Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry 15 (2007) 854–867
Bioorganic & Medicinal Chemistry

Synthesis and biological evaluation of several structural analogs of 2-arachidonoylglycerol, an endogenous cannabinoid receptor ligand

Yoshitomo Suhara,[a] Saori Oka,[b] Atsushi Kittaka,[a] Hiroaki Takayama,[a] Keizo Waku[b] and Takayuki Sugiura[b,*]

[a]*Department of Pharmaceutical Chemistry, Faculty of Pharmcaceutical Sciences, Teikyo University, Sagamihara, Kanagawa 199-0195, Japan*
[b]*Department of Molecular Health Science, Faculty of Pharmcaceutical Sciences, Teikyo University, Sagamihara, Kanagawa 199-0195, Japan*

Received 31 August 2006; revised 19 October 2006; accepted 20 October 2006
Available online 27 October 2006

Abstract—2-Arachidonoylglycerol (2-AG (**1**)) is an endogenous ligand for the cannabinoid receptors (CB1 and CB2). There is growing evidence that 2-arachidonoylglycerol plays important physiological and pathophysiological roles in various mammalian tissues and cells, though the details remain to be clarified. In this study, we synthesized several remarkable analogs of 2-arachidonoylglycerol, closely related in chemical structure to 2-arachidonoylglycerol: an analog containing an isomer of arachidonic acid with migrated olefins (2-AGA118 (**3**)), an analog containing a one-carbon shortened fatty acyl moiety (2-AGA113 (**4**)), an analog containing an one-carbon elongated fatty acyl moiety (2-AGA114 (**5**)), a hydroxy group-containing analog (2-AGA105 (**6**)), a ketone group-containing analog (2-AGA109 (**7**)), and a methylene-linked analog (2-AGA104 (**8**)). We evaluated their biological activities as cannabinoid receptor agonists using NG108-15 cells which express the CB1 receptor and HL-60 cells which express the CB2 receptor. Notably, these structural analogs of 2-arachidonoylglycerol exhibited only weak agonistic activities toward either the CB1 receptor or the CB2 receptor, which is in good contrast to 2-arachidonoylglycerol which acted as a full agonist at these cannabinoid receptors. These results clearly indicate that the structure of 2-arachidonoylglycerol is strictly recognized by the cannabinoid receptors (CB1 and CB2) and provide further evidence that the cannabinoid receptors are primarily the intrinsic receptors for 2-arachidonoylglycerol.

1. Introduction

2-Arachidonoylglycerol (2-AG) (**1**) is an arachidonic acid-containing 2-monoacylglycerol isolated from rat brain[1] and canine gut[2] as an endogenous ligand for the cannabinoid receptors. 2-AG binds to both the CB1 receptor, which is abundantly expressed in the nervous system including the brain, and the CB2 receptor, which is expressed in lymphoid organs such as the spleen, with high affinity and exhibits a variety of biological activities. For example, 2-AG induces the inhibition of adenylyl cyclase,[2] the inhibition of electrically evoked contractions of the mouse vas deferens,[2] a Ca^{2+} transient in neuroblastoma x glioma hybrid NG108-15 cells[3,4] and promyelocytic leukemia HL-60 cells,[5] the inhibition of a depolarization-induced elevation of the intracellular free Ca^{2+} concentration of neuroblastoma x glioma hybrid cells,[6] the inhibition of long-term potentiation in rat hippocampal slices,[7] the inhibition of synaptic transmission in hippocampal neurons,[8] the suppression or potentiation of lymphocyte proliferation,[9] hypotension,[10] and the migration of various types of leukocytes and microglia cells.[11–13] Based on the results of structure–activity relationship experiments, we have proposed that 2-AG rather than anandamide is the true natural ligand for the cannabinoid receptors.[3–5,14,15] Further detailed studies on 2-AG are thus essential for a better understanding of the physiological and pathophysiological roles of the endocannabinoid system in diverse mammalian tissues and cells.

In order to elucidate the pharmacological properties of the receptor molecules, it is indispensable to prepare a variety of structural analogs of endogenous ligands with agonistic and antagonistic activities. Previously, we[4,16] and Mechoulam et al.[10] developed an ether-linked analog of 2-AG (2-AG ether (**2**), HU-310). This compound exhibited appreciable cannabimimetic activities in vitro

* Corresponding author. Tel: +81 42 685 3746; fax: +81 42 685 1345; e-mail: sugiurat@pharm.teikyo-u.ac.jp

0968-0896/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2006.10.049

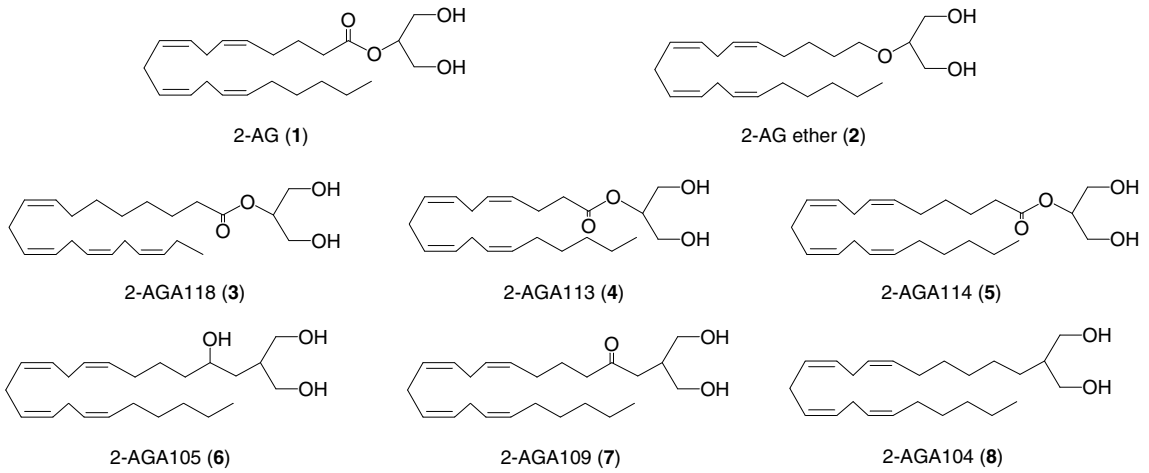


Figure 1. Chemical structures of 2-AG and its structural analogs.

