Bioorganic & Medicinal Chemistry 19 (2011) 2939–2949

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

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

Replacement of the hydrophobic part of 9-*cis*-retinoic acid with cyclic terpenoid moiety results in RXR-selective agonistic activity

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ARTICLE INFO

Article history:
Received 1 February 2011
Revised 14 March 2011
Accepted 15 March 2011
Available online 21 March 2011

Keywords:
Rexinoids
RXR
RAR
Agonist
9-*cis*-Retinoic acid

ABSTRACT

Retinoid X receptor (RXR) agonists are interesting candidates for the treatment of metabolic syndrome. 9-*Cis*-retinoic acid (9cRA: **1**) is a natural RXR agonist, that also works as a retinoic acid receptor (RAR) agonist. This fact prompted us to study the structure–activity relationship (SAR) of RXR agonists derived from **1**. Though **3** and **4**, in which the cyclohexene part of **1** is replaced with bulkier hydrophobic moieties, show RXR-selective agonistic activity, some analogs containing other ring structures show RAR agonistic activity. Thus, we were interested in establishing what kind of ring skeleton is required for RXR-selective agonistic activity. In this study, we systematically prepared **5** and **6**, in which the cyclohexene ring of **1** is replaced with various cyclic terpenoid moieties, and evaluated their RXR and RAR agonistic activities. Our previously reported CsF-promoted Stille coupling reaction was employed as a key step for the comprehensive synthesis of **5** and **6**. The results of transcriptional assay showed that compounds **5b**–**f**, which possess a menthane skeleton, exhibit RXR-selective agonistic activity. These results should be helpful for the design of superior RXR-selective agonists based on the structure of **1**.

1. Introduction

Retinoid X receptors (RXRs; isotypes α, β, and γ) are members of the nuclear receptor superfamily and form homodimers with themselves or heterodimers with other nuclear receptors, including retinoic acid receptors (RARs), peroxisome proliferator-activated receptors (PPARs), liver X receptors (LXRs), and farnesoid X receptors (FXRs).[1] These dimers can modulate gene transcription through ligand binding. RAR/RXR heterodimers cannot be activated by RXR agonists; this is the so-called non-permissive effect.[2] However, combined treatment of RAR/RXR heterodimers with RXR agonists and RAR agonists results in increased transcriptional activity compared with that in the presence of RAR agonist only (RXR subordination or retinoid synergist effect).[3] For other RXR heterodimers, including PPAR/RXR, LXR/RXR, and FXR/RXR, transcription can be activated by RXR agonists alone (permissive effect).[4] Therefore, RXR-selective agonists may not influence RAR/RXR directly, but can modulate metabolic functions through their effects on other RXR heterodimers, such as PPARs (associated with lipid metabolism), LXRs (associated with cholesterol metabolism), and FXRs (associated with bile acid production and lipid metabolism), and so they are interesting candidates for the treatment of metabolic syndrome.[5]

9-*Cis*-retinoic acid (9cRA: **1**, Fig. 1) is a stereoisomer of all-*trans*-retinoic acid (ATRA: **2**) and is a native agonist of RXRs. Unfortunately, **1** also acts as an RAR agonist, so it cannot be utilized as a drug to treat dyslipidemia and diabetes mellitus, because it induces retinoic acid syndrome as a side effect.[6] Thus, various studies aimed at finding RXR agonists based on structure–activity relationship (SAR) analysis of **1** have been carried out. For example, de Lera and co-workers examined the conformation of **1** in RXR and RAR; in RXR, **1** takes an L-shaped conformation with a twisted polyene side chain (PDB: 3LBD), while in RAR, it takes an I-shaped conformation (PDB: 1FBY).[2] This information indicates that the C6–C7 bond and C8–C9 bond of **1** are likely to twist with respect to the conjugative plane to minimize steric repulsion of the dimethyl group at the 1-position and the methyl group at the 5-position with the hydrogen at the 8-position of the polyene unit. Thus, de Lera and co-workers prepared 9cRA derivatives **3** and **4**, in which the cyclohexene unit of **1** is replaced with various sterically hindered aromatic rings, and they found that these analogs work as RXR-selective agonists.[7] However, the RXR agonistic activities of compounds **3** and **4** were 10-fold lower than that of **1**. Very recently, the authors also synthesized a series of 9cRA analogs **5** modified at the hydrophobic ring with a (bi)cyclohexenyl moiety derived from natural terpenes (they called these analogs

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doi:10.1016/j.bmc.2011.03.033

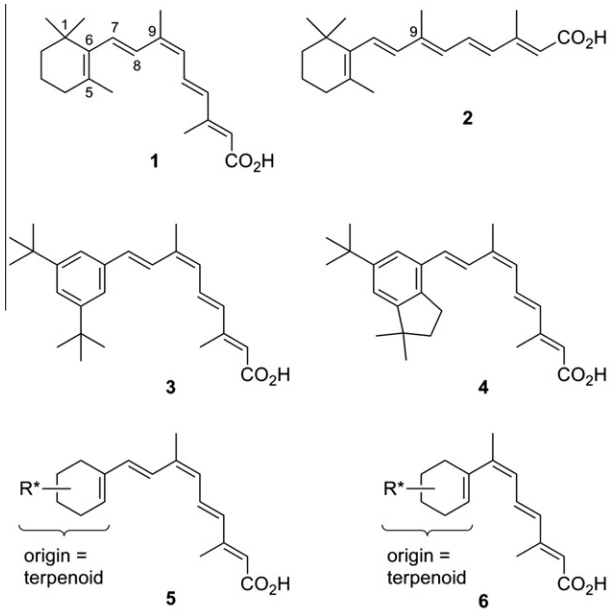


Figure 1. Chemical structures of 9-*cis*-retinoic acid (**1**), all-*trans*-retinoic acid (**2**) and rexinoids (**3**–**6**).

'terpene-retinoid' chimeras) and evaluated their transcriptional activities toward RARs (α, β, and γ) and RXRβ.[8] It was found that **5** exhibited a RAR pan-agonistic profile and was less active towards RXRβ than **1**. Thus, we were interested in discovering what type of hydrophobic ring in such 9cRA derivatives would produce potent and RXR-selective agonistic activity.

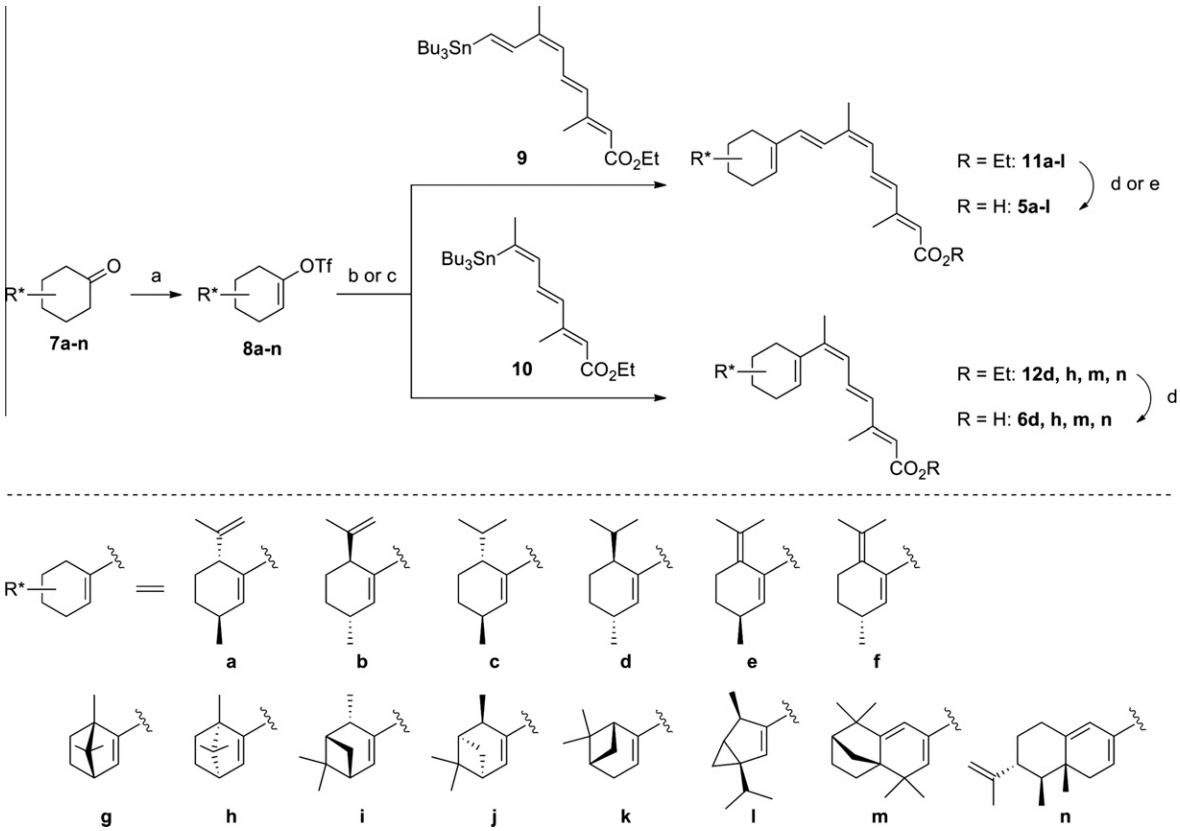
Recently, we developed a highly efficient and rapid total synthesis of **1**, and we adapted this synthetic methodology to the preparation of various analogs of **1**.[9] To understand the structural characteristics of 9cRA analogs exhibiting RXR-selective agonistic activity, we focused on the comprehensive synthesis of 9cRA derivatives using our method, followed by examination of their SAR.[10] In particular, we planned to synthesize a series of 9cRA derivatives **5** and **6** in which the cyclohexene ring of **1** is replaced with various cyclic terpenoid moieties **7**, because the menthane skeleton (a kind of terpenoid) seems to be an equivalent as a lipophilic part in the 3,5-di-*t*-butylphenyl structure of **3**. In addition, compounds **7** are optically active and can be readily modified utilizing an oxygenic functionality. Here, we describe the systematic preparation of 9cRA derivatives **5** and **6** according to our strategy, together with the results of an examination of their RXR and RAR activities.

2. Results and discussion

As mentioned above, we have reported an efficient synthesis of **1** and a convergent synthetic route to its derivatives by means of CsF-promoted Stille coupling as a key reaction.[9] Utilizing this methodology, the carbon framework of **1** can be constructed under mild conditions without *cis-trans* isomerization, and without tedious experimental procedures. To synthesize 9cRA derivatives **5** and **6**, we decided to use stannanyl ester **9**, which was developed by de Lera,[11] or the two-carbon-shortened derivative **10** (Scheme 1). The required vinyl triflates **8** were easily prepared in one step from the corresponding ketones **7**. Five enantiomeric pairs [isopulegone (**7a**, **7b**), menthone (**7c**, **7d**), pulegone (**7e**, **7f**), camphor (**7g**, **7h**), and isopinocamphenone (**7i**, **7j**)], and other terpenoid compounds such as (+)-nopinone (**7k**) and (−)-α-thujone (**7l**) were used as starting materials for comprehensive synthesis of 9cRA derivatives **5**. Furthermore, analogs **6**, which are shortened by two carbons (C7–C8 of **1**), were also synthesized from monoterpenes (−)-menthone (**7d**) and (+)-camphor (**7h**), and sesquiterpenes (−)-isolongifolen-9-one (**7m**) and (+)-nootkatone (**7n**). The CsF-promoted Stille coupling of **8** with **9** or **10** proceeded under our conditions[9] or Baldwin's conditions[12] to afford the coupled products **11** and **12** in good to high yields. Finally, esters **11** and **12** were hydrolyzed under basic conditions at 50 °C or under microwave irradiation to afford carboxylic acids **5** and **6**. Thus, we accomplished the synthesis of sixteen 9cRA derivatives **5** and **6**. Compounds **5** and **6** were relatively stable to light and could be stored for a long period in the freezer. Compared with de Lera's synthetic method,[8] ours has the advantage; (1) vinyl triflates **8**, coupling partners for Stille coupling, can be synthesized in one step from ketones **7** without epimerization of the chiral centers. On the other hand, boronic esters, which are needed for the previous method based on Suzuki coupling, had to be prepared from ketones via trisylhydrazones in two steps or via vinyl iodides in four steps. Furthermore, epimerization readily occurred during trisylhydrazone synthesis, so that the reactions had to be carefully monitored. (2) Stannanyl esters **9** and **10** can be used for Stille coupling, while the previous method requires transformation from stannanyl ester to the corresponding iodo-ester for Suzuki coupling. (3) Our experimental procedure for cross-coupling is therefore easier than the previous method.

