 (2C), 30.61, 31.29, 34.22, 34.76, 39.69, 126.69, 127.66, 136.83, 137.06, 137.23, 155.21, 190.06; HRMS (EI) *m*/*z*: Calcd C18H28O (M+) 260.2138. Found: 260.2139.

4.6.6. (2*Z*,4*E*)-3-Methyl-5-[6,6-dimethyl-2-(2-methylpropyl)cyclohexen-1-yl]-2,4-pentadienal (10′b)

This was prepared from the nonaflate (**7b**, 800 mg, 1.63 mmol) and *Z*-stannyl olefin (**8′**, 885 mg, 2.45 mmol) in 57% yield (276 mg, 2 steps) as a pale yellow oil. UV–vis λ_{max} nm: 276.8, 323.6; IR ν_{max} cm^{-1}: 1615, 1660; ^1H NMR (300 MHz) δ: 0.80 (6H, d, J = 6.3 Hz), 1.04 (6H, s), 1.43–1.51 (2H, m), 1.53–1.66 (2H, m), 1.69–1.87 (1H, m), 1.96 (2H, d, J = 7.5 Hz), 2.01 (2H, t, J = 3.0 Hz), 2.10 (3H, s), 5.84 (1H, d, J = 8.3 Hz), 6.62 (1H, d, J = 16.2 Hz), 7.03 (1H, d, J = 16.2 Hz), 10.15 (1H, d, J = 8.3 Hz); ^{13}C NMR (75 MHz) δ: 19.06, 21.14, 22.51 (2C), 27.26, 28.99 (2C), 30.92, 34.44, 39.84, 43.65, 126.97, 127.69, 135.88, 137.30, 138.30, 155.26, 190.14; HRMS (EI) *m*/*z*: Calcd C18H28O (M+) 260.2139. Found: 260.2137.

4.6.7. (2*Z*,4E)-5-(2-Benzyl-6,6-dimethylcyclohexen-1-yl)-3-methyl-2,4-pentadienal (10'c)

This was prepared from the nonaflate (**7c**, 547 mg, 1.28 mmol) and *Z*-stannyl olefin (**8′**, 693 mg, 1.92 mmol) in 58% yield (218 mg, 2 steps) as a pale yellow oil. UV–vis λ_{max} nm: 314.6; IR ν_{max} cm^{-1}: 1494, 1615, 1659, 3012; ^1H NMR (300 MHz) δ: 1.12 (6H, s), 1.52–1.58 (2H, m), 1.59–1.70 (2H, m), 2.04 (2H, t, J = 5.6 Hz), 3.49 (2H, s), 5.77 (1H, d, J = 8.4 Hz), 6.65 (1H, d, J = 16.8 Hz), 7.08 (1H, d, J = 16.8 Hz), 7.10 (2H, d, J = 7.2 Hz), 7.15–7.32 (3H, m), 9.65 (1H, d, J = 8.4 Hz); ^{13}C NMR (75 MHz) δ: 19.05, 20.94, 29.06 (2C), 30.98, 34.56, 39.37, 40.66, 125.95, 127.19, 128.03 (2C), 128.21, 128.48 (2C), 133.69, 135.92, 139.91, 140.67, 154.45, 190.20; HRMS (EI) *m*/*z*: Calcd C21H26O (M+) 294.1982. Found: 294.1990.

4.6.8. (2*Z*,4E)-3-Methyl-5-[6,6-dimethyl-2-(2-naphthylmethyl)cyclohexen-1-yl]-2,4-pentadienal (10′d)

This was prepared from the nonaflate (**7d**, 1.55 g, 4.44 mmol) and Z-stannyl olefin (**8′**, 2.41 g, 6.66 mmol) in 70% yield (1.07 g, 2 steps) as a pale yellow solid. UV–vis λ_{max} nm: 223.8, 274.8; IR ν_{max} cm^{-1}: 1508, 1616, 1661, 3013; ^1H NMR (300 MHz) δ: 1.15 (6H, s), 1.52–1.59 (2H, m), 1.60–1.69 (2H, m), 2.06 (2H, t, J = 8.6 Hz), 2.09 (3H, s), 3.64 (2H, s), 5.79 (1H, d, J = 8.6 Hz), 6.71 (1H, d, J = 15.9 Hz), 6.83 (1H, d, J = 15.9 Hz), 7.24 (1H, d, J = 8.9 Hz), 7.39–7.48 (2H, m), 7.53 (1H, s), 7.74–7.82 (3H, m), 9.80 (1H, d, J = 8.6 Hz); ^{13}C NMR (75 MHz) δ: 19.03, 21.05, 29.05 (2C), 30.81, 34.63, 39.40, 40.83, 125.27, 126.01, 126.33, 126.80, 127.35, 127.45, 127.62, 128.06, 128.16, 131.99, 133.52, 133.81, 136.15, 138.11, 139.90, 154.45, 190.00; HRMS (EI) *m*/*z*: Calcd $C_{25}H_{28}O$ (M^+) 344.2139. Found: 344.2145.