and in vivo. We also synthesized several structural analogs and homologs of 2-AG.[3–5] Among them, (5*Z*,8*Z*,11*Z*)-1,3-dihydroxypropan-2-yl icosa-5,8,11-trienoate (2-eicosatrienoylglycerol) and (5*Z*,8*Z*,11*Z*,14*Z*,17*Z*)-1,3-dihydroxypropan-2-yl icosa-5,8,11,14,17-pentaenoate (2-eicosapentaenoylglycerol) are particularly noteworthy because they possessed appreciable biological activities; these analogs are potentially valuable experimental tools in studies on the functions of the endocannabinoid system. Despite these previous reports, however, information concerning the structural analogs of 2-AG is scarce compared with the case of anandamide.

In this study, we synthesized several remarkable analogs of 2-AG closely related in chemical structure to 2-AG: an analog containing an arachidonic acid isomer with migrated olefins (**3**), an analog containing a one-carbon shortened acyl chain (**4**), an analog containing a one-carbon lengthened acyl chain (**5**), a hydroxy group-containing analog (**6**), a ketone group-containing analog (**7**), and a methylene-linked analog (**8**) (Fig. 1). We evaluated their biological activities as cannabinoid receptor agonists using NG108-15 cells which express the CB1 receptor and HL-60 cells which express the CB2 receptor and compared them with those of 2-AG.

2. Results and discussion

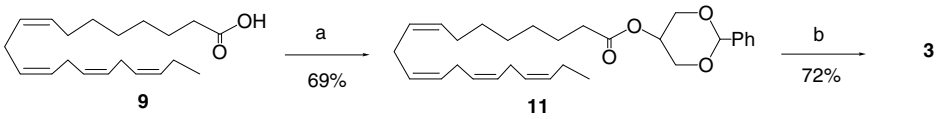
2.1. Chemistry

First, we synthesized (8*Z*,11*Z*,14*Z*,17*Z*)-1,3-dihydroxypropan-2-yl icosa-8,11,14,17-tetraenoate (2-AGA118) (**3**). As shown in Scheme 1, the synthesis of **3** was carried out from commercially available (8*Z*,11*Z*,14*Z*,17*Z*)-icosa-8,11,14,17-tetraenoic acid (**9**) and 1,3-benzylideneglycerol (**10**).[16] Esterification of **9** with alcohol **10** using *N*,*N*-dicyclohexylcarbodiimide (DCC) and 4-*N*,*N*-dimethylaminopyridine (DMAP) in dry toluene at room temperature for 6 h provided ester **11** in 69% yield. Deprotection of the benzylidene group was accomplished by dissolving ester **11** in trimethyl borate at room temperature followed by the addition of boric acid. The reaction mixture was stirred at 100 °C for 30 min, then cooled to room temperature. Chromatography on silica gel gave **3** in 72% yield. Compound **3** was further purified by borate-impregnated TLC to remove any contaminating migrated 1- or 3-isomer.

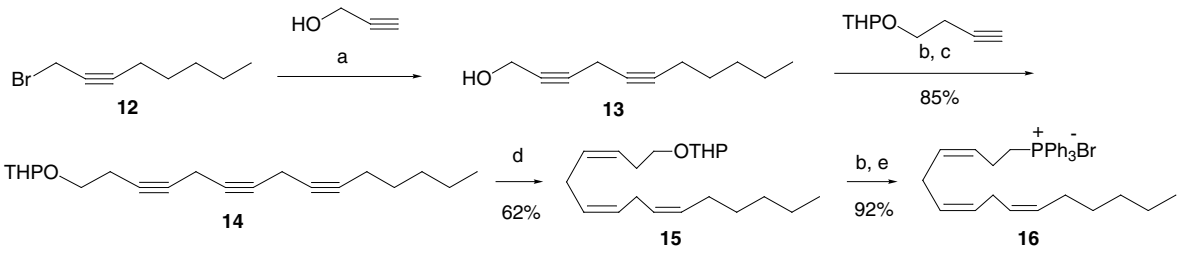
We next synthesized an analog of 2-AG containing a one carbon-shortened fatty acyl moiety (**4**) and another analog containing a one carbon-lengthened fatty acyl moiety (**5**). The synthesis of **4** and **5** is summarized in Schemes 2 and 3. An intermediate phosphonium salt **16**, and aldehyde synthons **19a**, **19b** were prepared and then combined using the Wittig reaction in a convergent and stereoselective manner to obtain the necessary precursors **20a**, **20b**. Chemical modification of these compounds was then successively carried out to give the desired compounds **4**, **5**.