After 9cRA derivatives **5** and **6** had been obtained, reporter gene assay was performed using transfected MG-63 cells for the evaluation of transcriptional activities toward RXRs and RARs. Transcriptional activities were evaluated toward retinoid X response elements (RXREs) present in the promoter region of rat cellular retinoic acid binding protein 2 (CRABP2) gene for RXRs, and toward retinoic acid response elements (RAREs) present in the promoter region of human RARβ gene for RARs. RXRα, β, and γ are distributed in different locations in the body. RXRα is expressed in liver, kidney, intestine, and epidermis; RXRβ is ubiquitously distributed; RXRγ is expressed in skeletal muscles, heart muscles, skin, and brain.[13] These assays do not provide information about the isotype-selectivity, but they are concise and efficient methods for the screening of many compounds. Table 1 shows relative ratios of transcriptional activities towards RXR and RAR for 9cRA derivatives **5** and **6**, with respect to that of **1**, whose transcriptional efficacy at 1 μM was defined as 1.0. Compounds **5** except for **5h** and **5i** induced stronger RXR-mediated gene expression than **1**, while compounds **6** exhibited weaker transcriptional activity than **1** toward both RXR and RAR. In addition, bicyclic compounds **5h** and **5i** showed RAR agonistic activity. These results indicated that the replacement of the cyclohexene unit of **1** with a sterically hindered hydrophobic ring system does not always enhance RXR-selective agonistic activity. However, **5a**–**f**, which have a menthane skeleton, showed a significant RXR transcriptional activity relative to that toward RAR. This outcome is reasonable, because the position of the isopropyl group of **5a**–**f** corresponds to that of the *t*-butyl group of **3** and **4** when the C–C bond between the hydrophobic ring domain and the tetraene unit rotates. The reason for the contrasting transcriptional activities of enantiomers **5i** and **5j** is not evident, but this phenomenon is intriguing.

To discover candidates for the treatment of metabolic syndrome, transcriptional activity toward RXRα, which is mainly expressed in liver, was examined for the selected compounds **5b**–**f**, which showed higher selectivity for RXR over RAR (Table 1). Each of compounds **5b**–**f** dose-dependently exhibited the transcriptional activity in a human RXRα-GAL4 assay (Fig. 2). Among the compounds tested, **5d** showed higher activity than that of **1** (the native ligand of RXR). The other compounds **5b**–**c** and **5e**–**f** were less potent than **1**, but at high concentrations (10^{-6} M), all compounds exhibited almost the same activity of **1**. It is noteworthy that **5d**, which showed the lower transcriptional activity for RXRE



Scheme 1. Reagents and conditions: (a) LDA, $PhNTf_2$, THF, −78 °C to rt, overnight; (b) $Pd_2(dba)_3{\cdot}CHCl_3$, Ph_3As, CsF, DMF, 45 °C, 0.5–4 h; (c) $Pd(Ph_3P)_4$, CuI, CsF, DMF, 45 °C, 0.5–1.5 h; (d) 10% KOH aq, EtOH, 50 °C, overnight; (e) 10% KOH aq, EtOH, microwave, 130 °C, 2–3 min.

than those of **5c** and **5e**–**f** in Table 1, exhibited the highest activity in RXRα-GAL4 assay. In addition, compound **5e**, which had the highest RXR/RAR selectivity ratio among the compounds in Table 1, showed lower potency than **1** at low concentration. These facts were understandable by the following considerations: It is well known that RARs and RXRs enhance their gene transcription by removal of co-repressors and subsequent binding to co-activators as heterodimer or homodimer.[14] Therefore, it can be speculated that RXRs bound to the analogs **5b**–**f** have the different interactions with co-repressors or co-activators than those of the natural ligand-binding RXRs. Thus, the RXR incorporating an analog **5d** may make a more fitted RXR co-activator complex than those of other compounds and exhibited high activity. On the contrary, the RXR incorporating other compounds may fail to make a RXR co-activator complex or seems to be difficult to bind to RXRE, thus, lower transcriptional activities were observed. de Lera reported that compound **5d** showed weaker RAR agonistic activity than the pan-RAR agonist TTNPB and weaker RXRβ agonistic activity than **1**.[8] Interestingly, the enantiomer **5c** was less potent than **5d**. To clarify the difference of RXRα agonistic activity between compound **5d** and its enantiomer **5c**, a docking study was performed with AutoDock4.0.[15] The results, shown in Figure 3, revealed that the binding modes of both **5d** and **5c** in the ligand-binding domain (LBD) of RXRα were twisted at the π-conjugative system, resulting in L-shaped conformations. It is remarkable that the mean binding energy of **5c** toward RXRα was the same as that of **5d** (data not shown), but the enatiomeric **5d** showed stronger biological activity than that of **5c**. This may be the reason why the hydrophobic interaction of the lipophilic part of **5d** with the LBD is stronger than that of **5c**. Actually, in this model isopropyl moiety of **5c** or **5d** showed a significant difference in the hydrophobic interactions with RXR. Especially, a strong interaction between valine 265 or isoleucine 268 in helix 2 of RXR and isopropyl moiety of **5d** was observed.

3. Conclusions

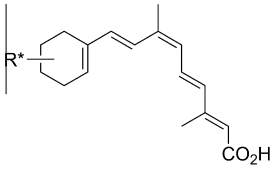
To understand what type of hydrophobic unit in 9cRA derivatives is required for RXR-selective agonistic activity, we carried out systematic synthesis and RXR and RAR agonistic activity evaluation of 9cRA derivatives **5** and **6**, in which the cyclohexene ring of **1** is replaced with various cyclic terpenoid moieties. Compounds **5b**–**f** bearing a menthane skeleton were found to show RXR-selective agonistic activity. On the other hand, bornane or pinane skeleton-fused derivatives **5g**–**j** exhibited not only RXR agonistic activity, but also RAR agonistic activity. These results indicate that the replacement of the cyclohexene unit of **1** with a sterically hindered hydrophobic ring system does not necessarily favor RXR-selective agonistic activity, but there seems to be a tendency that compounds bearing isopropyl or isopropenyl structure near the polyene linker work as RXR-selective agonists. This information will be useful as a guideline for the design of novel RXR-selective agonists.

4. Experimental

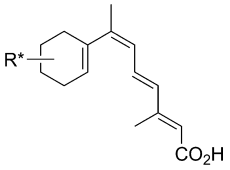
4.1. General methods

Optical rotations were measured on a JASCO DIP-370 polarimeter ($[\alpha]_D$ values are in units of 10^{-1} deg cm^2 g^{-1}). Melting points were determined on a micro melting point apparatus (Yanagimoto) and are uncorrected. UV–vis spectra were recorded on a JASCO Ubest-55 or JASCO V-650 instrument. IR spectra were measured on a

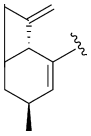
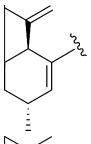
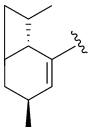
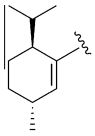
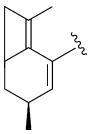
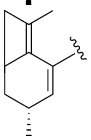
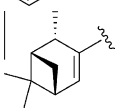
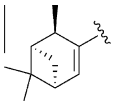
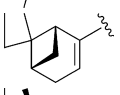
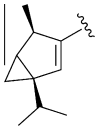
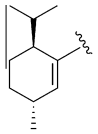
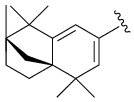
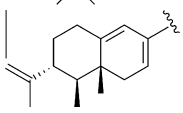
Table 1
Co-transfection data for pan-RXR/RAR agonist 9-*cis*-retinoic acid (**1**) and synthetic compounds **5** and **6** in MG-63 cells



5



6

Compound	R*	RXRE[a]	RARE[b]	RXR selectivity ratio[c]
1		1.00	1.00	1.00
5a		1.76	1.33	1.33
5b		1.07	0.35	3.06
5c		1.37	0.38	3.64
5d		1.12	0.25	4.44
5e		2.06	0.32	6.37
5f		1.50	0.50	3.00
5g		2.34	1.36	1.70
5h		0.66	1.99	0.33
5i		0.52	1.48	0.35
5j		1.30	0.89	1.45
5k		2.42	1.92	1.26
5l		2.52	1.03	2.45
6d		0.37	0.14	2.58
6h		0.18	0.04	4.63
6m		0.71	0.98	0.73
6n		0.23	0.10	2.27

[a] Transcriptional potency of **5** and **6** (10^{-6} M) on rat CRABP2-RXRE (transcriptional efficacy of **1** at 1 μM was defined as 1.0).
[b] Transcriptional potency of **5** and **6** (10^{-6} M) on human RARβ-RARE (transcriptional efficacy of **1** at 1 μM was defined as 1.0).
[c] RXR selectivity ratio was defined as the ratio of (transcriptional efficacy on RXRE)/(transcriptional efficacy on RARE) at the concentration of 10^{-6} M.

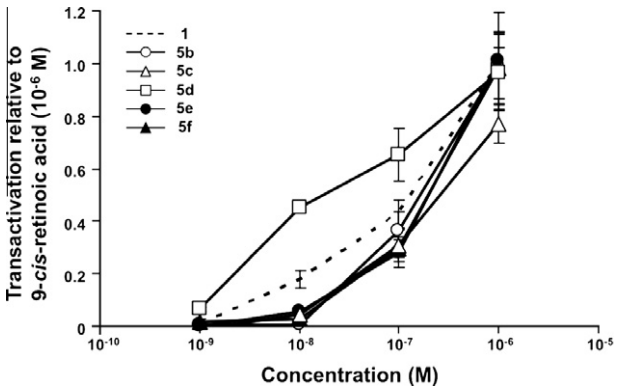


Figure 2. Results of RXRα reporter gene assay for **1** and compounds **5b–f** bearing a menthane skeleton.

Perkin Elmer FT-IR spectrometer, model Paragon 1000 or Horiba FT-IR spectrometer, model FT-720, using $CHCl_3$ unless otherwise noted. Chemical shifts (δ) are reported in ppm relative to tetramethylsilane as an internal reference ($CDCl_3$: δ = 0 ppm for ^1H) or residual solvent signals ($CDCl_3$: δ = 77 ppm for ^{13}C). *J*-Values are given in Hertz. Mass spectra were taken on a Hitachi M-4100 spectrometer. Column chromatography was performed using Kanto Silica Gel 60 N (spherical, neutral). All reagents were used as obtained commercially unless otherwise noted.

4.2. General procedure for the triflation of carbonyl compounds (GP-A)

A solution of *i*-Pr_2NH (1.1 equiv) in dry THF (1 M) was cooled at −78 °C and *n*-BuLi (1.1 equiv) was added dropwise under an argon atmosphere. The reaction mixture was stirred for 10 min at the same temperature, then for 30 min at 0 °C, and recooled to −78 °C. A solution of a carbonyl compound (1 equiv) in THF (1 M) was added and the reaction mixture was stirred for 2 h at the same temperature. $PhNTf_2$ (1.5 equiv) was added to the reaction mixture, which was then allowed to warm to room temperature in a water bath overnight. The solution was evaporated in

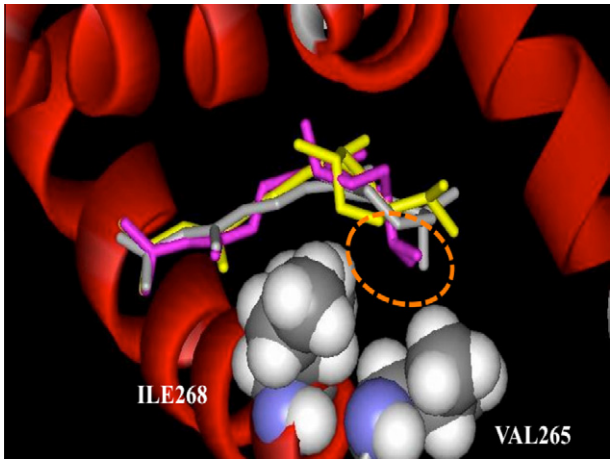


Figure 3. Docking models of compounds **1** (gray), **5c** (yellow), and **5d** (magenta) in the LBD of RXRα (PDB: 1FM9) using AutoDock4.0. The dashed-line circle shows the isopropyl moiety of **5d**.

vacuo, then the residue was purified by flash column chromatography on silica gel to give the vinyl triflate.