4.7. General procedure for the preparation of ethyl 5-substituted retinoate (12a–d and 12′a–d)

To a solution of diethyl 3-(methoxycarbonyl)-2-methyl-2-propenyl-phosphonate (*E*:*Z* = 4:1) (**11**, 391 mg, 2 mmol) and DMPU (4.0 equiv) in THF (5 mL) was added *n*-BuLi (1.6 M hexane solution, 1.25 mL, 2 mmol) at 0 °C. After stirring for 30 min, the solution was cooled at −78 °C and to this mixture was added a solution of the aldehyde (**10** or **10′**, ca. 0.7 mmol) in THF (5 mL). The resulting mixture was stirred for an additional 5 h and allowed to come to the room temperature. The reaction mixture was quenched with saturated NH_4Cl (5 mL) and extracted with ether followed by standard workup. The residue was purified by column choromatography on silica gel (ether:hexane = 1:9) to give the respective pentaenyl esters (**12** or **12′**) as a mixture of stereoisomers (**12** for all-*E* and 13*Z*-isomer, **12′** for 9*Z*- and 9*Z*,13*Z*-isomer).

4.7.1. Ethyl (2*E*,4*E*,6*E*,8*E*)-9-(2-Butyl-6,6-dimethylcyclohexen-1-yl)-3,7-dimethyl-2,4,6,8-nonatetraenoate and Ethyl (2*Z*,4*E*,6*E*,8*E*)-9-(2-butyl-6,6-dimethylcyclohexen-1-yl)-3,7-dimethyl-2,4,6,8-nonatetraenoate (12a)

This was prepared from the aldehyde (**10a,** 231 mg, 0.89 mmol), phosphonate (470.3 mg, 1.78 mmol), DMPU (776.7 mg, 3.56 mmol) and *n*-BuLi (1.19 mL, 1.78 mmol) in 94% yield (310 mg, all-*E*:13*Z* = 15:1) as a pale yellow oil.

all-E-Isomer: UV–vis λ_{max} nm: 354.3; IR ν_{max} cm^{-1}: 1584, 1698; ^1H NMR (300 MHz) δ: 0.87 (3H, t, J = 6.9 Hz), 1.02 (6H, s), 1.29 (3H, t, J = 7.1 Hz), 1.23–1.66 (8H, m), 1.99 (3H, s), 2.00–2.08 (4H, m), 2.35 (3H, s), 3.47 (2H, q, J = 7.1 Hz), 5.77 (1H, s), 6.11 (1H, d, J = 17.1 Hz), 6.12 (1H, d, J = 11.0 Hz), 6.27 (1H, d, J = 17.1 Hz), 6.28 (1H, d, J = 15.0 Hz), 6.99 (1H, dd, J = 11.0, 15.0 Hz); ^{13}C NMR (75 MHz) δ: 12.87, 13.78, 14.00, 14.29, 19.26, 22.69, 28.97 (2C), 30.44, 31.11, 34.28, 34.56, 39.79, 59.59, 118.51, 128.89, 129.35, 130.88, 134.46, 135.04, 136.25, 137.74, 139.53, 152.65, 167.17; HRMS (EI) *m*/*z*: Calcd $C_{25}H_{38}O_2$ (M^+) 370.2870. Found: 370.2866.

4.7.2. Ethyl (2*E*,4*E*,6*E*,8*E*)-3,7-dimethyl-9-[6,6-dimethyl-2-(2-methylpropyl)cyclohexen-1-yl]-2,4,6,8-nonatetraenoate and Ethyl (2*Z*,4*E*,6*E*,8*E*)-3,7-dimethyl-9-[6,6-dimethyl-2-(2-methylpropyl)cyclohexen-1-yl]-2,4,6,8-nonatetraenoate (12b)

This was prepared from the aldehyde (**10b,** 200 mg, 0.78 mmol), phosphonate (412.2 mg, 1.56 mmol), DMPU (680.7 mg, 3.12 mmol), and *n*-BuLi (1.04 mL, 1.56 mmol)in 92% yield (267 mg, all-*E*:13*Z* = 14:1) as a pale yellow oil.

all-E-Isomer: UV–vis λ_{max} nm: 242.4, 352.8; IR ν_{max} cm^{-1}: 1584, 1698; ^1H NMR (300 MHz) δ: 0.81 (6H, d, J = 6.6 Hz), 1.03 (6H, s), 1.28 (3H, t, J = 7.1H), 1.40–1.52 (2H, m), 1.55–1.63 (2H, m), 1.68–1.85 (1H, m), 1.96 (2H, d, J = 8.4 Hz), 1.98 (3H, s), 2.00 (2H, t, J = 6.6 Hz), 2.35 (3H, s), 4.17 (2H, q, J = 7.1 Hz), 5.76 (1H, s), 6.09 (1H, d, J = 16.2 Hz), 6.13 (1H, d, J = 10.7 Hz), 6.26 (1H, d, J = 16.2 Hz), 6.28 (1H, d, J = 15.0 Hz), 6.99 (1H, dd, J = 10.7, 15.0 Hz); ^{13}C NMR (75 MHz) δ: 12.87, 13.78, 14.29, 19.26, 22.49 (2C), 27.12, 28.97 (2C), 30.76, 34.51, 39.86, 43.56, 59.59, 118.50, 129.29, 129.38, 130.88, 133.28, 135.04, 136.51, 138.87, 139.55, 152.66, 167.17; HRMS (EI) m/z: Calcd $C_{25}H_{38}O_2$ (M^+) 370.2870. Found: 370.2871.