Scheme 2 summarizes the synthesis of the intermediate alkyl chain synthon of **4** and **5**. The common intermediate in the synthesis of all these analogs of **1**, namely 2-[(3*Z*,6*Z*,9*Z*)-pentadeca-3,6,9-trien-1-yl] triphenylphosphonium bromide (**16**), was prepared as follows. Commercially available 2-octyn-1-ol was brominated with phosphorus tribromide and the resulting halide **12** coupled with propargyl alcohol to give undeca-2,5-diyn-1-ol (**13**) by a reported method.[17] Diyne **13** was subsequently halogenated with 1,2-bis(diphenylphosphino)ethane tetrabromide and the resulting bromide was coupled with the tetrahydropyranyl ether of butynyl alcohol to yield the triyne **14** in 85% yield. Triyne **14** was then converted to *cis*-triene by controlled hydrogenation using a Lindlar catalyst. Traces of overreduced or underreduced products were removed by argentation chromatography utilizing 10% silver nitrate on silica gel to give **15** in 62% yield.[17,18] The resulting pure *cis* triene **15** was converted to the bromide using 1,2-bis(diphenylphosphino)ethane tetrabromide in one step and finally treated with triphenylphosphine to afford **16** in 92% yield as a colorless oil.

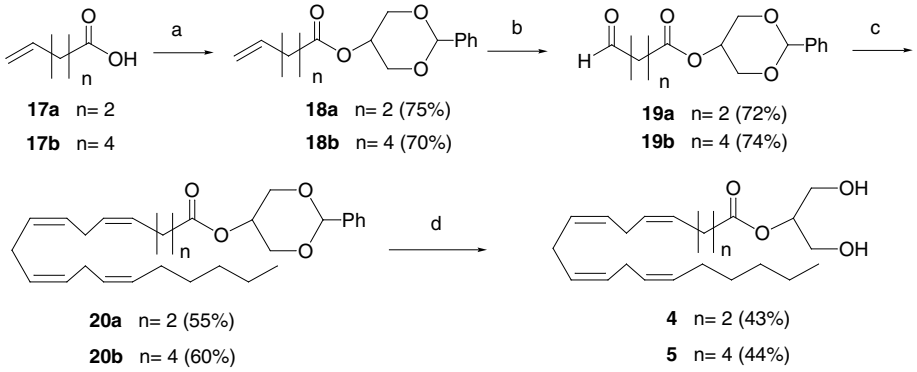
As shown in Scheme 3, we chose pentenoic acid (**17a**) and heptenoic acid (**17b**) as the starting materials to make the aldehyde synthons **19a** and **19b**. After coupling



Scheme 1. Synthesis of 2-AGA118 (**3**). Reagents: (a) 1, 3-benzylideneglycerol (**10**), DCC, DMAP, toluene; (b) boric acid, trimethyl borate.



Scheme 2. Synthesis of an intermediate **16**. Reagents and conditions: (a) EtMgBr, CuI; (b) $Ph_2PCH_2CH_2PPh_2 \cdot 2Br_2$; (c) EtMgBr, CuI; (d) H_2/Lindlar catalyst; (e) Ph_3P, reflux 36 h.



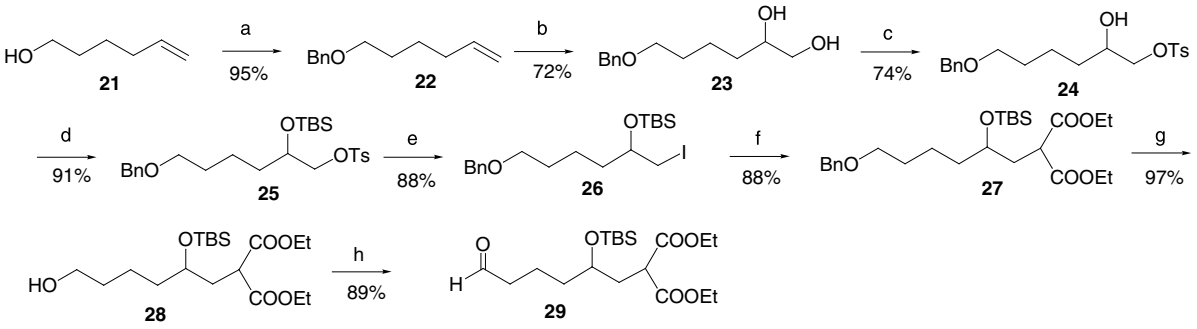
Scheme 3. Synthesis of 2-AGA113 (**4**) and 2-AGA114 (**5**). Reagents and conditions: (a) **10**, DCC, DMAP; (b) O_3 and then CH_3SCH_3; (c) **16**, LiHMDS, HMPA, −78 °C; (d) boric acid, trimethyl borate, 100 °C.

with 1,3-benzylideneglycerol (**10**) by the same method as used for **2**, ozonolysis of **18a** and **18b**, respectively, gave the desired aldehyde synthons **19a** and **19b** in good yield. Wittig coupling of the anion derived from phosphonate **16** with aldehydes **19a** and **19b** gave 55% and 60% yields of each adduct **20a** and **20b**. Finally, deprotection of the benzylidene group using boric acid and trimethyl borate as described in Scheme 3 gave **4** and **5** in good yields.