4.2.1. (3*S*,6*R*)-3-Methyl-6-(1-propen-2-yl)-1-cyclohexen-1-yl trifluoromethanesulfonate (8a)

According to general procedure (GP-A), **8a** (322 mg, 49%) was obtained as a colorless oil from *i*-Pr_2NH (359 μL, 2.54 mmol), *n*-BuLi (1.60 mL, 1.59 M in *n*-hexane, 2.54 mmol), (+)-isopulegone (**7a**) (351 mg, 2.30 mmol), $PhNTf_2$ (1.24 g, 3.46 mmol). Eluent: hexane. $[\alpha]_D^{24}$ −124 (*c* 0.884, MeOH); IR 2964, 1677, 1650, 1415, 1213, 1143, 1063 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 5.71 (br t, J = 2.0 Hz, 1H), 4.92 (quint, J = 1.5 Hz, 1H), 4.85 (t, J = 1.0 Hz, 1H), 3.17-3.13 (m, 1H), 2.43-2.38 (m, 1H), 1.94-1.88 (m, 1H), 1.82-1.76 (m, 1H), 1.68 (q, J = 1.0 Hz, 3H), 1.66-1.59 (m, 1H), 1.24-1.17 (m, 1H), 1.05 (d, J = 7.0 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 150.0, 142.9, 126.1, 114.6 (q, J_{CF} = 319 Hz), 46.4, 30.2, 28.6, 27.8, 20.9, 19.1; HR-EIMS calcd for $C_{11}H_{15}F_3O_3S$ (M^+) 284.0694, found 284.0708.

4.2.2. (3*R*,6*S*)-3-Methyl-6-(1-propen-2-yl)-1-cyclohexen-1-yl trifluoromethanesulfonate (8b)

According to general procedure (GP-A), **8b** (514 mg, 73%) was obtained as a colorless oil from *i*-Pr_2NH (384 μL, 2.71 mmol), *n*-BuLi (1.75 mL, 1.55 M in *n*-hexane, 2.71 mmol), (−)-isopulegone (**7b**) (376 mg, 2.47 mmol), $PhNTf_2$ (1.32 g, 3.70 mmol). Eluent: hexane. $[\alpha]_D^{25}$ +146 (*c* 1.17, MeOH). Anal. Calcd for $C_{11}H_{15}F_3O_3S$: C, 45.46; H, 2.67; F, 21.57. Found: C, 45.34; H, 2.81; F, 21.53; HR-EIMS calcd for $C_{11}H_{15}F_3O_3S$ (M^+) 284.0694, found 284.0710.

4.2.3. (3*S*,6*R*)-6-Isopropyl-3-methyl-1-cyclohexen-1-yl trifluoromethanesulfonate (8c)

According to general procedure (GP-A), **8c** (549 mg, 55%) was obtained as a colorless oil from *i*-Pr_2NH (531 μL, 3.82 mmol), *n*-BuLi (2.30 mL, 1.66 M in *n*-hexane, 3.82 mmol), (+)-menthone (**7c**) (525 mg, 3.47 mmol), $PhNTf_2$ (1.86 g, 5.20 mmol). Eluent: hexane. ^1H NMR (300 MHz, $CDCl_3$) δ 5.64 (br s, 1H), 2.50-2.44 (m, 1H), 2.36-2.28 (m, 1H), 2.21-2.10 (m, 1H), 1.86-1.77 (m, 2H), 1.48-1.36 (m, 1H), 1.21-1.07 (m, 1H), 1.04 (d, J = 7.2 Hz, 3H), 0.95 (d, J = 6.9 Hz, 3H), 0.82 (d, J = 6.9 Hz, 3H); HR-EIMS calcd for $C_{11}H_{17}F_3O_3S$ (M^+) 286.0850, found 286.0871. The ^1H NMR spectral data of this compound was identical with that of literature.[16]

4.2.4. (3*R*,6*S*)-6-Isopropyl-3-methyl-1-cyclohexen-1-yl trifluoromethanesulfonate (8d)

According to general procedure (GP-A), **8d** (1.10 g, 59%) was obtained as a colorless oil from *i*-Pr_2NH (1.00 mL, 7.13 mmol), *n*-BuLi (4.32 mL, 1.65 M in *n*-hexane, 7.13 mmol), (−)-menthone (**7d**) (1.00 g, 6.48 mmol), $PhNTf_2$ (3.47 g, 9.72 mmol). Eluent: hexane. The ^1H NMR spectral data of this compound was identical with that of literature.[16]

4.2.5. (*S*)-3-Methyl-6-(propan-2-ylidene)-1-cyclohexen-1-yl trifluoromethanesulfonate (8e)

According to general procedure (GP-A), **8e** (671 mg, 46%) was obtained as an inseparable mixture of its isomer from *i*-Pr_2NH (956 μL 6.76 mmol), *n*-BuLi (4.25 mL, 1.59 M in *n*-hexane, 6.76 mmol), (−)-pulegone (**7e**) (936 mg, 6.14 mmol), $PhNTf_2$ (3.29 g, 9.22 mmol). Eluent: hexane. Here the optical rotation was not shown due to the contamination of isomer of **8e**. ^1H NMR (300 MHz, $CDCl_3$) δ 5.58 (d, J = 3.3 Hz, 1H), 2.57-2.49 (m, 2H), 2.28-2.24 (m, 1H), 1.93 (s, 3H), 1.90-1.82 (m, 1H), 1.78 (s, 3H), 1.34-1.22 (m, 1H), 1.08 (d, J = 6.9 Hz, 3H); HR-EIMS calcd for $C_{11}H_{15}F_3O_3S$ (M^+) 284.0694, found 284.0711.

4.2.6. (*R*)-3-Methyl-6-(propan-2-ylidene)-1-cyclohexen-1-yl trifluoromethanesulfonate (8f)

According to general procedure (GP-A), **8f** (1.67 g, 89%) was obtained as an inseparable mixture of its isomer from *i*-Pr_2NH (1.02 mL 7.23 mmol), *n*-BuLi (4.66 mL, 1.55 M in *n*-hexane, 7.23 mmol), (+)-pulegone (1.00 g, 6.57 mmol), $PhNTf_2$ (3.52 g, 9.85 mmol). Eluent: hexane. Here the optical rotation was not shown due to the contamination of isomer of **8f**. ^1H NMR (300 MHz, $CDCl_3$) δ 5.58 (d, J = 4.5 Hz, 1H), 2.56-2.49 (m, 2H), 2.30-2.23 (m, 1H), 1.93 (s, 3H), 1.90-1.83 (m, 1H), 1.78 (s, 3H), 1.34-1.22 (m, 1H), 1.08 (d, J = 6.9 Hz, 3H); HR-EIMS calcd for $C_{11}H_{15}F_3O_3S$ (M^+) 284.0694, found 284.0703.

4.2.7. (1*S*,4*S*)-1,7,7-Trimethylbicyclo[2.2.1]hept-2-en-2-yl trifluoromethanesulfonate (8g)

According to general procedure (GP-A), **8g** (468 mg, 73%) was obtained as a colorless oil from *i*-Pr_2NH (351 μL, 2.48 mmol), *n*-BuLi (1.56 mL, 1.59 M in *n*-hexane, 2.48 mmol), (−)-camphor (**7g**) (312 mg, 2.26 mmol), $PhNTf_2$ (1.21 g, 3.39 mmol). Eluent: hexane. ^1H NMR (300 MHz, $CDCl_3$) δ 5.67 (d, J = 3.9 Hz, 1H), 2.45 (t, J = 3.9 Hz, 1H), 1.93 (ddd, J = 15.6, 8.7, 3.3 Hz, 1H), 1.65 (ddd, J = 12.3, 8.7, 3.6 Hz, 1H), 1.37-1.29 (m, 1H), 1.14 (ddd, J = 12.3, 8.7, 3.6 Hz, 1H), 1.03 (s, 3H), 0.92 (s, 3H), 0.79 (s, 3H); HR-EIMS calcd for $C_{11}H_{15}F_3O_3S$ (M^+) 284.0694, found 284.0699. The ^1H NMR spectral data of this compound was identical with that of literature.[17]

4.2.8. (1*R*,4*R*)-1,7,7-Trimethylbicyclo[2.2.1]hept-2-en-2-yl trifluoromethanesulfonate (8h)

According to general procedure (GP-A), **8h** (605 mg, 65%) was obtained as a colorless oil from *i*-Pr_2NH (511 μL, 3.61 mmol), *n*-BuLi (2.27 mL, 1.59 M in *n*-hexane, 3.61 mmol), (+)-camphor (500 mg, 3.29 mmol), $PhNTf_2$ (1.76 g, 4.93 mmol). Eluent: hexane. The ^1H NMR spectral data of this compound was identical with that of literature.[17]

4.2.9. (1*R*,4*S*,5*S*)-4,6,6-Trimethylbicyclo[3.1.1]hept-2-en-3-yl trifluoromethanesulfonate (8i)

According to general procedure (GP-A), **8i** (930 mg, quant.) was obtained as an inseparable mixture of its isomer from *i*-Pr_2NH (511 μL, 3.61 mmol), *n*-BuLi (2.33 mL, 1.55 M in *n*-hexane, 3.61 mmol), (+)-isopinocamphenone (**7i**) (500 mg, 3.29 mmol), $PhNTf_2$ (1.76 g, 4.93 mmol). Eluent: hexane. Here the optical rotation was not shown due to the contamination of isomer of **8i**. ^1H NMR (300 MHz, $CDCl_3$) δ 6.14 (dd, J = 5.1, 2.1 Hz, 1H), 2.96-2.92 (m, 1H), 2.56-2.48 (m, 1H), 2.27-2.16 (m, 2H), 1.40 (d, J = 9.3 Hz, 1H), 1.33 (s, 3H), 1.23 (d, J = 7.2 Hz, 3H), 1.06 (s, 3H); HR-EIMS calcd for $C_{11}H_{15}F_3O_3S$ (M^+) 284.0694, found 284.0701.

4.2.10. (1*S*,4*R*,5*R*)-4,6,6-Trimethylbicyclo[3.1.1]hept-2-en-3-yl trifluoromethanesulfonate (8j)

According to general procedure (GP-A), **8j** (1.56 g, 81%) was obtained as an inseparable mixture of its isomer from *i*-Pr_2NH (1.03 mL, 7.44 mmol), *n*-BuLi (4.49 mL, 1.66 M in *n*-hexane, 7.44 mmol), (−)-isopinocamphenone (**7j**) (1.03 g, 6.76 mmol), $PhNTf_2$ (3.63 g, 10.15 mmol). Eluent: hexane. HR-EIMS calcd for $C_{11}H_{15}F_3O_3S$ (M^+) 284.0694, found 284.0698.

4.2.11. (1*R*,5*S*)-6,6-Dimethylbicyclo[3.3.1]hept-2-en-2-yl trifluoromethanesulfonate (8k)

According to general procedure (GP-A), **8k** (1.47 g, 75%) was obtained as a colorless oil from *i*-Pr_2NH (1.10 mL, 7.96 mmol), *n*-BuLi (4.80 mL, 1.66 M in *n*-hexane, 7.96 mmol), (1*R*)-(+)-nopinone (**7k**) (1.00 g, 7.24 mmol), $PhNTf_2$ (3.88 g, 10.9 mmol). Eluent: hexane. ^1H NMR (300 MHz, $CDCl_3$) δ 5.55-5.52 (m, 1H), 2.56 (dt, J = 15.0, 8.5 Hz, 1H), 2.42-2.25 (m, 3H), 2.17-2.12 (m, 1H), 1.38 (d, J = 9.3 Hz, 1H), 1.34 (s, 3H), 0.93 (s, 3H). The ^1H NMR spectral data of this compound was identical with that of literature.[15]

4.2.12. (1*S*,4*R*,5*R*)-1-Isopropyl-4-methylbicyclo[3.1.0]hex-2-en-3-yl trifluoromethanesulfonate (8l)

According to general procedure (GP-A), **8l** (961 mg, quant.) was obtained as a colorless oil from *i*-Pr_2NH (511 μL, 3.61 mmol), *n*-BuLi (2.33 mL, 1.55 M in *n*-hexane, 3.61 mmol), (−)-α-thujone (**7l**) (500 mg, 3.29 mmol), $PhNTf_2$ (1.76 g, 4.93 mmol). Eluent: hexane. $[\alpha]_D^{29}$ −0.969 (*c* 1.03, MeOH); IR 3020, 2963, 1639, 1421, 1216, 1141, 1085 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 5.78 (s, 1H), 2.72-2.65 (m, 1H), 1.39 (quint, J = 6.9 Hz, 1H), 1.16 (d, J = 7.2 Hz, 3H), 1.07 (dd, J = 7.8, 4.5 Hz, 1H), 1.01 (d, J = 6.9 Hz, 3H), 0.91 (d, J = 6.9 Hz, 3H), 0.86 (dd, J = 8.1, 4.8 Hz, 1H), 0.33 (t, J = 4.5 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 150.5, 120.7, 116.4, (q, J_{CF} = 319 Hz), 41.7, 35.9, 30.8, 23.9, 21.5, 20.6, 20.5, 19.1, 37.6. Anal. Calcd for $C_{11}H_{15}F_3O_3S$: C, 45.46; H, 2.67; F, 21.57. Found: C, 45.34; H, 2.81; F, 21.53; HR-EIMS calcd for $C_{11}H_{15}F_3O_3S$ (M^+) 284.0694, found 284.0709.