4.7.3. Ethyl (2*E*,4*E*,6*E*,8*E*)-9-(2-benzyl-6,6-dimethylcyclohexen-1-yl)-3,-dimethyl-2,4,6,8-nonatetraenoate and Ethyl (2*Z*,4*E*,6*E*,8*E*)-9-(2-benzyl-6,6-dimethylcyclohexen-1-yl)-3,7-dimethyl-2,4,6,8-nonatetraenoate (12c)

This was prepared from the aldehyde (**10c,** 130 mg, 0.44 mmol), phosphonate (232 mg, 0.88 mmol), DMPU (384.0 mg, 1.76 mmol), and *n*-BuLi (0.59 ml, 0.88 mmol) in 95% yield (170 mg, all-*E*:13*Z* = 41:1) as a pale yellow oil.

all-E-Isomer: UV–vis λ_{max} nm: 350.8; IR ν_{max} cm^{-1}: 1608, 1699, 3020; ^1H NMR (500 MHz) δ: 1.07 (6H, s), 1.26 (3H, t, J = 7.3 Hz), 1.471.50 (2H, m), 1.551.60 (2H, m), 1.92 (2H, t, J = 6.3 Hz), 1.95 (3H, s), 2.31 (3H, s), 3.45 (2H, s), 4.15 (2H, q, J = 7.3 Hz), 5.73 (1H, s), 6.02 (1H, d, J = 11.5 Hz), 6.17 (1H, d, J = 16.0 Hz), 6.23 (1H, d, J = 15.0 Hz), 6.31 (1H, d, J = 16.0 Hz), 6.93 (1H, dd, J = 11.5,15.0 Hz), 7.10 (2H, d, J = 7.4 Hz), 7.16 (1H, t, J = 7.4 Hz), 7.25 (2H, t, J = 7.4 Hz); ^{13}C NMR (125 MHz) δ: 12.91, 13.80, 14.34, 19.19, 29.03 (2C), 30.41, 34.67, 39.56, 40.63, 59.64, 118.71, 125.62, 128.24 (2C), 128.41 (2C), 128.46, 129.94, 130.72, 131.78, 135.40, 136.82, 139.13, 140.21, 141.27, 152.59, 167.17; HRMS (EI) m/z: Calcd $C_{28}H_{36}O_2$ (M^+) 404.2713. Found: 404.2721.

4.7.4. Ethyl (2*E*,4*E*,6*E*,8*E*)-3,7-dimethyl-9-[6,6-dimethyl-2-(2-naphthylmethyl)cyclohexen-1-yl]-2,4,6,8-nonatetraenoate and Ethyl (2*Z*,4*E*,6*E*,8*E*)-3,7-dimethyl-9-[6,6-dimethyl-2-(2-naphthylmethyl)cyclohexen-1-yl]-2,4,6,8-nonatetraenoate (12d)

This was prepared from the aldehyde (**10d,** 650 mg, 1.89 mmol) , phosphonate (998 mg, 3.78 mmol), DMPU (1.65 g, 7.56 mmol), and *n*-BuLi (2.52 mL, 3.78 mmol) in 89% yield (760 mg, all-*E*:13*Z* = 50>:1) as a pale yellow solid.

all-E-Isomer: UV–vis λ_{max} nm: 224.0, 353.0; IR ν_{max} cm^{-1}: 1508, 1608, 1699, 3011; ^1H NMR (300 MHz) δ: 1.11 (6H, s), 1.28 (3H, t, J = 7.0 Hz), 1.48–1.56 (2H, m), 1.57–1.63 (2H, m), 1.952.00 (2H, m), 1.98 (3H, s), 2.32 (3H, s), 3.63 (2H, s), 4.16 (2H, q, J = 7.0 Hz), 5.73 (1H, s), 6.03 (1H, d, J = 11.5 Hz), 6.22 (1H, d, J = 15.1 Hz), 6.23 (1H, d, J = 16.2 Hz), 6.37 (1H, d, J = 16.2 Hz), 6.94 (1H, dd, J = 11.5, 15.1 Hz), 7.27 (1H, d, J = 8.4 Hz), 7.39–7.47 (2H, m), 7.54 (1H, s), 7.74–7.82 (3H, m); ^{13}C NMR (75 MHz) δ: 12.88, 13.75, 14.29, 19.17, 29.02 (2C), 30.78, 34.69, 39.51, 40.81, 59.59, 118.69, 125.01, 125.77, 126.50, 127.22, 127.42 (2C), 127.54, 127.72, 128.39, 129.97, 130.64, 131.66, 131.94, 135.42, 136.94, 138.84, 139.03, 140.41, 152.51, 167.11; HRMS (EI) m/z: Calcd $C_{32}H_{38}O_2$ (M^+) 454.2870. Found: 454.2878.

4.7.5. Ethyl (2*E*,4*E*,6*Z*,8*E*)-9-(2-butyl-6,6-dimethylcyclohexen-1-yl)-3,7-dimethyl-2,4,6,8-nonatetraenoateand ethyl (2*Z*,4*E*,6*Z*,8*E*)-9-(2-butyl-6,6-dimethylcyclohexen-1-yl)-3,7-dimethyl-2,4,6,8-nonatetraenoate (12′a)

This was prepared from the aldehyde (**10'a,** 276 mg, 1.06 mmol), phosphonate (560.2 mg, 2.12 mmol), DMPU (925.1 mg, 4.24 mmol), and *n*-BuLi (1.41 mL, 2.12 mmol) in 92% yield (359 mg, 9*Z*: 9Z,13*Z* = 13:1) as a pale yellow oil.