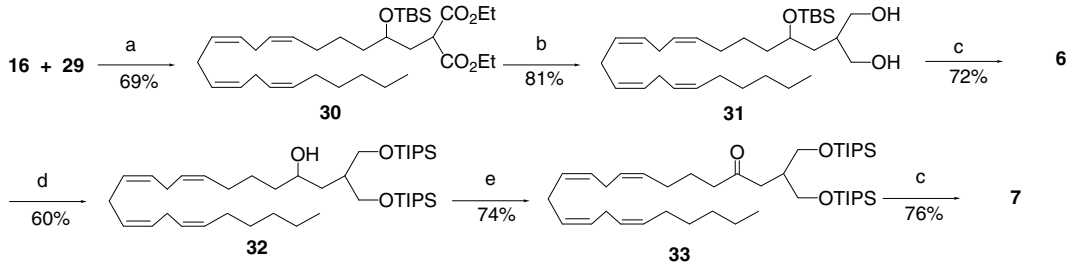
We then modified the bond between the glycerol backbone and arachidonic acid in compound **1**. Previously, an ether analog of 2-AG, 2-[(5*Z*,8*Z*,11*Z*,14*Z*)-icosa-5,8,11,14-tetraenyloxy]propane-1,3-diol (2-AG ether, HU-310) (**2**), was synthesized by us[4,15] and by Mechoulam et al.[10] The agonistic activities of this compound at the cannabinoid receptors have already been evaluated.[4,10,15] However, no other hydrolysis-resistant analogs have been reported so far. Here, we synthesized two structurally related analogs of 2-AG, that is, (8*Z*,11*Z*,14*Z*,17*Z*)-2-(hydroxymethyl)tricosa-8,11,14,17-tetraene-1,4-diol (2-AGA105) (**6**) and (8*Z*,11*Z*,14*Z*,17*Z*)-1-hydroxy-2-(hydroxymethyl)tricosa-8,11,14,17-tetraen-4-one (2-AGA109) (**7**) (Fig. 1). Compound **6** is a hydroxy group-containing analog whose ester bond was substituted by a methylene bond. Compound **7** is a ketone group-containing analog. We first prepared a racemic **6** which was then converted to the ketone group-containing analog **7** through oxidation of the hydroxy group.

The key aldehyde synthon **29** for the synthesis of **6** and **7** was prepared from commercially available 5-hexen-1-ol (**21**) as illustrated in Scheme 4. Protection of alcohol **21** with benzyl bromide using sodium hydride (NaH) followed by oxidation with osmium tetraoxide (OsO_4) gave diol **23** in 72%. After selective tosylation of the primary alcohol of **23**, the *tert*-butyldimethylsilyl (TBS) group was introduced into the secondary alcohol of **24** to afford **25** in good yield. The tosylate was then converted to iodide **26** with lithium iodide in dry acetone in 88% yield. Alkylation of **26** with diethyl malonate employing NaH gave dicarboxylate **27** in 88% yield. The benzyl group of **27** was hydrogenated using palladium-activated carbon (Pd/C) in ethanol to afford the alcohol **28** in 97% yield. Swern oxidation of **28** afforded the desired aldehyde **29** in 89% yield after purification by column chromatography.

The preparation of the 2-AG analogs **6** and **7** proceeded as follows (Scheme 5). Wittig coupling of phosphonate **16** with aldehyde **29** gave a 69% yield of coupling prod-



Scheme 4. Synthesis of an intermediate **29**. Reagents: (a) NaH, BnBr; (b) OsO_4, 4-methylmorpholine *N*-oxide; (c) TsCl, pyridine; (d) TBSCl, imidazole; (e) LiI, acetone; (f) NaH, $CH_2(CO_2Et)_2$; (g) H_2, Pd/C; (h) Swern oxidation.



Scheme 5. Synthesis of a 2-AGA109 (**7**). Reagents and conditions: (a) LiHMDS, HMPA, −78 to 0 °C; (b) $LiAlH_4$; (c) TBAF; (d) TIPSCl, imidazole, DMF; (e) PDC.

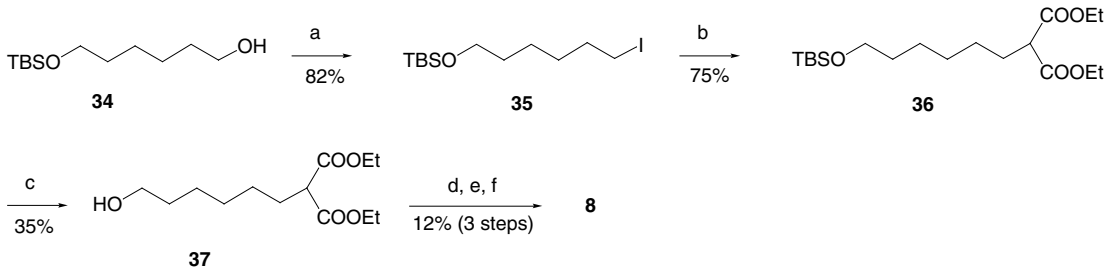
uct **30**. No trace of the *trans* isomer was produced as evidenced by the NMR spectrum. Reduction of the diethyl ester of **30** with lithium aluminum hydride afforded the diol **31** in 81% yield. The TBS-protecting group of **31** was removed by treatment with tetrabutylammonium fluoride (TBAF) to produce the triol **6** in 72% yield. Selective protection of the primary alcohol **6** using a triisopropylsilyl (TIPS) group followed by oxidation of the secondary alcohol **32** with pyridinium dichromate (PDC); deprotection of the TIPS group then gave the ketone analog **7** in good yield.

Finally, we synthesized a methylene-linked analog of **1** (2-AGA104) (**8**) (Scheme 6). This compound contains a methylene bond instead of an ester bond between the glycerol moiety and the fatty chain. For the synthesis of **8**, we chose 6-(*tert*-butyldimethylsilanyloxy)-hexan-1-ol (**34**) as a starting material. The alcohol **34** was halogenated with methyltriphenoxyphosphonium iodide in *N*,*N*-dimethylformamide (DMF) at room temperature in good yield. Alkylation of the resulting iodide **35** via a similar procedure as used for **27** gave dicarboxylate **36** in 75% yield. Although deprotection of the TBS group with TBAF resulted in the alcohol **37**, it was difficult to remove the solvent completely after purification by silica gel column chromatography because the boiling point of **37** was close to that of the solvent used. When we tried to remove the solvent completely from the solution, compound **37** was also evaporated. This is why the resulting compound **37** was crude and contained a small amount of the solvent. The compound **37** was converted to an aldehyde, and then Wittig coupling with **16** followed by reduction of the dicarboxylate gave **8** in 12% overall yield in three steps.