4.2.13. (2*S*,4*aR*)-1,2,3,4,4a,5-Hexahydro-1,1,5,5-tetramethyl-2,4a-methanonaphthalen-7-yl trifluoromethanesulfonate (8m)

According to general procedure (GP-A), **8m** (469 mg, 83%) was obtained as a yellow oil from *i*-Pr_2NH (247 μL, 1.76 mmol), *n*-BuLi (1.14 mL, 1.55 M in *n*-hexane, 1.76 mmol), (−)-isolongifolen-9-one (350 mg, 1.60 mmol), $PhNTf_2$ (859 mg, 2.41 mmol). Eluent: hexane. $[\alpha]_D^{29}$ −290 (*c* 0.980, MeOH); IR 1662, 1613, 1417, 1142 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 5.40 (d, J = 2.1 Hz, 1H), 5.19 (d, J = 2.1 Hz, 1H), 1.93 (t, J = 2.1 Hz, 1H), 1.79-1.74 (dq, J = 7.8, 2.1 Hz, 1H), 1.72-1.60 (m, 2H), 1.55-1.43 (m, 1H), 1.28 (dd, J = 6.9, 0.6 Hz, 1H), 1.19-1.10 (m, 1H), 1.13 (s, 3H), 1.12 (s, 3H), 1.05 (s, 3H), 0.99 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.4, 146.2, 121.9, 118.6 (q, J_{CF} = 319 Hz), 106.4, 56.7, 46.6, 43.7, 35.5, 34.1, 28.6, 27.1, 24.4, 24.3, 24.0, 23.9. Anal. Calcd for $C_{16}H_{21}F_3O_3S$: C, 54.84; H, 6.04; F, 16.27. Found: C, 55.55; H, 6.09; F, 16.13; HR-EIMS calcd for $C_{16}H_{21}F_3O_3S$ (M^+) 350.1163, found 350.1159.

4.2.14. (4*R*,4*aS*,6*R*)-4,4a,5,6,7,8-Hexahydro-4,4a-dimethyl-6-(1-methyl)ethenylnaphthalen-2-yl trifluoromethanesulfonate (8n)

According to general procedure (GP-A), **8n** (1.08 g, 84%) was obtained as a colorless oil from *i*-Pr_2NH (565 μL, 4.03 mmol), *n*-BuLi (2.44 mL, 1.65 M in *n*-hexane, 4.03 mmol), (+)-nootkatone (800 mg, 3.66 mmol), $PhNTf_2$ (1.96 g, 5.50 mmol). Eluent: hexane. $[\alpha]_D^{28}$ +152 (*c* 1.14, MeOH); IR 1655, 1607, 1418, 1142 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 5.53 (s, 1H), 5.27 (t, J = 2.4 Hz, 1H), 4.74-4.72 (m, 2H), 2.59-2.52 (m, 1H), 2.40-2.32 (m, 2H), 2.17 (tt, J = 12.3, 3.0 Hz, 1H), 1.92 (dt, J = 12.9, 2.4 Hz, 1H), 1.87-1.79 (m, 1H), 1.74 (t, J = 1.2 Hz, 3H), 1.36-1.09 (m, 2H), 1.07 (d, J = 7.8 Hz, 3H), 0.90 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 152.1, 149.4, 144.7, 118.6 (q, J_{CF} = 319 Hz), 117.8, 114.2, 109.0, 45.2, 41.4, 41.0, 38.9, 31.4, 30.8, 20.8, 14.4, 13.5. Anal. Calcd for $C_{16}H_{21}F_3O_3S$: C, 54.84; H, 6.04; F, 16.27. Found: C, 54.78; H, 6.31; F, 16.07; HR-EIMS calcd for $C_{16}H_{21}F_3O_3S$ (M^+) 350.1163, found 350.1178.

4.3. General procedure for the Stille coupling

4.3.1. Method A (GP-B-1)

A mixture of the vinyl triflate **8** (1.1 equiv), stannanyl ester **9** or **10** (1.0 equiv), $Pd_2(dba)_3{\cdot}CHCl_3$ (4 mol %), and $AsPh_3$ (16 mol %) was dissolved in dry DMF (0.1 M), then CsF (2.0 equiv) was added. The flask was evacuated and refilled with argon five times. The mixture was stirred at 45 °C for the required time, then cooled to room temperature, quenched with water, and extracted with Et_2O. The organic phase was dried over Na_2SO_4, filtered, and evaporated in vacuo. The residue was purified by column chromatography using neutralized SiO_2/powdered KF (9:1) to give the coupling product.

4.3.2. Method B (GP-B-2)

According to Baldwin's procedure,[12] a mixture of the vinyl triflate **8** (1.0 equiv) and the stannanyl ester **9** or **10** (1.3 equiv) was dissolved in dry DMF (0.1 M), then CsF (2.0 equiv), $Pd(PPh_3)_4$ (10 mol %), and CuI (20 mol %) were added. The flask was evacuated and refilled with argon five times. The mixture was stirred at 45 °C for the required time, then cooled to room temperature, and diluted with CH_2Cl_2 and water. After vigorous stirring, the mixture was filtered through Celite with CH_2Cl_2/AcOEt (1:1). The organic layer was separated, dried over Na_2SO_4, filtered, and evaporated in vacuo. The residue was purified by column chromatography using neutralized SiO_2/powdered KF (9:1) to give the coupling product.

4.3.3. Ethyl (2*E*,4*E*,6*Z*,8*E*)-3,7-dimethyl-9-((3*S*,6*R*)-3-methyl-6-(1-propen-2-yl)-1-cyclohexen-1-yl)-2,4,6,8-nonatetraenoate (11a)

According to Method A (GP-B-1), **11a** (189 mg, 76%) was obtained as a yellow oil from vinyl triflate **8a** (227 mg, 0.799 mmol), stannanyl ester **9** (360 mg, 0.726 mmol), $Pd_2(dba)_3{\cdot}CHCl_3$ (30.0 mg, 29.0 μmol), $AsPh_3$ (28.2 mg, 0.116 mmol), and CsF (221 mg, 1.45 mmol). Eluent: hexane/Et_2O = 40:1. $[\alpha]_D^{24}$ −87.7 (*c* 1.53, $CHCl_3$); IR 2960, 1698, 1610 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.05 (dd, J = 15.0, 11.4 Hz, 1H), 6.88 (d, J = 15.9 Hz, 1H), 6.23 (d, J = 15.9 Hz, 1H), 6.19 (d, J = 15.0 Hz, 1H), 6.01 (d, J = 11.4 Hz, 1H), 5.89 (d, J = 3.0 Hz, 1H), 5.75 (s, 1H), 4.89-4.88 (m, 1H), 4.80-4.79 (m, 1H), 4.17 (q, J = 6.9 Hz, 2H), 3.14-3.11 (m, 1H), 2.32 (d, J = 0.9 Hz, 3H), 2.33-2.32 (m, 1H), 1.94 (s, 3H), 1.84-1.79 (m, 2H), 1.73 (s, 3H), 1.56-1.53 (m, 1H), 1.29 (t, J = 6.9 Hz, 3H), 1.22-1.14 (m, 1H), 1.03 (d, J = 7.2 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 167.2, 152.7, 148.1, 139.6, 138.5, 136.7, 134.1, 133.6, 130.0, 128.3, 123.1, 118.6, 112.0, 59.6, 43.2, 30.6, 27.6, 26.3, 21.2, 20.4, 20.3, 14.3, 13.6; HR-EIMS calcd for $C_{23}H_{32}O_2$ (M^+) 340.2402, found 340.2429.

4.3.4. Ethyl (2*E*,4*E*,6*Z*,8*E*)-3,7-dimethyl-9-((3*R*,6*S*)-3-methyl-6-(1-propen-2-yl)-1-cyclohexen-1-ly)-2,4,6,8-nonatetraenoate (11b)

According to Method A (GP-B-1), **11b** (151 mg, 67%) was obtained as a yellow oil from vinyl triflate **8b** (208 mg, 0.730 mmol), stannanyl ester **9** (329 mg, 0.664 mmol), $Pd_2(dba)_3{\cdot}CHCl_3$ (27.5 mg, 26.6 μmol), $AsPh_3$ (32.5 mg, 106 μmol), and CsF (203 mg, 1.33 mmol). Eluent: hexane/Et_2O = 40:1. $[\alpha]_D^{25}$ +98.7 (*c* 1.50, MeOH); HR-EIMS calcd for $C_{23}H_{32}O_2$ (M^+) 340.2402, found 340.2428.

4.3.5. Ethyl (2*E*,4*E*,6*Z*,8*E*)-3,7-dimethyl-9-((3*R*,6*S*)-6-isopropyl-3-methyl-1-cyclohexen-1-yl)-2,4,6,8-nonatetraenoate (11c)

According to Method A (GP-B-1), **11c** (47.3 mg, 75%) was obtained as a yellow oil from vinyl triflate **8c** (58.1 mg, 0.203 mmol), stannanyl ester **9** (91.3 mg, 0.184 mmol), $Pd_2(dba)_3{\cdot}CHCl_3$ (7.6 mg, 7.36 μmol), $AsPh_3$ (9.02 mg, 29.4 μmol), and CsF (55.9 mg, 0.368 mmol). Eluent: hexane/Et_2O = 40:1. $[\alpha]_D^{26}$ −102 (*c* 1.09, $CHCl_3$); IR 2960, 1698, 1609 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.11 (dd, *J* = 15.0, 11.4 Hz, 1H), 6.80 (d, *J* = 15.6 Hz, 1H), 6.26 (d, *J* = 15.6 Hz, 1H), 6.22 (d, *J* = 15.0 Hz, 1H), 6.04 (d, *J* = 11.4 Hz, 1H), 5.82 (s, 1H), 5.77 (s, 1H), 4.17 (q, *J* = 7.2 Hz, 2H), 2.44-2.42 (m, 1H), 2.35 (d, *J* = 1.2 Hz, 3H), 2.23-2.06 (m, 2H), 1.97 (s, 3H), 1.85-1.67 (m, 2H), 1.54-1.43 (m, 1H), 1.29 (t, *J* = 7.2 Hz, 3H), 1.14-1.06 (m, 1H), 1.01 (d, *J* = 7.2 Hz, 3H), 0.98 (d, *J* = 6.9 Hz, 3H), 0.73 (d, *J* = 6.9 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 167.1, 152.6, 139.1, 138.3, 137.6, 134.3, 133.9, 129.7, 128.1, 122.7, 118.6, 59.6, 40.0, 30.8, 29.6, 29.5, 21.8, 21.3, 21.0, 20.8, 16.9, 14.3, 13.7; HR-EIMS calcd for $C_{23}H_{34}O_2$ (M^+) 342.2559, found 342.2570.

4.3.6. Ethyl (2*E*,4*E*,6*Z*,8*E*)-3,7-dimethyl-9-((3*R*,6*S*)-6-isopropyl-3-methyl-1-cyclohexen-1-yl)-2,4,6,8-nonatetraenoate (11d)

According to Method A (GP-B-1), **11d** (118 mg, 70%) was obtained as a yellow oil from vinyl triflate **8d** (155 mg, 0.540 mmol), stannanyl ester **9** (243 mg, 0.491 mmol), $Pd_2(dba)_3{\cdot}CHCl_3$ (20.3 mg, 19.6 μmol), $AsPh_3$ (24.1 mg, 78.6 μmol), and CsF (149 mg, 0.982 mmol). Eluent: hexane/Et_2O = 40:1. $[\alpha]_D^{26}$ +97.8 (*c* 1.38, MeOH); HR-EIMS calcd for $C_{23}H_{34}O_2$ (M^+) 342.2559, found 342.2567.