9Z-Isomer: UV–vis λ_{max} nm: 349.4; IR ν_{max} cm^{-1}: 1591, 1699; ^1H NMR (300 MHz) δ: 0.85 (3H, t, J = 6.9 Hz), 1.01 (6H, s), 1.26 (3H, t, J = 7.1 Hz), 1.312.10 (12H, m), 1.97 (3H, s), 2.31 (3H, s), 4.15 (2H, q, J = 7.1 Hz), 5.74 (1H, s), 6.02 (1H, d, J = 11.5 Hz), 6.20 (1H, d, J = 15.2 Hz), 6.25 (1H, d, J = 15.6 Hz), 6.61 (1H, d, J = 15.6 Hz), 7.06 (1H, dd, J = 11.5, 15.2 Hz); ^{13}C NMR (75 MHz) δ: 13.73, 14.04, 14.29, 19.26, 20.82, 22.79, 29.06 (2C), 30.41, 31.52, 34.24, 34.82, 39.63, 59.59, 118.82, 127.82, 128.42, 129.65, 130.34, 134.36, 134.46, 138.03, 138.27, 152.65, 167.16; HRMS (EI) m/z: Calcd $C_{25}H_{38}O_2$ (M^+) 370.2870. Found: 370.2872.

4.7.6. Ethyl (2E,4E,6Z,8E)-3,7-dimethyl-9-[6,6-dimethyl-2-(2-methylpropyl)cyclohexen-1-yl]-2,4,6,8-Nonatetraenoate and Ethyl (2*Z*,4*E*,6*Z*,8*E*)-3,7-Dimethyl-9-[6,6-dimethyl-2-(2-methylpropyl)cyclohexen-1-yl]-2,4,6,8-nonatetraenoate (12′b)

This was prepared from the aldehyde (**10'b,** 230 mg, 0.89 mmol), phosphonate (470.3 mg, 1.78 mmol), DMPU (776.7 mg, 3.56 mmol), and *n*-BuLi (1.19 mL, 1.78 mmol) in 93% yield (307 mg, 9*Z*: 9Z,13*Z* = 12:1) as a pale yellow oil.

9Z-Isomer: UV–vis λ_{max} nm: 348.4; IR ν_{max} cm^{-1}: 1592, 1698; ^1H NMR (300 MHz) δ: 0.82 (6H, d, J = 6.6 Hz), 1.03 (6H, s), 1.26 (3H, t, J = 7.1 Hz), 1.43–1.52 (2H, m), 1.53–1.68 (2H, m), 1.71–1.86 (1H, m), 1.97 (3H, s), 1.98 (2H, d, J = 8.4 Hz), 2.02 (2H, t, J = 7.5 Hz), 2.31 (3H, s), 4.15 (2H, q, J = 7.1 Hz), 5.74 (1H, s), 6.03 (1H, d, J = 11.4 Hz), 6.20 (1H, d, J = 15.0 Hz), 6.23 (1H, d, J = 16.0 Hz), 6.59 (1H, d, J = 16.0 Hz), 7.06 (1H, dd, J = 11.4,15.0 Hz); ^{13}C NMR (75 MHz) δ: 13.76, 14.28, 19.24, 20.84, 22.52 (2C), 27.04, 29.05 (2C), 30.64, 34.45, 39.75, 43.60, 59.57, 118.50, 127.77, 128.83, 129.76, 130.84, 13.22, 134.30, 138.30, 139.11, 152.69, 167.14; HRMS (EI) m/z: Calcd $C_{25}H_{38}O_2$ (M^+) 370.2870. Found: 370.2876.

4.7.7. Ethyl (2*E*,4*E*,6*Z*,8*E*)-9-(2-Benzyl-6,6-dimethylcyclohexen-1-yl)-3,7-dimethyl-2,4,6,8-nonatetraenoate and Ethyl (2*Z*,4*E*,6*Z*,8*E*)-9-(2-Benzyl-6,6-dimethylcyclohexen-1-yl)-3,7-dimethyl-2,4,6,8-nonatetraenoate (12′c)

This was prepared from the aldehyde (**10'c,** 218 mg, 0.74 mmol), phosphonate (391.1 mg, 1.48 mmol), DMPU (645.8 mg, 2.96 mmol), and *n*-BuLi (0.99 mL, 1.48 mmol) in 89% yield (265 mg, 9*Z*: 9Z,13*Z* = 26:1) as a pale yellow oil.

9Z-Isomer: UV–vis λ_{max} nm: 350.8; IR ν_{max} cm^{-1}: 1494, 1591, 1702, 3026; ^1H NMR (300 MHz) δ: 1.12 6H, s), 1.28 (3H, t, J = 7.1 Hz), 1.50–1.57 (2H, m), 1.59–1.68 (2H, m), 1.97 (3H, s), 1.99 (2H, t, J = 5.5 Hz), 2.04 (3H, s), 3.52 (2H, s), 4.15 (2H, q, J = 7.1 Hz), 5.68 (1H, s), 5.97 (1H, d, J = 11.5 Hz), 6.11 (1H, d, J = 15.2 Hz), 6.32 (1H, d, J = 16.0 Hz), 6.65 (1H, dd, J = 11.5,15.2 Hz), 6.72 (1H, d, J = 16.0 Hz), 7.127.33 (5H, m); ^{13}C NMR (75 MHz) δ: 13.82, 14.31, 19.17, 20.75, 29.18 (2C), 30.61, 34.56, 39.42, 40.81, 59.54, 118.39, 125.87, 128.16 (2C), 128.24 (2C), 128.41, 128.48, 129.47, 129.52, 131.46, 134.65, 137.76, 140.66, 141.16, 152.85, 167.13; HRMS (EI) m/z: Calcd $C_{28}H_{36}O_2$ (M^+) 404.2713. Found: 404.2710.