2.2. Biological activity

First, we examined the effects of 2-AG (**1**) and its structural analogs (2-AG ether, 2-AGA118 (**3**), 2-AGA113 (**4**), 2-AGA114 (**5**), 2-AGA105 (**6**), 2-AGA109 (**7**), and 2-AGA104 (**8**)) on the intracellular free Ca^{2+} concentration ($[Ca^{2+}]_i$) in NG108-15 cells which express the CB1 receptor. As illustrated in Figure 2, 2-AG induced a rapid transient increase in $[Ca^{2+}]_i$ in NG108-15 cells. The response was detectable from a concentration as low as 0.3 nM, and the EC_{50} value was calculated to be 23 nM. The effect of 2-AG was mediated via the CB1 receptor; treatment of the cells with SR141716A, a CB1 receptor-specific antagonist, abolished the response induced by 2-AG (data not shown). 2-AG ether, an ether-linked analog of 2-AG, also induced a Ca^{2+} transient, although its activity was apparently weaker than that of 2-AG. Notably, an analog of 2-AG containing an isomer of arachidonic acid with migrated olefins (2-AGA118) (**3**) exhibited only weak agonistic activity. Two types of 2-AG analogs with one carbon-shortened and one carbon-lengthened fatty acyl moieties, 2-AGA113 (**3**) and 2-AGA114 (**4**), also possessed marginal agonistic activities. Similar markedly lower levels of activity compared with that of 2-AG (**1**) were observed with 2-AGA105 (**6**), 2-AGA109 (**7**), and 2-AGA 104 (**8**), which lack an ester linkage between the glycerol backbone and the arachidonoyl moiety.

Figure 3 shows the effects of 2-AG and its structural analogs on $[Ca^{2+}]_i$ in HL-60 cells which express the CB2 receptor. 2-AG induced a rapid increase in $[Ca^{2+}]_i$ in HL-60 cells as in the case of NG108-15 cells. The response was detectable from 1 nM and the EC_{50} value



Scheme 6. Synthesis of a 2-AGA104 (**8**). Reagents and conditions: (a) $CH_3P(OC_6H_5)_3I$, DMF; (b) NaH, $CH_2(CO_2Et)_2$; (c) TBAF in THF; (d) Swern oxidation; (e) **16**, LiHMDS, HMPA, −78 to 0 °C; (f) $LiAlH_4$.

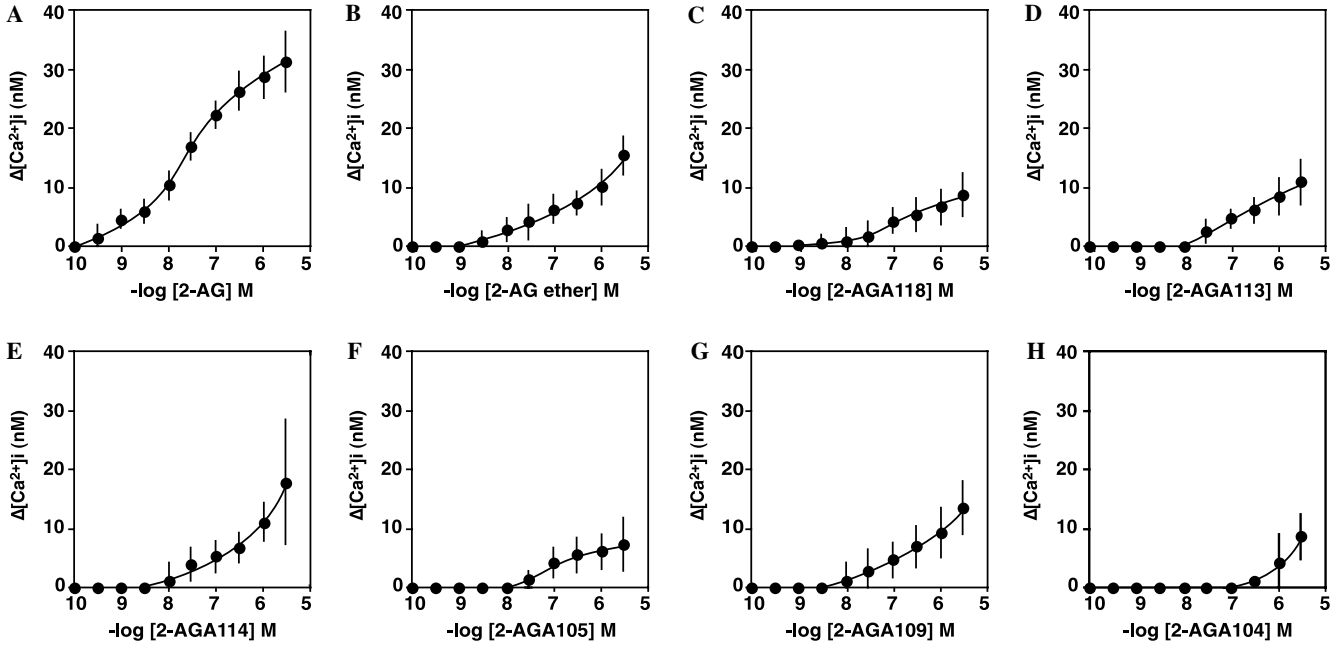


Figure 2. Effects of 2-AG and its structural analogs on $[Ca^{2+}]_i$ in NG108-15 cells. A, 2-AG (**1**); B, 2-AG ether (**2**); C, 2-AGA118 (**3**); D, 2-AGA113 (**4**); E, 2-AGA114 (**5**); F, 2-AGA105 (**6**); G, 2-AGA109 (**7**); H, 2-AGA104 (**8**). The data are means ± SD from five to six determinations.