4.3.7. Ethyl (2*E*,4*E*,6*Z*,8*E*)-3,7-dimethyl-9-((*S*)-3-methyl-6-(propan-2-ylidene)-1-cyclohexen-1-yl)-2,4,6,8-nonatetraenoate (11e)

According to Method A (GP-B-1), **11e** (183 mg, 75%) was obtained as a yellow oil from vinyl triflate **8e** (222 mg, 0.781 mmol), stannanyl ester **9** (356 mg, 0.710 mmol), $Pd_2(dba)_3{\cdot}CHCl_3$ (29.4 mg, 28.4 μmol), $AsPh_3$ (34.8 mg, 114 μmol), and CsF (216 mg, 1.42 mmol). Eluent: hexane/Et_2O = 40:1. $[\alpha]_D^{24}$ −16.5 (*c* 1.76, $CHCl_3$); IR 2926, 1699, 1609 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.10 (dd, *J* = 15.0, 11.4 Hz, 1H), 6.79 (d, *J* = 15.9 Hz, 1H), 6.34 (d, *J* = 15.9 Hz, 1H), 6.21 (d, *J* = 15.0 Hz, 1H), 6.03 (d, *J* = 11.4 Hz, 1H), 5.79 (d, *J* = 3.6 Hz, 1H), 5.76 (s, 1H), 4.17 (q, *J* = 7.2 Hz, 2H), 2.39-2.36 (m, 1H), 2.34 (d, *J* = 1.2 Hz, 3H), 2.27-2.17 (m, 1H), 1.98 (s, 3H), 1.95-1.85 (m, 2H), 1.78 (s, 3H), 1.71 (s, 3H), 1.31-1.28 (m, 1H), 1.28 (t, *J* = 7.2 Hz, 3H), 1.07 (d, *J* = 7.2 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 167.2, 152.7, 138.5, 138.3, 137.0, 134.3, 134.0, 129.8, 129.2, 128.1, 127.1, 123.3, 118.5, 59.6, 32.4, 31.7, 27.6, 24.4, 21.4, 21.2, 20.8, 14.3, 13.7; HR-EIMS calcd for $C_{23}H_{32}O_2$ (M^+) 340.2402, found 340.2411.

4.3.8. Ethyl (2*E*,4*E*,6*Z*,8*E*)-3,7-dimethyl-9-((*R*)-3-methyl-6-(propan-2-ylidene)-1-cyclohexen-1-yl)-2,4,6,8-nonatetraenoate (11f)

According to Method A (GP-B-1), **11f** (183 mg, 75%) was obtained as a yellow oil from vinyl triflate **8f** (222 mg, 0.781 mmol), stannanyl ester **9** (356 mg, 0.710 mmol), $Pd_2(dba)_3{\cdot}CHCl_3$ (29.4 mg, 28.4 μmol), $AsPh_3$ (34.8 mg, 114 μmol), and CsF (216 mg, 1.42 mmol). Eluent: hexane/Et_2O = 40:1. $[\alpha]_D^{24}$ +30.6 (*c* 1.80, $CHCl_3$); HR-EIMS calcd for $C_{23}H_{32}O_2$ (M^+) 340.2402, found 340.2419.

4.3.9. Ethyl (2*E*,4*E*,6*Z*,8*E*)-3,7-dimethyl-9-((1*S*,4*S*)-1,7,7-trimethylbicyclo[2.2.1]hept-2-en-2-yl)-2,4,6,8-nonatetraenoate (11g)

According to Method B (GP-B-2), **15g** (110 mg, 61%) was obtained as a yellow oil from vinyl triflate **8g** (150 mg, 0.530 mmol), stannanyl ester **9** (340 mg, 0.686 mmol), CsF (160 mg, 1.06 mmol), $Pd(PPh_3)_4$ (61.0 mg, 52.8 μmol), and CuI (20.1 mg, 106 μmol). Eluent: hexane/Et_2O = 40:1. $[\alpha]_D^{24}$ +161 (*c* 1.10, $CHCl_3$); IR 2959, 1697, 1605 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.12 (dd, *J* = 15.3, 11.7 Hz, 1H), 7.09 (d, *J* = 16.2 Hz, 1H), 6.23 (d, *J* = 15.6 Hz, 3H), 6.05 (d, *J* = 11.7 Hz, 1H), 5.77 (s, 1H), 4.17 (q, *J* = 7.2 Hz, 2H), 2.36 (d, *J* = 0.9 Hz, 3H), 2.36-2.32 (m, 1H), 1.97 (s, 3H), 1.95-1.88 (m, 1H), 1.62-1.56 (m, 1H) 1.29 (t, *J* = 6.9 Hz, 3H), 1.17 (s, 3H), 1.10-0.96 (m, 2H), 0.81 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 167.2, 152.7, 147.4, 138.3, 134.5, 133.0, 129.6, 128.6, 125.9, 123.7, 118.6, 59.6, 56.7, 54.0, 51.7, 31.6, 25.4, 20.6, 19.6, 19.5, 14.3, 13.8, 12.6; HR-EIMS calcd for $C_{23}H_{32}O_2$ (M^+) 340.2402, found 340.2382.

4.3.10. Ethyl (2*E*,4*E*,6*Z*,8*E*)-3,7-dimethyl-9-((1*R*,4*R*)-1,7,7-trimethylbicyclo[2.2.1]hept-2-en-2-yl)-2,4,6,8-nonatetraenoate (11h)

According to Method B (GP-B-2), **11h** (120 mg, 70%) was obtained as a yellow oil from vinyl triflate **8h** (143 mg, 0.503 mmol), stannanyl ester **9** (324 mg, 0.654 mmol), CsF (153 mg, 1.01 mmol), $Pd(PPh_3)_4$ (58.1 mg, 50.3 μmol), and CuI (19.2 mg, 101 μmol). Eluent: hexane/Et_2O = 40:1. $[\alpha]_D^{24}$ −180 (*c* 1.03, $CHCl_3$); HR-EIMS calcd for $C_{23}H_{32}O_2$ (M^+) 340.2402, found 340.2397.

4.3.11. Ethyl (2*E*,4*E*,6*Z*,8*E*)-3,7-dimethyl-9-((1*R*,4*S*,5*S*)-4,6,6-trimethylbicyclo[3.1.1]hept-2-en-3-yl)-2,4,6,8-nonatetraenoate (11i)

According to Method A (GP-B-1), **11i** (225 mg, 73%) was obtained as an inseparable mixture of its isomer from vinyl triflate **8i** (281 mg, 0.991 mmol), stannanyl ester **9** (446 mg, 0.901 mmol), $Pd_2(dba)_3{\cdot}CHCl_3$ (37.3 mg, 36.0 μmol), $AsPh_3$ (44.2 mg, 0.144 mmol), and CsF (274 mg, 1.80 mmol). Eluent: hexane/Et_2O = 40:1. Here the optical rotation was not shown due to the contamination of isomer of **11i**. IR 2941, 1697, 1608 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.11 (dd, *J* = 15.3, 11.7 Hz, 1H), 6.79 (d, *J* = 15.9 Hz, 1H), 6.40 (d, *J* = 6.3 Hz, 1H), 6.34 (d, *J* = 15.9 Hz, 1H), 6.22 (d, *J* = 15.3 Hz, 1H), 6.05 (d, *J* = 11.7 Hz, 1H), 5.77 (s, 1H), 4.17 (q, *J* = 7.2 Hz, 2H), 2.99-2.96 (m, 1H), 2.48 (dt, *J* = 8.4, 5.4 Hz, 1H), 2.35 (s, 3H), 2.25 (q, *J* = 8.7 Hz, 1H), 2.19 (td, *J* = 8.7, 2.4 Hz, 1H), 2.00 (s, 3H), 1.33 (d, *J* = 7.5 Hz, 3H), 1.32 (s, 3H), 1.29 (d, *J* = 7.2 Hz, 3H), 1.21 (d, *J* = 8.7 Hz, 1H), 1.01 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 167.2, 152.7, 139.2, 138.4, 134.3, 132.0, 129.6, 128.1, 122.4, 118.5, 59.6, 48.6, 43.0, 39.5, 38.5, 34.4, 27.4, 24.2, 20.8, 18.2, 14.3, 13.8; HR-EIMS calcd for $C_{23}H_{32}O_2$ (M^+) 340.2420, found 340.2414.

4.3.12. Ethyl (2*E*,4*E*,6*Z*,8*E*)-3,7-dimethyl-9-((1*S*,4*R*,5*R*)-4,6,6-trimethylbicyclo[3.1.1]hept-2-en-3-yl)-2,4,6,8-nonatetraenoate (11j)

According to Method A (GP-B-1), **11j** (231 mg, 74%) was obtained as an inseparable mixture of its isomer from vinyl triflate **8j** (289 mg, 1.02 mmol), stannanyl ester **9** (457 mg, 0.923 mmol), $Pd_2(dba)_3{\cdot}CHCl_3$ (38.3 mg, 37.3 μmol), $AsPh_3$ (45.2 mg, 0.148 mmol), and CsF (280 mg, 2.03 mmol). Eluent: hexane/Et_2O = 40:1. Here the optical rotation was not shown due to the contamination of isomer of **11j**. HR-EIMS calcd for $C_{23}H_{32}O_2$ (M^+) 340.2420, found 340.2420.

4.3.13. Ethyl (2*E*,4*E*,6*Z*,8*E*)-3,7-dimethyl-9-((1*R*,5*S*)-6,6-dimethylbicyclo[3.1.1]hept-2-en-2-yl)-2,4,6,8-nonatetraenoate (11k)

According to Method B (GP-B-2), **11k** (52.7 mg, 73%) was obtained as a yellow oil from vinyl triflate **8k** (59.5 mg, 0.220 mmol), stannanyl ester **9** (142 mg, 0.286 mmol), CsF (66.9 mg, 0.440 mmol), $Pd(PPh_3)_4$ (25.4 mg, 22.0 μmol), and CuI (8.4 mg, 44.0 μmol). Eluent: hexane/Et_2O = 40:1. ^1H NMR (300 MHz, $CDCl_3$) δ 7.10 (dd, *J* = 14.7, 11.4 Hz, 1H), 6.72 (d, *J* = 15.9 Hz, 1H), 6.39 (d,

J = 15.9 Hz, 1H), 6.22 (d, J = 14.7 Hz, 1H), 6.05 (d, J = 11.4 Hz, 1H), 5.77 (s, 1H), 5.73 (s, 1H), 4.17 (q, J = 7.2 Hz, 2H), 2.67 (br t, J = 4.2 Hz, 1H), 2.51 (dt, J = 8.7, 5.7 Hz, 1H), 2.42-2.39 (m, 2H), 2.38 (d, J = 1.2 Hz, 3H), 2.17-2.15 (m, 1H), 1.99 (s, 3H), 1.38 (s, 3H), 1.29 (t, J = 7.2 Hz, 3H), 1.18 (d, J = 8.7 Hz, 1H), 0.83 (s, 3H). The ^{1}H NMR spectral data of this compound was identical with that of literature.[9]

4.3.14. Ethyl (2*E*,4*E*,6*Z*,8*E*)-3,7-dimethyl-9-((1*S*,4*R*,5*R*)-1-isopropyl-4-methylbicyclo[3.1.0]hex-2-en-3-ly)-2,4,6,8-nonatetraenoate (11l)

According to Method A (GP-B-1), **11l** (130 mg, 66%) was obtained as a yellow oil from vinyl triflate **8l** (180 mg, 0.633 mmol), stannanyl ester **9** (285 mg, 0.576 mmol), $Pd_2(dba)_3{\cdot}CHCl_3$ (23.8 mg, 23.0 μmol), $AsPh_3$ (28.2 mg, 922 μmol), and CsF (175 mg, 1.15 mmol). Eluent: hexane/Et_2O = 40:1. $[\alpha]_D^{25}$ +27.7 (c 1.30, MeOH); IR 2960, 1698, 1608 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.05 (dd, J = 15.0, 11.7 Hz, 1H), 6.68 (d, J = 15.9 Hz, 1H), 6.34 (d, J = 15.9 Hz, 1H), 6.20 (d, J = 15.0 Hz, 1H), 6.02 (d, J = 11.7 Hz, 1H), 5.96 (s, 1H), 5.75 (s, 1H), 4.16 (q, J = 7.2 Hz, 2H), 2.89 (q, J = 7.2 Hz, 1H), 2.35 (s, 3H), 1.93 (s, 3H), 1.44 (quint, J = 6.6 Hz 1H), 1.27 (t, J = 7.2 Hz, 3H) 1.20 (d, J = 6.9 Hz, 3H), 1.17-1,13 (m, 1H), 1.03 (d, J = 6.9 Hz, 3H), 0.92 (d, J = 6.9 Hz, 3H), 0.80 (dd, J = 8.1, 3.9 Hz, 1H), 0.13-0.11 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 167.0, 152.5, 144.5, 138.1, 136.7, 134.5, 129.4, 128.4, 127.6, 123.7, 118.6, 59.5, 41.2, 41.1, 30.6, 27.8, 22.3, 21.8, 21.0, 20.8, 20.6, 14.3, 13.7; HR-EIMS calcd for $C_{23}H_{32}O_2$ (M^+) 340.2402, found 340.2424.