4.7.8. Ethyl (2*E*,4*E*,6*Z*,8*E*)-3,7-dimethyl-9-[6,6-dimethyl-2-(2-naphthylmethyl)cyclohexen-1-yl]-2,4,6,8-nonatetraenoate and Ethyl (2*Z*,4*E*,6*Z*,8*E*)-3,7-dimethyl-9-[6,6-dimethyl-2-(2-naphthylmethyl)cyclohexen-1-yl]-2,4,6,8-nonatetraenoate (12′d)

This was prepared from the aldehyde (**10'd,** 650 mg, 1.89 mmol), phosphonate (998.8 mg, 3.78 mmol), DMPU (1.65 g, 7.56 mmol), and *n*-BuLi (2.52 mL, 3.78 mmol) in 89% yield (762 mg, 9*Z*: 9Z,13*Z* = 50>:1) as a pale yellow solid.

9Z-Isomer: UV–vis λ_{max} nm: 223.6, 273.6, 350.2; IR ν_{max} cm^{-1}: 1508, 1591, 1699, 3009; ^1H NMR (300 MHz) δ: 1.15 (6H, s), 1.25 (3H, t, J = 7.1 Hz), 1.53–1.59 (2H, m, 2-CH_2), 1.59 (3H, s), 1.62–1.69 (2H, m), 1.97 (3H, s), 2.04 (2H, t, J = 4.5 Hz), 3.68 (2H, s), 4.12 (2H, q, J = 7.1 Hz), 5.60 (1H, s), 5.94 (1H, d, J = 11.4 Hz), 6.04 (1H, d, J = 15.0 Hz), 6.36 (1H, d, J = 15.9 Hz), 6.53 (1H, dd, J = 11.4, 15.0 Hz), 6.78 (1H, d, J = 15.9 Hz), 7.31 (1H, d, J = 8.6 Hz), 7.37–

7.47 (2H, m), 7.57 (1H, s), 7.73–7.81 (3H, m); ^{13}C NMR (75 MHz) δ: 12.88, 14.26, 19.18, 20.75, 29.22 (2C), 30.69, 34.62, 39.43, 41.02, 59.50, 118.45, 125.24, 125.95, 126.09, 127.13, 127.36, 127.62, 127.95, 128.24, 128.56, 129.30, 129.45, 131.34, 132.05, 133.61, 134.75, 137.64, 138.76, 140.90, 152.78, 167.02; HRMS (EI) *m*/*z*: Calcd $C_{32}H_{38}O_2$ (M^+) 454.2870. Found: 4542871.

4.8. General procedure for the preparation of retinoic acid analogs (1b–e and 2b–e)

A mixture of the ester (**12 or 12′**, ca.200 mg, 0.5 mmol) and 10% KOH solution (3 mL) in methanol (6 mL) was heated at 50 °C for 2 h. After cooling, the reaction mixture was made acidic with 5% HCl, and the organics were extracted with ethyl acetate followed by standard workup. The residue was purified by CC (ethyl acetate: hexane = 3:1) and recrystallization afforded the stereoisomeric pure acid (**1 or 2**) as a yellow solid.

4.8.1. (2*E*,4*E*,6*E*,8*E*)-9-(2-Butyl-6,6-dimethylcyclohexen-1-yl)-3,7-dimethyl-2,4,6,8-nonatetraenoic acid (1b)

This was prepared from the ester (**12a**, 246 mg, 0.66 mmol) in 82% yield (193 mg). mp: 83–86 °C (ether–hexane); UV–vis λ_{max} nm (ε): 200.6, 339.4 (36300); IR ν_{max} cm^{-1}: 1583, 1689, 2600-3500; ^{1}H NMR (500 MHz) δ: 0.86 (3H, t, *J* = 7.3 Hz), 1.01 (6H, s), 1.221.29 (2H, m), 1.301.37 (2H, m), 1.43–1.45 (2H, m), 1.56–1.62 (2H, m), 1.99 (3H, s), 2.01 (2H, t, *J* = 5.5 Hz), 2.04 (2H, t, *J* = 7.8 Hz), 2.35 (3H, s), 5.78 (1H, s), 6.11 (1H, d, *J* = 16.3 Hz), 6.13 (1H, d, *J* = 16.0 Hz), 6.28 (1H, d, *J* = 16.3 Hz), 6.30 (1H, d, *J* = 13.3 Hz), 7.03 (1H, dd, *J* = 13.3, 16.0 Hz), COOH signal was not observed; ^{13}C NMR (125 MHz) δ: 12.96, 14.02, 14.06, 19.31, 22.75, 29.03 (2C), 30.53, 31.18, 34.35, 34.62, 39.85, 116.99, 129.29, 129.35, 131.89, 134.70, 134.75, 136.24, 137.76, 140.34, 155.33, 170.30; HRMS (EI) *m*/*z*: Calcd $C_{23}H_{34}O_2$ (M^+) 342.2557. Found: 342.2555.