was 17 nM. The response induced by 2-AG was abrogated by treatment of the cells with SR144528, a CB2 receptor-specific antagonist (data not shown), indicating that the CB2 receptor is involved in the response. On the other hand, the activities of various structural analogs of 2-AG examined here (2-AG ether, 2-AGA118 (**3**), 2-AGA113 (**4**), 2-AGA114 (**5**), 2-AGA105 (**6**), 2-AGA109 (**7**), and 2-AGA104 (**8**)) were markedly weaker than that of 2-AG as in the case of NG108-15 cells. The magnitude of the response induced by each of these compounds was rather small even at 3 μM.

2.3. Discussion

Previously, we examined in detail the biological activities of 2-AG and various 2-AG analogs using NG108-15 cells and HL-60 cells.[3,4] We found 2-AG to have the highest level of activity among the various structural analogs. For example, saturated or monoenoic 2-monoacylglycerols, such as 2-palmitoylglycerol and 2-oleoylglycerol, did not exhibit any agonistic activity. The activities of 2-linoleoylglycerol, 2-γ-linolenoylglycerol, and 2-docosahexaenoylglycerol were also found to be negligible. 2-Eicosapentaenoylglycerol possessed appreciable agonistic activity, yet its activity was markedly weaker than that of 2-AG. Thus, the presence of the arachidonoyl moiety in the molecule is crucial to the activity. We also found that the isopropanol-type analog of 2-AG did not exhibit any agonistic activity. The activity levels of the ethyleneglycol-type analog of 2-AG (arachidonoylethyleneglycol) and 2-hydroxypropyl arachidonate were lower than that of 2-AG, indicating that the presence of the glycerol moiety is also essential. These results strongly suggested that the structure of 2-AG is strictly recognized by the receptor molecules.[3–5]

In the present study, we synthesized several novel structural analogs of 2-AG, closely related in chemical structure to 2-AG, and compared their biological activities to

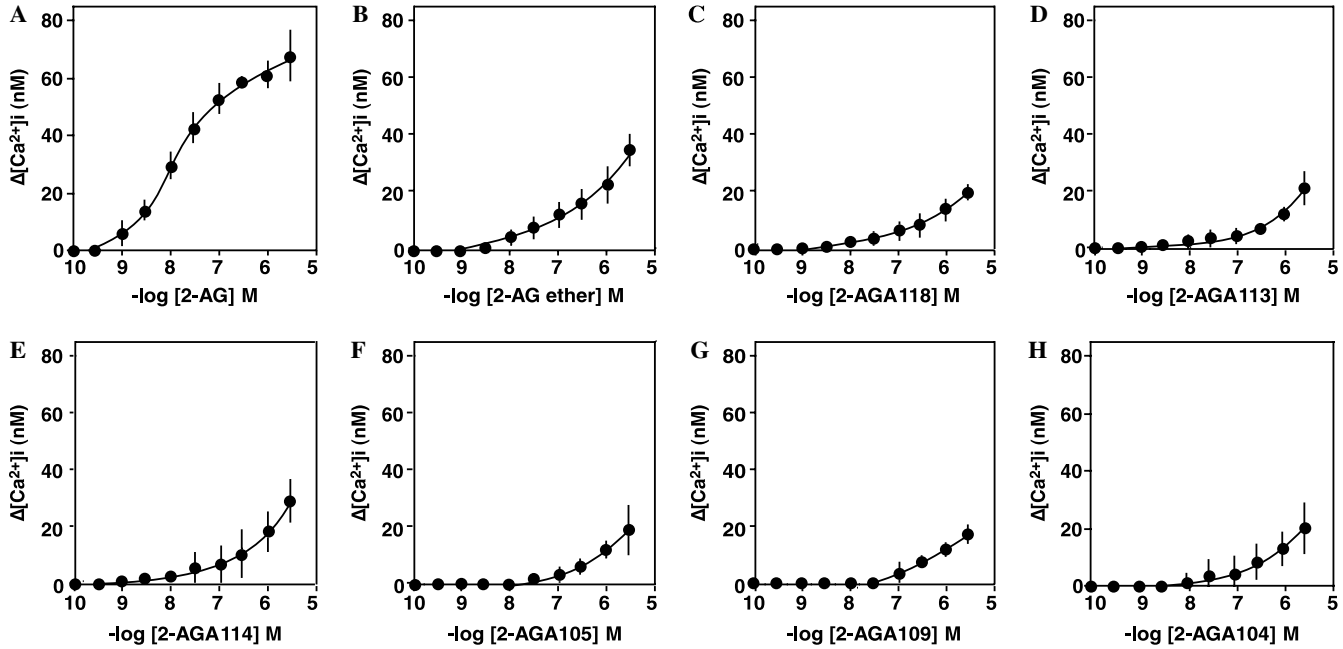


Figure 3. Effects of 2-AG and its structural analogs on $[Ca^{2+}]_i$ in HL-60 cells. A, 2-AG (**1**); B, 2-AG ether (**2**); C, 2-AGA118 (**3**); D, 2-AGA113 (**4**); E, 2-AGA114 (**5**); F, 2-AGA105 (**6**); G, 2-AGA109 (**7**); H, 2-AGA104 (**8**). The data are means ± SD from five to six determinations.