4.3.15. Ethyl (2*E*,4*E*,6*Z*)-3-methyl-7-((3*R*,6*S*)-6-(1-methylethyl)-3-methyl-1-cyclohexen-1-yl)- 2,4,6-octatrienoate (12d)

According to Method A (GP-B-1), **12d** (243 mg, 77%) was obtained as a yellow oil from vinyl triflate **8d** (310 mg, 1.08 mmol), stannanyl ester **10** (465 mg, 0.991 mmol), $Pd_2(dba)_3{\cdot}CHCl_3$ (41.0 mg, 39.6 μmol), $AsPh_3$ (48.5 mg, 0.158 mmol), and CsF (300 mg, 1.97 mmol). Eluent: hexane/Et_2O = 30:1. $[\alpha]_D^{26}$ +215 (c 0.858, MeOH); IR 1698, 1600 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.98 (dd, J = 15.3, 11.1 Hz, 1H), 6.15 (d, J = 15.3 Hz, 1H), 5.97 (d, J = 10.8 Hz, 1H), 5.72 (s, 1H), 5.42 (s, 1H), 4.15 (q, J = 7.2 Hz, 2H), 2.34-2.18 (m, 2H), 2.27 (d, J = 1.2 Hz, 3H), 1.88 (s, 3H), 1.88-1.69 (m, 3H), 1.47-1.34 (m, 1H), 1.27 (t, J = 7.2 Hz, 3H), 1.12-0.99 (m, 1H), 0.96 (d, J = 7.2 Hz, 3H), 0.90 (d, J = 7.2 Hz, 3H), 0.67 (d, J = 6.6 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 167.3, 153.3, 146.8, 141.1, 135.1, 133.1, 132.7, 126.3, 117.6, 59.5, 41.7, 31.2, 30.8, 28.9, 23.9, 22.0, 21.20, 21.16, 16.6, 14.3, 13.8; HR-EIMS calcd for $C_{21}H_{32}O_2$ (M^+) 316.2402, found 316.2420.

4.3.16. Ethyl (2*E*,4*E*,6*Z*)-3-methyl-7-((1*R*,4*R*)-1,7,7-trimethylbicyclo[2.2.1]hept-2-en-2-yl)-2,4,6-octatrienoate (12h)

According to Method B (GP-B-2), **12h** (81.6 mg, 76%) was obtained as a yellow oil from vinyl triflate **8h** (98.0 mg, 0.345 mmol), stannanyl ester **10** (210 mg, 0.448 mmol), CsF (300 mg, 1.97 mmol), $Pd(PPh_3)_4$ (39.9 mg, 34.5 μmol), and CuI (13.1 mg, 68.9 μmol). Eluent: hexane/Et_2O = 40:1. $[\alpha]_D^{26}$ −185 (c 1.63, MeOH); IR 1698, 1600 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.91 (dd, J = 15.0, 11.0 Hz, 1H), 6.16 (d, J = 15.0 Hz, 1H), 6.07 (d, J = 11.0 Hz, 1H), 5.76 (d, J = 3.5 Hz, 1H), 5.71 (s, 1H), 4.15 (q, J = 7.0 Hz, 2H), 2.38 (t, J = 3.5 Hz, 1H), 2.24 (d, J = 1.0 Hz, 3H), 1.94-1.89 (m, 1H), 1.91 (s, 3H), 1.60 (ddd, J = 12.0, 8.5, 3.5 Hz, 1H), 1.28 (t, J = 7.0 Hz, 3H), 1.20 (ddd, J = 12.5, 9.0, 3.5 Hz, 1H), 1.07 (ddd, J = 12.0, 9.0, 3.5 Hz, 1H), 1.01 (s, 3H), 0.93 (s, 3H), 0.80 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 167.3, 153.3, 148.8, 141.9, 133.3, 133.03, 132.97, 128.0, 117.8, 59.5, 56.9, 55.8, 51.9, 31.5, 25.5, 23.9, 19.78, 19.76, 14.3, 13.7, 12.2; HR-EIMS calcd for $C_{21}H_{30}O_2$ (M^+) 314.2246, found 314.2242.

4.3.17. Ethyl (2*E*,4*E*,6*Z*)-3-methyl-7-((2*S*,4*aR*)-1,2,3,4,4a,5-hexahydro-1,1,5,5-tetramethyl-2,4a-methanonaphthalen-7-yl)-2,4,6-octatrienoate (12m)

According to Method A (GP-B-1), **12m** (42.3 mg, 75%) was obtained as a yellow oil from vinyl triflate **8m** (56.9 mg, 0.162 mmol), stannanyl ester **10** (69.3 mg, 0.148 mmol), $Pd_2(dba)_3{\cdot}CHCl_3$ (6.1 mg, 5.91 μmol), $AsPh_3$ (7.2 mg, 23.6 μmol), and CsF (44.9 mg, 0.295 mmol). Eluent: hexane/Et_2O = 40:1. $[\alpha]_D^{27}$ −164 (c 1.04, MeOH); IR 1698, 1603 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.90 (dd, J = 15.3, 11.1 Hz, 1H), 6.14 (d, J = 15.3 Hz, 1H), 6.00 (d, J = 11.1 Hz, 1H), 5.72 (s, 1H), 5.42 (d, J = 0.6 Hz, 1H), 5.11 (d, J = 1.2 Hz, 1H), 4.15 (q, J = 7.2 Hz, 2H), 2.24 (d, J = 0.9 Hz, 3H), 1.95-1.89 (m, 1H), 1.93 (s, 3H), 1.79-1.75 (m, 1H), 1.70-1.62 (m, 2H), 1.54-1.46 (m, 1H), 1.30-1.25 (m, 4H), 1.16-1.06 (m, 1H), 1.13 (s, 3H), 1.09 (s, 3H), 1.04 (s, 3H), 0.97 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 167.3, 159.0, 153.2, 144.7, 134.0, 133.44, 133.40, 132.6, 126.0, 117.8, 111.1, 59.5, 56.0, 46.6, 43.2, 35.8, 33.7, 28.9, 27.5, 24.9, 24.8, 24.3, 24.2, 24.0, 14.3, 13.6. Anal. Calcd for $C_{26}H_{36}O_2$: C, 82.06; H, 9.53. Found: C, 81.80; H, 9.70; HR-EIMS calcd for $C_{26}H_{36}O_2$ (M^+) 380.2715, found 380.2727.

4.3.18. Ethyl (2*E*,4*E*,6*Z*)-7-((4*R*,4*aS*,6*R*)-4,4a,5,6,7,8-hexahydro-4,4a-dimethyl-6-(1-methyl)ethenylnaphthalen-2-yl)-3-methyl-2,4,6-octatrienoate (12n)

According to Method B (GP-B-2), **12n** (72.3 mg, 84%) was obtained as a yellow oil from vinyl triflate **8n** (79.0 mg, 0.225 mmol), stannanyl ester **10** (138 mg, 0.293 mmol), CsF (68.5 mg, 0.451 mmol), $Pd(PPh_3)_4$ (26.1 mg, 22.6 μmol), and CuI (8.6 mg, 45.1 μmol). Eluent: hexane/Et_2O = 40:1. $[\alpha]_D^{25}$ +81.9 (c 1.44, MeOH); IR 1698, 1601 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.89 (dd, J = 15.5, 11.0 Hz, 1H), 6.13 (d, J = 15.5 Hz, 1H), 5.99 (d, J = 11.0 Hz, 1H), 5.70 (s, 1H), 5.58 (s, 1H), 5.23 (s, 1H), 4.71 (s, 2H), 4.13 (q, J = 7.0 Hz, 2H), 2.37 (td, J = 7.5, 2.5 Hz, 1H), 2.32 (t, J = 2.5 Hz, 2H), 2.22 (d, J = 1.0 Hz, 3H), 2.16 (tt, J = 12.5, 2.5 Hz, 1H), 1.93-1.90 (m, 1H), 1.91 (s, 3H), 1.80 (dq, J = 12.5, 2.5 Hz, 1H), 1.73 (s, 3H), 1.29-1.24 (m, 1H), 1.26 (t, J = 7.0 Hz, 3H), 1.15 (t, J = 12.5 Hz, 1H), 1.03 (d, J = 7.5 Hz, 3H), 0.87 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 167.2, 153.1, 150.2, 146.1, 143.4, 135.3, 133.1, 132.9, 129.7, 126.2, 119.8, 118.0, 108.6, 59.5, 45.7, 42.2, 41.4, 38.4, 31.6, 31.4, 23.8, 20.9, 14.3 (2C), 13.64, 13.60; HR-EIMS calcd for $C_{26}H_{36}O_2$ (M^+) 380.2715, found 380.2707.

4.4. General procedure for the hydrolysis of ester compounds

4.4.1. Standard conditions for hydrolysis (GP-C-1)

A mixture of an ester (1.0 equiv) and 10% KOH (0.133 M) aqueous solution in EtOH (0.08 M) was heated at 50 °C overnight. After cooling, the reaction mixture was made acidic or neutral by addition of 5% HCl aqueous solution at 0 °C. The mixture was extracted with AcOEt, and the organic layer was washed with brine, dried over Na_2SO_4, filtered, and evaporated in vacuo. The residue was purified by flash column chromatography on silica gel to give the carboxylic acid.

4.4.2. Microwave conditions for hydrolysis (GP-C-2)

A mixture of an ester (1.0 equiv) and 10% KOH (0.133 M) aqueous solution in EtOH (0.08 M) was microwave-irradiated at 130 °C for 3 min. After cooling, the reaction mixture was made acidic or neutral by addition of 5% HCl aqueous solution at 0 °C. The mixture was extracted with AcOEt, and the organic layer was washed with brine, dried over Na_2SO_4, filtered, and evaporated in vacuo. The residue was purified by flash column chromatography on silica gel to give the carboxylic acid.

4.4.3. (2*E*,4*E*,6*Z*,8*E*)-3,7-Dimethyl-9-((3*S*,6*R*)-3-methyl-6-(1-propen-2-yl)-1-cyclohexen-1-yl)-2,4,6,8-nonatetraenoic acid (5a)

According to general procedure (GP-C-1), **5a** (151 mg, 92%) was obtained as yellow crystals from ester **11a** (179 mg, 0.525 mmol),

10% KOH (3.9 mL), 5% HCl (9.2 mL). Eluent: hexane/AcOEt = 3:1. $[\alpha]_D^{23}$ −115 (*c* 0.898, $CHCl_3$); mp: 159-163 °C; UV–vis λ_{max} nm (ε): 342 (31500); IR 3500-2800, 1678, 1608 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.10 (dd, *J* = 15.0, 11.7 Hz, 1H), 6.89 (d, *J* = 15.9 Hz, 1H), 6.25 (d, *J* = 15.9 Hz, 1H), 6.22 (d, *J* = 15.0 Hz, 1H), 6.03 (d, *J* = 11.7 Hz, 1H), 5.90 (d, *J* = 3.3 Hz, 1H), 5.78 (s, 1H), 4.89 (br s, 1H), 4.80 (br s, 1H), 3.13 (br t, *J* = 5.1 Hz, 1H), 2.33 (d, *J* = 0.9 Hz, 3H), 2.35-2.34 (m, 1H), 1.95 (s, 3H), 1.85-1.80 (m, 2H), 1.74 (s, 3H), 1.62-1.53 (m, 1H), 1.26-1.15 (m, 1H), 1.04 (d, *J* = 7.2 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.4, 155.3, 148.1, 139.9, 139.3, 136.7, 133.9, 133.8, 131.0, 128.2, 123.0, 117.6, 112.0, 43.2, 30.6, 27.6, 26.4, 21.3, 20.5, 20.3, 13.8; HR-EIMS calcd for $C_{21}H_{28}O_2$ (M^+) 312.2089, found 312.2093.

4.4.4. (2*E*,4*E*,6*Z*,8*E*)-3,7-Dimethyl-9-((3*R*,6*S*)-3-methyl-6-(1-propen-2-yl)-1-cyclohexen-1-yl)-2,4,6,8-nonatetraenoic acid (5b)

According to general procedure (GP-C-1), **5b** (120 mg, 87%) was obtained as yellow crystals from ester **11b** (150 mg, 0.441 mmol), 10% KOH (3.4 mL), 5% HCl (7.7 mL). Eluent: hexane/AcOEt = 3:1. $[\alpha]_D^{24}$ +120 (*c* 0.934, $CHCl_3$); mp: 165-167 °C; UV–vis λ_{max} nm (ε): 347 (30500); HR-EIMS calcd for $C_{21}H_{28}O_2$ (M^+) 312.2089, found 312.2096.