4.8.2. (2*E*,4*E*,6*E*,8*E*)-3,7-Dimethyl-9-[6,6-dimethyl-2-(2-methylpropyl)cyclohexen-1-yl]-2,4,6,8-nonatetraenoic acid (1c)

This was prepared from the ester (**12b**, 265 mg, 0.72 mmol) in 75% yield (193 mg). mp: 105–108 °C (ether–hexane); UV–vis λ_{max} nm (ε): 337.4 (44800); IR ν_{max} cm^{-1}: 1582, 1681, 2600–3500; ^{1}H NMR (500 MHz) δ: 0.80 (6 H, d, *J* = 6.5 Hz), 1.01 (6H, s), 1.44–1.47 (2H, m), 1.56–1.62 (2H, m), 1.71–1.77 (1H, m), 1.95 (2H, d, *J* = 7.5 Hz), 1.98 (2H, t, *J* = 3.7 Hz), 1.99 (3H, s), 2.35 (3H, s), 5.78 (1H, s), 6.09 (1H, d, *J* = 15.3 Hz), 6.13 (1H, d, *J* = 11.5 Hz), 6.27 (1H, d, *J* = 15.3 Hz), 6.30 (1H, d, *J* = 15.0 Hz), 7.03 (1H, dd, *J* = 11.5, 15.0 Hz), COOH signal was not observed; ^{13}C NMR (125 MHz) δ: 12.97, 14.03, 19.30, 22.55 (2C), 27.19, 29.04 (2C), 30.83, 34.57, 39.94, 43.62, 117.02, 129.22, 129.85, 131.90, 133.51, 134.74, 136.49, 138.88, 140.36, 155.37, 170.56; HRMS (EI) *m*/*z*: Calcd $C_{23}H_{34}O_2$ (M^+) 342.2557. Found: 342.2557. Anal. Calcd for $C_{23}H_{34}O_2$: C, 80.65; H, 10.01. Found: C, 80.37; H, 9.67.

4.8.3. (2*E*,4*E*,6*E*,8*E*)-9-(2-Benzyl-6,6-dimethylcyclohexen-1-yl)-3,7-dimethyl-2,4,6,8-nona- tetraenoic acid (1d)

This was prepared from the ester (**12c**, 157 mg, 0.39 mmol) in 69% yield (100 mg). mp: 147–149 °C (ether–hexane); UV–vis λ_{max} nm (ε): 340.2 (38900); IR ν_{max} cm^{-1}: 1582, 1682, 2600–3500; ^{1}H NMR (500 MHz) δ: 1.07 (6H, s), 1.46–1.50 (2H, m), 1.55–1.60 (2H, m), 1.92 (2H, t, *J* = 5.3 Hz), 1.96 (3H, s), 2.32 (3H, s), 3.45 (2H, s), 5.76 (1H, s), 6.03 (1H, d, *J* = 11.0 Hz), 6.18 (1H, d, *J* = 16.0 Hz), 6.25 (1H, d, *J* = 15.0 Hz), 6.33 (1H, d, *J* = 16.0 Hz), 6.98 (1H, dd, *J* = 11.0, 15.0 Hz), 7.10 (2H, d, *J* = 7.2 Hz), 7.16 (1H, t, *J* = 7.2 Hz), 7.25 (2H, t, *J* = 7.2 Hz), COOH signal was not observed; ^{13}C NMR (125 MHz) δ: 12.69, 14.00, 19.19, 29.04 (2 C), 30.44, 34.68, 39.56, 40.64, 117.24, 125.65, 128.26 (2C), 128.41(2C), 128.86, 129.83, 131.67, 131.97, 135.07, 136.75, 139.87, 140.18, 141.24, 155.19, 170.49; HRMS (EI) *m*/*z*: Calcd $C_{26}H_{32}O_2$ (M^+) 376.2400. Found: 376.2407.

4.8.4. (2*E*,4*E*,6*E*,8*E*)-3,7-Dimethyl-9-[6,6-dimethyl-2-(2-naphthylmethyl)cyclohexen-1-yl]-2,4,6,8-nonatetraenoic acid (1e)

This was prepared from the ester (**12d**, 390 mg, 0.86 mmol) in 79% yield (299 mg). mp: 169–171 °C (ether–hexane); UV–vis λ_{max} nm (ε): 223.8, 340.0 (44300); IR ν_{max} cm^{-1}: 1583, 1694, 2600–3500; ^{1}H NMR (500 MHz) δ: 1.10 (6H, s), 1.49–1.52 (2H, m), 1.57–1.60 (2H, m), 1.94–1.99 (2H, m), 1.97 (3H, s), 2.31 (3H, s), 3.61 (2H, s), 5.74 (1H, s), 6.02 (1H, d, *J* = 11.5 Hz), 6.22 (1H, d, *J* = 15.8 Hz), 6.23 (1H, d, *J* = 14.8 Hz), 6.37 (1H, d, *J* = 14.8 Hz), 6.97 (1H, dd, *J* = 11.5, 15.8 Hz), 7.25 (1H, d, *J* = 8.8 Hz), 7.38–7.45 (2H, m), 7.52 (1H, s), 7.72–7.80 (3H, m), COOH signal was not observed; ^{13}C NMR (125 MHz) δ: 12.97, 13.98, 19.21, 29.08 (2C), 30.55, 34.75, 39.55, 40.86, 117.25, 125.07, 125.82, 126.53, 127.26, 127.47, 127.58, 127.79, 128.82, 129.91, 131.60, 131.82, 131.98, 133.58, 135.13, 136.91, 138.85, 139.79, 140.43, 155.11, 170.32; HRMS (EI) *m*/*z*: Calcd $C_{30}H_{34}O_2$ (M^+) 426.2557. Found: 426.2557.