that of 2-AG. As shown in Figures 2 and 3, 2-AG acted as a full agonist and exhibited the most potent agonistic effect among the various structural analogs examined here. Importantly, any modification to the structure of 2-AG, even if it appeared to be minor, markedly reduced the agonistic activity of 2-AG. It is apparent, therefore, that the presence of the arachidonoyl moiety, the glycerol backbone, and the ester linkage is indispensable for exhibiting strong agonistic activity. These results reinforce the hypothesis that the cannabinoid receptors (CB1 and CB2) are primarily the '2-AG receptors'; various analogs of 2-AG employed here, closely related in chemical structure to 2-AG, are thus valuable experimental tools in the elucidation of the structure and properties of the cannabinoid receptor molecules.

Several remarkable analogs of 2-AG have also been synthesized by other investigators. Parkkari et al.[19] recently reported that a dimethylheptyl derivative of 2-AG is a weak CB1 receptor agonist, and Bobrov et al.[20] demonstrated that α,α-dimethyl arachidonoylethyleneglycol acts as a weak CB1 and CB2 receptor agonist. Parkkari et al.[21] also reported the synthesis of an α-methylated analog of 2-AG and a fluorine-containing analog of 2-AG. Interestingly, the α-methylated analog of 2-AG was much more stable toward hydrolytic enzymes than 2-AG, which is in agreement with the assertion by other investigators that the α-methylated analog of 1-AG is metabolically rather stable and can be used as an inhibitor of monoacylglycerol lipase.[22,23] Of note, the α-methylated analog of 2-AG possessed biological activity only slightly weaker than that of 2-AG, while a fluorine-containing analog of 2-AG did not exhibit any biological activity. The α-methylated analog of 2-AG may become a useful experimental tool in the elucidation of the physiological and pathophysiological roles of 2-AG, especially those in vivo.

In relation to this, it should be noted that 2-AG ether exhibits appreciable biological activities comparable to those of 2-AG in vivo[10,24] and in prolonged incubation experiments in vitro,[25] although its level of activity was apparently lower than that of 2-AG estimated in short-term incubation experiments in vitro.[4,5] This is probably due to the fact that 2-AG ether is metabolically stable compared with 2-AG, especially in vivo and in prolonged incubation experiments in vitro. By the way, several of the 2-AG analogs examined in the present study are also metabolically rather stable. For example, 2-AGA105 (**6**), 2-AGA109 (**7**), and 2-AGA104 (**8**) are resistant to hydrolytic enzymes due to the lack of an ester bond in the molecule. It is possible, therefore, that these compounds may exhibit appreciable cannabimimetic activities in vivo, like 2-AG ether, and can be used as valuable tools in exploring the physiological and pathophysiological significance of 2-AG. Further detailed studies are thus essential to evaluate the exact biological activities of these novel 2-AG analogs in vivo.

3. Conclusion

We synthesized several remarkable analogs of 2-AG (2-AGA118 (**3**), 2-AGA113 (**4**), 2-AGA114 (**5**), 2-AGA105 (**6**), 2-AGA109 (**7**), and 2-AGA104 (**8**)) which are closely related in chemical structure to 2-AG. Notably, these structural analogs exhibited only weak agonistic activities toward either the CB1 receptor or the CB2 receptor, while 2-AG acted as a full agonist toward these cannabinoid receptors. A modification of the structure of 2-AG, even a minor one, markedly reduced the agonistic activity of 2-AG. It is apparent, therefore, that an intact arachidonoyl moiety, glycerol backbone, and ester linkage are crucially important for having potent agonistic activities. Based on these experimental results

and those in previous studies, we concluded that both the CB1 receptor and the CB2 receptor are the intrinsic-specific receptors for 2-AG.

Not much attention has thus far been directed to 2-AG compared with anandamide,[14,15] yet it is becoming evident that 2-AG plays pivotal roles as the true natural ligand for the cannabinoid receptors in various mammalian tissues and cells. Thus, further intensive studies on 2-AG are indispensable for a thorough elucidation of the physiological and pathophysiological roles of the endocannabinoid system. In order to promote such studies, it is essential to develop a number of structural analogs of 2-AG which may mimic the functions of 2-AG: metabolically stable 2-AG analogs would become valuable experimental tools, especially in prolonged incubation experiments and in vivo experiments, as receptor agonists or as inhibitors of monoacylglycerol lipase and the thioester bond-containing analog of 2-AG may be useful as a synthetic substrate in the measurement of monoacylglycerol lipase activity. Nevertheless, the number of available 2-AG analogs is still limited. Further detailed studies are needed to synthesize and provide a variety of novel 2-AG analogs. The development of various 2-AG analogs with different chemical structures, characteristics, and agonistic or antagonistic activities would greatly contribute to the thorough elucidation of the physiological and pathophysiological significance of 2-AG and the cannabinoid receptors in mammalian tissues.

4. Experimental

4.1. General

^{1}H NMR spectra were recorded on a JEOL GSX-400 spectrometer at 400 MHz and ^{13}C NMR spectra were recorded at 100 MHz using $CDCl_3$ as a solvent unless otherwise specified. Chemical shifts are given in ppm (δ) using tetramethylsilane (TMS) as the internal standard. MS spectra (EIMS) and high-resolution MS spectra (HREIMS) were recorded on a JEOL JMX-SX 102 A mass spectrometer. Column chromatography was carried out on silica gel 60 (70–230 mesh) and preparative thin layer chromatography (TLC) was run on silica gel $60F_{254}$. Unless otherwise noted, all reagents were purchased from commercial suppliers and used as received.