4.4.5. (2*E*,4*E*,6*Z*,8*E*)-3,7-Dimethyl-9-((3*S*,6*R*)-6-Isoprpoyl-3-methyl-1-cyclohexen-1-yl)-2,4,6,8-nonatetraenoic acid (5c)

According to general procedure (GP-C-1), **5c** (101 mg, 87%) was obtained as yellow crystals from ester **11c** (127 mg, 0.370 mmol), 10% KOH (2.8 mL), 5% HCl (6.5 mL). Eluent: hexane/AcOEt = 3:1. $[\alpha]_D^{26}$ −221 (*c* 0.820, $CHCl_3$); mp: 130-133 °C; UV–vis λ_{max} nm (ε): 341 (32400); IR 3500-2800, 1678, 1607 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.16 (dd, *J* = 15.0, 11.4 Hz, 1H), 6.80 (d, *J* = 15.9 Hz, 1H), 6.28 (d, *J* = 15.9 Hz, 1H), 6.25 (d, *J* = 15.0 Hz, 1H), 6.06 (d, *J* = 11.4 Hz, 1H), 5.84 (br s, 1H), 5.80 (s, 1H), 2.45-2.43 (m, 1H), 2.36 (d, *J* = 0.6 Hz, 3H), 2.22-2.05 (m, 2H), 1.98 (s, 3H), 1.88-1.67 (m, 2H), 1.55-1.43 (m, 1H), 1.14-1.05 (m, 1H), 1.01 (d, *J* = 7.2 Hz, 3H), 0.98 (d, *J* = 6.9 Hz, 3H), 0.74 (d, *J* = 6.9 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.4, 155.2, 139.14, 139.10, 137.9, 134.3, 134.1, 130.6, 128.0, 122.7, 117.7, 40.0, 30.8, 29.6, 29.5, 21.8, 21.3, 21.0, 20.4, 16.9, 14.0; HR-EIMS calcd for $C_{21}H_{32}O_2$ (M^+) 314.2246, found 314.2265.

4.4.6. (2*E*,4*E*,6*Z*,8*E*)-3,7-Dimethyl-9-((3*R*,6*S*)-6-isopropyl-3-methyl-1-cyclohexen-1-yl)-2,4,6,8-nonatetraenoic acid (5d)

According to general procedure (GP-C-1), **5d** (95.0 mg, 50%) was obtained as yellow crystals from ester **11d** (130 mg, 0.380 mmol), 10% KOH (2.9 mL), 5% HCl (6.7 mL). Eluent: hexane/AcOEt = 3:1. $[\alpha]_D^{26}$ +221 (*c* 1.13, $CHCl_3$); mp: 130-133 °C; UV–vis λ_{max} nm (ε): 343 (36200); HR-EIMS calcd for $C_{21}H_{30}O_2$ (M^+) 314.2246, found 314.2225.

4.4.7. (2*E*,4*E*,6*Z*,8*E*)-3,7-Dimethyl-9-((*S*)-3-methyl-6-(propan-2-ylidene)-1-cyclohexen-1-yl)-2,4,6,8-nonatetraenoic acid (5e)

According to general procedure (GP-C-2), **5e** (79.8 mg, 75%) was obtained as yellow crystals from ester **11e** (116 mg, 0.342 mmol), 10% KOH (2.0 mL), 5% HCl (4.7 mL). Eluent: hexane/AcOEt = 3:1. $[\alpha]_D^{24}$ −28.8 (*c* 0.798, $CHCl_3$); mp: 169-171 °C; UV–vis λ_{max} nm (ε): 347 (38000); IR 3500-2800, 1679, 1607 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.15 (dd, *J* = 15.0, 11.4 Hz, 1H), 6.79 (d, *J* = 15.9 Hz, 1H), 6.36 (d, *J* = 15.9 Hz, 1H), 6.24 (d, *J* = 15.0 Hz, 1H), 6.05 (d, *J* = 11.4 Hz, 1H), 5.81 (s, 1H), 5.79 (s, 1H), 2.36 (d, *J* = 0.9 Hz, 3H), 2.36-2.35 (m, 1H), 2.27-2.17 (m, 1H), 1.99 (s, 3H), 1.97-1.85 (m, 2H), 1.78 (s, 3H), 1.71 (s, 3H), 1.35-1.23 (m, 1H), 1.07 (d, *J* = 7.2 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.5, 155.3, 139.3, 138.3, 137.2, 134.3, 134.0, 130.7, 129.2, 128.1, 127.2, 123.3, 117.6, 32.4, 31.7, 27.6, 24.4, 21.4, 21.2, 20.9, 14.0; HR-EIMS calcd for $C_{21}H_{28}O_2$ (M^+) 312.2089, found 312.2077.

4.4.8. (2*E*,4*E*,6*Z*,8*E*)-3,7-Dimethyl-9-((*R*)-3-methyl-6-(propan-2-ylidene)-1-cyclohexen-1-yl)-2,4,6,8-nonatetraenoic acid (5f)

According to general procedure (GP-C-2), **5f** (92.8 mg, 93%) was obtained as yellow crystals from ester **11f** (109 mg, 0.319 mmol), 10% KOH (2.0 mL), 5% HCl (4.3 mL). Eluent: hexane/AcOEt = 3:1. $[\alpha]_D^{24}$ +34.8 (*c* 0.978, $CHCl_3$); mp: 167-169 °C; UV–vis λ_{max} nm (ε): 347 (39900); IR 3500-2800, 1679, 1608 cm^{-1}; HR-EIMS calcd for $C_{21}H_{28}O_2$ (M^+) 312.2089, found 312.2100.

4.4.9. (2*E*,4*E*,6*Z*,8*E*)-3,7-Dimethyl-9-((1*S*,4*S*)-1,7,7-trimethylbicyclo[2.2.1]hept-2-en-2-yl)-2,4,6,8-nonatetraenoic acid (5g)

According to general procedure (GP-C-2), **5g** (55.6 mg, 55%) was obtained as yellow crystals from ester **11g** (110 mg, 0.323 mmol), 10% KOH (2.0 mL), 5% HCl (4.7 mL). Eluent: hexane/AcOEt = 3:1. $[\alpha]_D^{24}$ +196 (*c* 1.39, $CHCl_3$); mp: 180-183 °C; UV–vis λ_{max} nm (ε): 358 (40700); IR 3500-2800, 1679, 1605 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.17 (dd, *J* = 15.0, 11.5 Hz, 1H), 7.09 (d, *J* = 16.0 Hz, 1H), 6.25 (d, *J* = 16.0 Hz, 1H), 6.25 (d, *J* = 15.0 Hz, 1H), 6.21 (d, *J* = 3.5 Hz, 1H), 6.05 (d, *J* = 11.5 Hz, 1H), 5.80 (s, 1H), 2.37 (d, *J* = 1.0 Hz, 3H), 2.36 (t, *J* = 3.5 Hz, 1H), 1.99 (s, 3H), 1.94-1.86 (m, 1H), 1.62-1.57 (m, 1H), 1.17 (s, 3H), 1.09-0.99 (m, 2H), 0.81 (s, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.1, 155.2, 147.4, 139.1, 134.3, 133.2, 130.5, 128.5, 126.2, 123.6, 117.4, 56.8, 54.0, 51.8, 31.6, 25.3, 20.7, 19.7, 19.5 14.0, 12.6; HR-EIMS calcd for $C_{21}H_{28}O_2$ (M^+) 312.2089, found 312.2105.

4.4.10. (2*E*,4*E*,6*Z*,8*E*)-3,7-Dimethyl-9-((1*R*,4*R*)-1,7,7-trimethylbicyclo[2.2.1]hept-2-en-2-yl)-2,4,6,8-nonatetraenoic acid (5h)

According to general procedure (GP-C-2), **5h** (36.0 mg, 75%) was obtained as yellow crystals from ester **11h** (52.0 mg, 0.153 mmol), 10% KOH (0.95 mL), 5% HCl (2.7 mL). Eluent: hexane/AcOEt = 3:1. $[\alpha]_D^{24}$ −220 (*c* 0.900, $CHCl_3$); mp: 180- 183 °C; UV–vis λ_{max} nm (ε): 356 (44100); IR 3500-2800, 1678, 1609 cm^{-1}; HR-EIMS calcd for $C_{21}H_{28}O_2$ (M^+) 312.2089, found 312.2086.

4.4.11. (2*E*,4*E*,6*Z*,8*E*)-3,7-Dimethyl-9-((1*R*,4*S*,5*S*)-4,6,6-trimethylbicyclo[3.1.1]hept-2-en-3-yl)-2,4,6,8-nonatetraenoic acid (5i)

According to general procedure (GP-C-1), **5i** (126 mg, 64%) was obtained as red crystals from ester **11i** (225 mg, 0.631 mmol), 10% KOH (5.0 mL), 5% HCl (11.0 mL). Eluent: hexane/AcOEt = 3:1. $[\alpha]_D^{24}$ +65.4 (*c* 0.872, $CHCl_3$); mp: 170-171 °C; UV–vis λ_{max} nm (ε): 350 (42800); IR 3500-2700, 1678, 1608 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.16 (dd, *J* = 15.0, 11.4 Hz, 1H), 6.80 (d, *J* = 15.9 Hz, 1H), 6.42 (d, *J* = 6.3 Hz, 1H), 6.36 (d, *J* = 15.9 Hz, 1H), 6.25 (d, *J* = 15.0 Hz, 1H), 6.07 (d, *J* = 11.4 Hz, 1H), 5.80 (s, 1H), 2.99-2.97 (m, 1H), 2.48 (dt, *J* = 8.4, 5.4 Hz, 1H), 2.36 (s, 3H), 2.26 (q, *J* = 6.0 Hz, 1H), 2.19 (td, *J* = 8.4, 2.7 Hz, 1H), 2.01 (s, 3H), 1.33 (s, 3H), 1.30 (d, *J* = 7.5 Hz, 3H), 1.23-1.20 (m, 1H), 1.01 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.4, 155.2, 139.4, 139.1, 138.3, 134.1, 132.3, 130.5, 128.1, 122.3, 117.7, 48.5, 43.0, 39.5, 38.5, 34.4, 27.4, 24.2, 20.8, 18.2, 14.0; HR-EIMS calcd for $C_{21}H_{28}O_2$ (M^+) 312.2089, found 312.2091.

4.4.12. (2*E*,4*E*,6*Z*,8*E*)-3,7-Dimethyl-9-((1*S*,4*R*,5*R*)-4,6,6-trimethylbicyclo[3.1.1]hept-2-en-3-yl)-2,4,6,8-nonatetraenoic acid (5j)

According to general procedure (GP-C-1), **5j** (106 mg, 70%) was obtained as red crystals from ester **11j** (165 mg, 0.485 mmol), 10% KOH (3.6 mL), 5% HCl (8.5 mL). Eluent: hexane/AcOEt = 3:1. $[\alpha]_D^{25}$ −60.0 (*c* 1.30, $CHCl_3$); mp: 168-170 °C; UV–vis λ_{max} nm (ε): 349 (45000); IR 3500-2700, 1678, 1608 cm^{-1}; HR-EIMS calcd for $C_{21}H_{28}O_2$ (M^+) 312.2089, found 312.2100.

4.4.13. (2*E*,4*E*,6*Z*,8*E*)-3,7-Dimethyl-9-((1*R*,5*S*)-6,6-dimethylbicyclo[3.1.1]hept-2-en-2-yl)-2,4,6,8-nonatetraenoic acid (5k)

According to general procedure (GP-C-1), **5k** (105 mg, 82%) was obtained as yellow crystals from ester **11k** (140 mg, 0.429 mmol), 10% KOH (3.2 mL), 5% HCl (7.5 mL). Eluent: hexane/AcOEt = 3:1. $[\alpha]_D^{26}$ +131 (*c* 0.832, $CHCl_3$); mp: 175-178 °C; UV–vis λ_{max} nm (ε): 360 (43900); IR 3500-2800, 2960, 1678, 1605 cm^{-1}, ^1H NMR (300 MHz, $CDCl_3$) δ 7.15 (dd, *J* = 15.0, 11.7 Hz, 1H), 6.72 (d, *J* = 15.9 Hz, 1H), 6.40 (d, *J* = 15.9 Hz, 1H), 6.25 (d, *J* = 15.0 Hz, 1H), 6.07 (d, *J* = 11.7 Hz, 1H), 5.80 (s, 1H), 5.74 (br s, 1H), 2.68 (br t, *J* = 4.5 Hz, 1H), 2.51 (dt, *J* = 8.7, 5.7 Hz, 1H), 2.43-2.40 (m, 2H), 2.39 (s, 3H), 2.17-2.15 (m, 1H), 2.00 (s, 3H), 1.39 (s, 3H), 1.18 (d, *J* = 8.7 Hz, 1H), 0.83 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.3, 155.3, 146.9, 138.9, 137.8, 134.4, 132.8, 130.5, 128.6, 127.5, 120.8, 117.6, 41.2, 40.9, 37.9, 32.3, 31.4, 26.4, 21.1, 20.9, 14.2; HR-EIMS calcd for $C_{20}H_{26}O_2$ (M^+) 298.1933, found 298.1952.