4.8.5. (2*E*,4*E*,6*Z*,8*E*)-9-(2-Butyl-6,6-dimethylcyclohexen-1-yl)-3,7-dimethyl-2,4,6,8-nonatetraenoic acid (2b)

This was prepared from the ester (**12′a**, 300 mg, 0.81 mmol) in 70% yield (202 mg). mp: 124–126 °C (ether–hexane); UV–vis λ_{max} nm (ε): 335.2 (35800); IR ν_{max} cm^{-1}: 1589, 1682, 2600–3550; ^{1}H NMR (500 MHz) δ: 0.86 (3H, t, *J* = 7.4 Hz), 1.02 (6H, s), 1.27 (2H, sext, *J* = 7.4 Hz), 1.38 (2H, tt, *J* = 7.4, 8.0 Hz), 1.451.48 (2H, m), 1.58–1.64 (2H, m), 1.98 (3H, s), 2.03 (2H, t, *J* = 6.0 Hz), 2.06 (2H, t, *J* = 8.0 Hz), 2.32 (3H, s), 5.78 (1H, s), 6.04 (1H, d, *J* = 11.3 Hz), 6.23 (1H, d, *J* = 15.3 Hz), 6.27 (1H, d, *J* = 16.0 Hz), 6.62 (1H, d, *J* = 16.0 Hz), 7.11 (1H, dd, *J* = 11.3, 15.3 Hz), COOH signal was not observed; ^{13}C NMR (125 MHz) δ: 14.20, 14.32, 19.52, 21.15, 23.08, 29.34 (2C), 30.71, 31.81, 34.53, 35.09, 39.90, 117.43, 128.00, 128.63, 130.84, 130.96, 134.34, 134.85, 138.29, 139.30, 155.48, 170.99; HRMS (EI) *m*/*z*: Calcd $C_{23}H_{34}O_2$ (M^+) 342.2557. Found: 342.2552. Anal. Calcd for $C_{23}H_{34}O_21/2H_2O$: C, 78.59; H, 10.04. Found: C, 78.96; H, 9.81.

4.8.6. (2*E*,4*E*,6*Z*,8*E*)-3,7-Dimethyl-9-[6,6-dimethyl-2-(2-methylpropyl)cyclohexen-1-yl]-2,4,6,8-nonatetraenoic acid (2c)

This was prepared from the ester (**12′b**, 277 mg, 0.75 mmol) in 75% yield (200 mg). mp: 135–138 °C (ether–hexane); UV–vis λ_{max} nm (ε): 334.8 (35800); IR ν_{max} cm^{-1}: 1589, 1682, 2600–3600; ^{1}H NMR (500 MHz) δ: 0.83 (6H, d, *J* = 6.5 Hz), 1.03 (6H, s), 1.46–1.49 (2H, m), 1.59–1.64 (2H, m), 1.73–1.81 (1H, m), 1.98 (3H, s), 1.98 (2H, t, *J* = 3.5 Hz), 2.01 (2H, d, *J* = 4.0 Hz), 2.32 (3H, s), 5.78 (1H, s), 6.04 (1H, d, *J* = 11.3 Hz), 6.23 (1H, d, *J* = 15.0 Hz), 6.26 (1H, d, *J* = 15.8 Hz), 6.59 (1H, d, *J* = 15.8 Hz), 7.11 (1H, dd, *J* = 11.3, 15.0 Hz), COOH signal was not observed; ^{13}C NMR (125 MHz) δ: 14.03, 19.29, 20.94, 22.59 (2C), 27.11, 29.10 (2C), 30.69, 34.52, 39.79, 43.66, 117.20, 127.71, 128.80, 130.72, 131.24, 133.37, 134.06, 139.12, 139.15, 155.33, 170.87; HRMS (EI) *m*/*z*: Calcd $C_{23}H_{34}O_2$ (M^+) 342.2557. Found: 342.2544. Anal. Calcd for $C_{23}H_{34}O_2$: C, 80.65; H, 10.01. Found: C, 80.27; H, 9.71.

4.8.7. (2*E*,4*E*,6*Z*,8*E*)-9-(2-Benzyl-6,6-dimethylcyclohexen-1-yl)-3,7-dimethyl-2,4,6,8-nonatetraenoic acid (2d)

This was prepared from the ester (**12′c**, 170 mg, 0.42 mmol) in 86% yield (136 mg). mp: 146–148 °C (ether-hexane); UV–vis λ_{max} nm (ε): 266.0, 377.6 (37200); IR ν_{max} cm^{-1}: 1587, 1678, 2600–3550; ^{1}H NMR (500 MHz) δ: 1.10 (6H, s), 1.51–1.55 (2H, m), 1.59–1.65 (2H, m), 1.96 (3H, s), 1.98 (2H, t, *J* = 5.5 Hz), 2.03 (3H, s), 3.51 (2H, s), 5.69 (1H, s), 5.97 (1H, d, *J* = 11.5 Hz), 6.12 (1H, d, *J* = 14.5 Hz), 6.32 (1H, d, *J* = 15.8 Hz), 6.68 (1H, dd, *J* = 11.5,14.5 Hz), 6.71 (1H, d, *J* = 15.8 Hz), 7.12–7.19 (3H, m), 7.23–7.29 (2H,

m), COOH signal was not observed; ^{13}C NMR (125 MHz) δ: 14.06, 19.18, 20.82, 29.22 (2C), 30.67, 34.60, 39.44, 40.84, 117.11, 125.91, 128.13, 128.20 (2C), 128.39, 128.45 (2C), 129.86, 130.45, 131.59, 134.40, 138.56, 140.69, 141.20, 155.43, 170.83; HRMS (EI) m/z: Calcd $C_{26}H_{32}O_2$ (M^+) 376.2400. Found: 376.2396.

4.8.8. (2*E*,4*E*,6*Z*,8*E*)-3,7-Dimethyl-9-[6,6-dimethyl-2-(2-naphthylmethyl)cyclohexen-1-yl]-2,4,6,8- nonatetraenoic acid (2e)

This was prepared from the ester (**12′d**, 1.0 g, 2.20 mmol) in quantitative yield (965 mg). mp: 162–164 °C (ether-hexane); UV–vis λ_{max} nm (ε): 223.6, 268.6, 338.0 (35700); IR ν_{max} cm^{-1}: 1589, 1679, 2600–3500; ^{1}H NMR (500 MHz) δ: 1.14 (6H, s, *gem* -Me), 1.54-1.57 (2H, m), 1.62 (3H, s), 1.62-1.67 (2H, m), 1.97 (3H, s), 2.03 (2H, t, J = 5.5 Hz,), 3.66 (2H, s), 5.62 (1H, s), 5.95 (1H, d, J = 6.8 Hz), 6.06 (1H, d, J = 8.9 Hz, 12-CH), 6.37 (1H, d, J = 9.6 Hz), 6.57 (1H, dd, J = 6.8, 8.9 Hz), 6.76 (1H, d, J = 9.6 Hz), 7.29 (1H, d, J = 8.5 Hz), 7.39–7.45 (2H, m), 7.55 (1H, s), 7.74–7.80 (3H, m), COOH signal was not observed; ^{13}C NMR (125 MHz) δ: 13.15, 19.22, 20.85, 29.25 (2C), 30.74, 34.68, 39.45, 41.07, 117.11, 125.30, 126.02, 126.16, 127.16, 127.40, 127.67, 127.99, 128.27, 128.50, 129.87, 130.23, 131.51, 132.08, 133.64, 134.51, 138.42, 138.79, 140.91, 155.32, 170.46; HRMS (EI) m/z: Calcd $C_{30}H_{34}O_2$ (M^+) 426.2557. Found: 426.2562.

4.9. Biological assay

4.9.1. HL-60. cells and synchronization of cell cycle at S phase by excess amounts of thymidine

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. Louis, 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 washing with Ca, Mg-free phosphate-buffered saline (PBS) [PBS(−)] twice, the synchronization of cell cycle was repeated in the same manner, and the cells thus obtained were used in the biological assays.

4.10. Analysis of cell cycle distribution by flow cytometry

Cells (10^5 cells/well) were placed in 24-well tissue culture plates and cultured for 3 days with retinoids (10^{-10}–10^{-6} 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 FACS analysis. Each group of cells was collected in PBS(−). Then, the cells were resuspended in PBS(−) containing 0.2% Triton-X and 1 mg/mL RNase, and 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 mg/mL) at 4 °C for 20 min. The cells were analyzed with a flow cytometer euipped with an argon laser (488 nm, Becton Dickinson FACScan™) and cell cycle distribution was analyzed by ModiFiT LT (Verity).

4.11. Cell surface antigen expression analysis

Cells (10^5 cells/well) were placed in 24-well tissue culture plates, and cultured for 3 days in RPMI-1640 medium with retinoids (10^{-10}-10^{-6} M) under the same conditions as described in the flow cytometry. Each group of cells was then collected and washed with PBS(-) once. Then, the cells (2×10^5 cells) were resuspended in 100 mL diluent solution containing 1% bovine serum albumin (BSA) and 1% sodium azide and incubated with 10 mL human monoclonal FITC conjugated CD 11 b antibody and CD14 antibody (Sigma) for 30 min at room temperature. The cells were washed once with diluent solution and then fixed in 300 mL of PBS(−) containing 2% paraformaldehyde. Fluorescence was detected on a Becton Dickinson FACScan™ at excitation wavelength of 490 nm and emission wavelength of 520 nm. Results were recorded as the mean fluorescence index, which is the product of the% fluorescence and the mean fluorescence intensity, with 10^4 cells being counted per treatment.

4.12. DNA fragmentation assay

For assessment of quantitative DNA fragmentation (laddering), DNA was isolated from cells of each culture, and was examined for fragmentation. DNA was subjected to electrophoresis in a 2% agarose gel that was stained with ethidium bromide for observation under ultraviolet light.

4.13. Transfection and luciferase activity assay

Human osteosarcoma MG-63 cells, which are positive for RXR gene expression, were maintained in Dulbecco's modification 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 so that they were confluent the 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 RARbpromoter (59/33: GGGTAAAGTTCACCGAAAGTTCACTCG) or the RXRE from the rat CRBPII promoter (639/605: GCTGTCACAGGTCA-CAGGTCACAGGTCACAGTTCA) in the pGL3 vector.8,9). The pRL-CMV vector was an internal control using the Tfx-50 reagent. After transfection, the cells were incubated with retinoids (10^{-6} μM) for 2 days. Luciferase activity of the cell lysates was measured with a luciferase assay system (Toyo Ink Co. Ltd), according to the manufactures' instructions. Transactivation determined from the luciferase activity was standardized with 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 are presented in terms of fold induction as means SE.

4.14. Statistical analysis

Statistical significances were determined using Dunnett's test and expressed as means ± SE. The data were compared to EtOH-treated control cells, and levels of significance were determined as, $^{***}p < 0.001$, $^{**}p < 0.01$ and $^{*}p < 0.05$.

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