4.4.14. (2*E*,4*E*,6*Z*,8*E*)-3,7-Dimethyl-9-((1*S*,4*R*,5*R*)-1-isopropyl-4-methylbicyclo[3.1.0]hex-2-en-3-yl)-2,4,6,8-nonatetraenoic acid (5l)

According to general procedure (GP-C-1), **5l** (109 mg, 92%) was obtained as orange crystals from ester **11l** (130 mg, 0.382 mmol), 10% KOH (3.0 mL), 5% HCl (6.7 mL). Eluent: hexane/AcOEt = 3:1. $[\alpha]_D^{24}$ +128 (*c* 1.06, $CHCl_3$); mp: 161-163 °C; UV–vis λ_{max} nm (ε): 352 (40800); IR 3500-2800, 1678, 1606 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.11 (dd, *J* = 15.0, 11.4 Hz, 1H), 6.69 (d, *J* = 15.9 Hz, 1H), 6.37 (d, *J* = 15.9 Hz, 1H), 6.24 (d, *J* = 15.0 Hz, 1H), 6.05 (d, *J* = 11.4 Hz, 1H), 5.98 (s, 1H), 5.80 (s, 1H), 2.90 (q, *J* = 6.9 Hz, 1H), 2.36 (s, 3H), 1.96 (s, 3H), 1.46 (quint, *J* = 6.6 Hz, 1H), 1.21 (d, *J* = 6.6 Hz, 3H), 1.18-1.15 (m, 1H), 1.04 (d, *J* = 6.9 Hz, 3H), 0.93 (d, *J* = 6.6 Hz 3H), 0.82 (dd, *J* = 7.5, 3.6 Hz, 1H), 0.15-0.12 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.4, 155.2, 144.6, 139.0, 137.1, 134.3, 130.4, 128.4, 128.0, 123.7, 117.8, 41.3, 41.2, 30.6, 27.9, 22.4, 21.9, 21.1, 20.9, 20.8, 14.0; HR-EIMS calcd for $C_{21}H_{28}O_2$ (M^+) 312.2089, found 312.2105.

4.4.15. (2*E*,4*E*,6*Z*)-7-[(3*R*,6*S*)-6-(1-Methylethyl)-3-methylcyclohexen-1-yl]-3-methyl-2,4,6-octatrienoic acid (6d)

According to general procedure (GP-C-1), **6d** (120 mg, 92%) was obtained as a yellow crystals from ester **12d** (143 mg, 0.452 mmol), 10% KOH (3.4 mL), 5% HCl (7.9 mL). Eluent: hexane/AcOEt = 5:1. $[\alpha]_D^{24}$ +229 (*c* 0.700, $CHCl_3$); mp: 128-130 °C; UV–vis λ_{max} nm (ε): 310 (28100); IR 3018, 1677, 1596 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.05 (dd, *J* = 15.0, 10.8 Hz, 1H), 6.18 (d, *J* = 15.6 Hz, 1H), 5.99 (d, *J* = 11.4 Hz, 1H), 5.74 (s, 1H), 5.42 (d, *J* = 1.2 Hz, 1H), 2.33-2.17 (m, 2H), 2.27 (s, 3H), 1.89 (s, 3H), 1.85-1.70 (m, 3H), 1.48-1.35 (m, 1H), 1.13-1.01 (m, 1H), 0.97 (d, *J* = 7.2 Hz, 3H), 0.91 (d, *J* = 7.2 Hz, 3H), 0.68 (d, *J* = 6.9 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.6, 156.0, 147.7, 141.1, 135.3, 134.1, 132.6, 126.3, 116.7, 41.7, 31.2, 30.9, 28.9, 23.9, 22.0, 21.2, 16.6, 14.1; HR-EIMS calcd for $C_{19}H_{28}O_2$ (M^+) 288.2089, found 288.2099.

4.4.16. (2*E*,4*E*,6*Z*)-3-Methyl-7-((1*R*,4*R*)-1,7,7-trimethylbicyclo[2.2.1]hept-2-en-2-yl)-2,4,6-octatrienoic acid (6h)

According to general procedure (GP-C-1), **6h** (97.0 mg, 87%) was obtained as yellow crystals from ester **12h** (123 mg, 0.391 mmol), 10% KOH (2.9 mL), 5% HCl (6.8 mL). Eluent: hexane/AcOEt = 3:1. $[\alpha]_D^{25}$ −217 (*c* 0.728, $CHCl_3$); mp: 150-153 °C; UV–vis λ_{max} nm (ε): 311 (24600); IR 3025, 1682, 1596 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 6.97 (dd, *J* = 15.0, 11.1 Hz, 1H), 6.18 (d, *J* = 15.0 Hz, 1H), 6.08 (d, *J* = 11.1 Hz, 1H), 5.77 (d, *J* = 3.3 Hz, 1H), 5.74 (s, 1H), 2.39 (t, *J* = 3.3 Hz, 1H), 2.24 (d, *J* = 0.9 Hz, 3H), 1.98-1.88 (m, 1H), 1.92 (s, 3H), 1.60 (ddd, *J* = 12.0, 8.4, 3.3 Hz, 1H), 1.20 (ddd, *J* = 12.0, 9.0, 3.3 Hz, 1H), 1.07 (ddd, *J* = 12.0, 9.0, 3.3 Hz, 1H), 1.02 (s, 3H), 0.93 (s, 3H), 0.80 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.5, 155.9, 148.8, 142.7, 134.2, 133.2, 132.7, 128.0, 116.9, 56.9, 55.8, 51.9, 31.5, 25.5, 23.9, 19.8, 19.7, 13.9, 12.2; HR-EIMS calcd for $C_{19}H_{26}O_2$ (M^+) 286.1933, found 286.1939.

4.4.17. (2*E*,4*E*,6*Z*)-7-((2*S*,4*aR*)-1,2,3,4,4a,5-Hexahydro-1,1,5,5-tetramethyl-2,4a-methanonaphthalen-7-yl)-3-methyl-2,4,6-octatrienoic acid (6m)

According to general procedure (GP-C-1), **6m** (140 mg, 98%) was obtained as yellow crystals from ester **12m** (154 mg, 0.405 mmol), 10% KOH (3.0 mL), 5% HCl (7.0 mL). Eluent: hexane/AcOEt = 3:1. $[\alpha]_D^{26}$ −189 (*c* 1.04, $CHCl_3$); mp: 145-148 °C; UV–vis λ_{max} nm (ε): 294 (24000) nm; IR 2961, 1678, 1595 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 6.95 (dd, *J* = 15.3, 11.1 Hz, 1H), 6.17 (d, *J* = 15.3 Hz, 1H), 6.02 (d, *J* = 11.1 Hz, 1H), 5.74 (s, 1H), 5.43 (d, *J* = 0.9 Hz, 1H), 5.12 (d, *J* = 1.2 Hz, 1H), 2.25 (d, *J* = 0.9 Hz, 3H), 1.94 (s, 3H), 1.90-1.89 (m, 1H), 1.80-1.76 (m, 1H), 1.72-1.61 (m, 2H), 1.54-1.43 (m, 1H), 1.30-1.27 (m, 1H), 1.16-1.06 (m, 1H), 1.13 (s, 3H), 1.09 (s, 3H), 1.05 (s, 3H), 0.98 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.6, 159.1, 155.9, 145.6, 134.4, 134.1, 133.6, 132.4, 126.0, 116.9, 111.0, 56.0, 46.6, 43.2, 35.8, 33.7, 28.9, 27.5, 24.9, 24.8, 24.3, 24.2, 24.0, 13.9. Anal. Calcd for $C_{24}H_{32}O_2$: C, 81.77; H, 9.15. Found: C, 81.54; H, 9.31; HR-EIMS calcd for $C_{24}H_{32}O_2$ (M^+) 352.2402, found 352.2422.

4.4.18. (2*E*,4*E*,6*Z*)-7-((4*R*,4*aS*,6*R*)-4,4a,5,6,7,8-Hexahydro-4,4a-dimethyl-6-(1-methyl)ethenylnaphthalen-2-yl)-3-methyl-2,4,6-octatrienoic acid (6n)

According to general procedure (GP-C-1), **6n** (103 mg, 94%) was obtained as yellow crystals from ester **12n** (118 mg, 0.310 mmol), 10% KOH (2.3 mL), 5% HCl (5.4 mL). Eluent: hexane/AcOEt = 3:1. $[\alpha]_D^{23}$ +93.5 (*c* 0.930, $CHCl_3$); mp: 106-110 °C; UV–vis λ_{max} nm (ε): 296 (30000); IR 2966, 1678, 1597 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 6.97 (dd, *J* = 15.0, 11.1 Hz, 1H), 6.18 (d, *J* = 15.0 Hz, 1H), 6.03 (d, *J* = 11.1 Hz, 1H), 5.75 (s, 1H), 5.60 (s, 1H), 5.26 (s, 1H), 4.73 (d, *J* = 0.6 Hz, 1H), 2.44-2.33 (m, 3H), 2.26 (d, *J* = 0.9 Hz, 3H), 2.23-2.14 (m, 1H), 1.96-1.91 (m, 1H), 1.95 (s, 3H), 1.85-1.80 (m, 1H), 1.75 (s, 3H), 1.37-1.25 (m, 1H), 1.17 (t, *J* = 12.6 Hz, 1H), 1.06 (d, *J* = 7.5 Hz, 3H), 0.89 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.5, 155.7, 150.2, 146.2, 144.2, 135.3, 134.1, 132.7, 129.8, 126.2, 119.8, 117.1, 108.6, 45.7, 42.2, 41.4, 38.4, 31.6, 31.4, 23.8, 20.9, 14.3, 13.9, 13.6; HR-EIMS calcd for $C_{24}H_{32}O_2$ (M^+) 352.2402, found 352.2410.

4.5. Transfection and luciferase activity assay (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 the day of transfection. The retinoid-responsive luciferase reporter construct, rat CRBPII-RXRE-SV40-Luc, was generated by cloning three copies of the RXRE from the rat CRBPII promoter (639/605: GCTGTCACAGGTCACAGGTCACAGGTCACAGTTCA) in the pGL3 vector. The pRL-CMV vector was used as an internal control. After transfection using the Tfx-50 reagent, 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 manufacturer's 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 (Toyo Ink Co., Ltd) as a control. Each set of experiments was repeated at least three times, and the results are presented in terms of fold induction as mean ± S.E.

4.6. Transfection and luciferase activity assay (RARE)

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 the day of transfection. The retinoid-responsive luciferase reporter construct, human RARβ-RARE3-SV40-Luc, was generated by cloning three copies of the RARE from the RARβ promoter (59/33: GGGTAAAGTTCACCGAAAGTTCACTCG). The pRL-CMV vector was used an internal control. After transfection using the Tfx-50 reagent, the cells were incubated with retinoid (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 manufacturer's 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 (Toyo Ink Co., Ltd) as a control. Each set of experiments was repeated at least three times, and the results are presented in terms of fold induction as mean ± S.E.

4.7. Transfection and luciferase activity assay (RXRα-GAL4)

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). Cells (2×10^5) were suspended in 2 mL of medium and transfected with 1.0 μg of a one-hybrid plasmid (pM vector, Promega Corp., Madison, WI, USA) containing a human RXRα cDNA linked with a yeast GAL4 DNA-binding domain cDNA (GAL-DBD), 0.5 μg of luciferase reporter plasmid (pGVP2 vector, Toyo Ink Co., Ltd) containing GAL4 binding site (GAL-BS) and pRL-CMV vector as an internal control, using the Tfx-50 reagent (Promega Corp.). The cells were incubated with retinoids (10^{-6} M) for 2 days. The luciferase activities of the cell lysates were measured with a luciferase assay system (Toyo Ink Co., Ltd), according to the manufacturer's instructions. Transactivation measured by luciferase activity was standardized with the luciferase activity of the same cells determined by the Sea Pansy luciferase assay system (Toyo Ink Co., Ltd) as a control. Each set of experiments was repeated at least three times, and the results are presented in terms of fold induction as mean ± S.E.

4.8. Docking study

The crystal structure of human RXRα-ligand binding domain (PDB code: 1FM9) was retrieved from the Brookhaven Protein Data Bank: http://www.rcsb.org/pdb/Welcome.do. Polar hydrogen atoms were added to both the protein and the ligand. United atom Kollman charges were assigned for the protein. The 3D structures of ligands used for the docking study were constructed by using Chem3D Pro 7.0 software [Molecular Modeling and Analysis; Cambridge Soft Corporation, USA]. These ligands were energetically minimized by using Molecular Mechanics (MM). The AutoDock4.0 molecular docking program[15] was employed by using a genetic algorithm with local search (GALS). One hundred individual GA runs, 150 chromosomes, a crossover ratio of 0.80, a rate of gene mutation of 0.02, and an elitism ratio of 0.10 were used for each ligand. Accelrys ViewerLite Version 4.2 [Accelrys Inc., San Diego, CA] was used for molecular modeling.

Acknowledgments

This work was supported by a Grant-in-Aid for Encouragement of Young Scientists from the Ministry of Education, Culture, Sports, Science and Technology and by a grant from the Science Research Promotion Fund of the Japanese Private School Promotion Foundation.

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