4.2. (8*Z*,11*Z*,14*Z*,17*Z*)-2-Phenyl-1,3-dioxan-5-yl icosa-8,11,14,17-tetraenoate (11)

To a solution of 1,3-benzylidene glycerol (**10**) (4.4 mg, 24.6 μmol) and (8*Z*,11*Z*,14*Z*,17*Z*)-icosa-8,11,14,17-tetraenoic acid (**9**) (5 mg, 16.4 μmol) in dry toluene (1 mL) were added DCC (6.8 mg, 33.0 μmol) and DMAP (200 μg, 1.6 μmol) at 0 °C under an argon atmosphere. After stirring at 0 °C for 2 h and then at room temperature for 12 h, the reaction mixture was diluted with ethyl acetate (5 mL). The mixture was washed with water (3 mL) and brine (3 mL), and then the organic layer was separated, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified with preparative TLC (hexane/ethyl acetate, 5:1, v/v) to give **11** (5.2 mg, yield 69%) as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.52–7.50 (m, 2H), 7.39–7.36 (m, 3H), 5.56 (s, 1H), 5.42–5.29 (m, 8H), 4.72 (br s, 1H), 4.28 (d, 2H, J = 12.6 Hz), 4.17 (d, 2H, J = 13.2 Hz), 2.85–2.81 (m, 6H), 2.44–2.40 (m, 2H), 2.09–2.04 (m, 4H), 1.82–1.60 (m, 2H), 1.39–1.28 (m, 6H), 0.98 (br t, 3H, J = 7.7 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.8, 137.8, 130.2, 129.1, 128.5, 128.4, 128.3, 128.02, 127.96, 127.8, 127.1, 126.0, 101.2, 69.2, 65.7, 34.0, 32.8, 30.9, 29.5, 29.0, 27.2, 26.4, 25.6, 24.7, 22.5, 14.0; MS 466 $[M]^+$; HRMS calcd for $[C_{30}H_{42}O_4]$ 466.3084; found 466.3073.

4.3. (8*Z*,11*Z*,14*Z*,17*Z*)-1,3-Dihydroxypropan-2-yl icosa-8,11,14,17-tetraenoate (3)

A suspension of **11** (2.5 mg, 5.4 μmol) and boric acid (662 μg, 10.7 mmol) in trimethyl borate (1 mL) was refluxed at 100 °C for 20 min. After being cooled to room temperature, the reaction mixture was diluted with ethyl acetate (5 mL) and successively washed with 3 mL of water and brine. The organic layer was dried ($MgSO_4$), and the solvent was evaporated in vacuo. Preparative TLC ($CHCl_3$/MeOH, 20:1, v/v) of the crude residue provided the product **3** (1.5 mg, yield 72%) as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.38–5.37 (m, 8H), 4.92 (tt, 1H, J = 4.8, 4.8 Hz), 3.84 (d, 4H, J = 4.9 Hz), 2.85–2.80 (m, 6H), 2.38 (t, 2H, J = 7.5 Hz), 2.21 (br s, 2H), 2.08–2.05 (m, 4H), 1.71–1.68 (m, 2H), 1.39–1.31 (m, 6H), 0.98 (br t, 3H, J = 7.5 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.0, 132.0, 130.2, 128.5, 128.4, 128.03, 127.97, 127.8, 127.1, 75.0, 62.5, 34.3, 29.4, 29.0, 28.9, 27.2, 25.6, 24.9, 20.6, 14.3; MS 378 $[M]^+$; HRMS calcd for $[C_{23}H_{38}O_4]$ 378.2770; found 378.2779.

4.4. Undeca-2,5-diyn-1-ol (13)

Compound **13** was prepared from 2-octyn-1-ol and 1-bromooct-2-yne (**12**) by a reported method.[17]

4.5. (Pentadeca-3,6,9-triyloxy)tetrahydro-2*H*-pyran (14)

A solution of bromine (3.33 mL, 64.6 mmol) in CH_2Cl_2 (30 mL) was added slowly dropwise to a solution of 1,2-bis(diphenylphosphino)ethane (14.2 g, 35.6 mmol) in CH_2Cl_2 (150 mL) under an argon atmosphere at 0 °C. Diyne **13** (5.3 g, 32.3 mmol) in CH_2Cl_2 (30 mL) was added to this mixture, and the resultant solution was stirred at room temperature for 2 h. The volume of the reaction mixture was reduced in vacuo to 15 mL and diethyl ether (300 mL) was added. Pentane (600 mL) was added to the diethyl ether solution and the organic solution was dried over $MgSO_4$. The mixture was filtered through a pad of silica gel (Merck, 40–60 μm). The eluate was concentrated in vacuo to afford 1-bromo-2,5-undecadiyne (7.2 g, yield 99%) as a colorless oil. Next, a solution of bromoethane (2.64 mL, 35.6 mmol) in dry tetrahydrofuran (THF) (20 mL) was added dropwise to magnesium (788 mg, 32.4 mmol) in THF (40 mL) at 0 °C under an atmosphere of argon. After the addition was completed, the reaction mixture was stirred at ambient temperature until all the magnesium dissolved. A solution of 2-(3-

butynyloxy)tetrahydro-2*H*-pyran (5.0 g, 32.4 mmol) in THF (15 mL) was slowly added to the cooled ethylmagnesium bromide solution. After stirring at 23 °C for 10 h, copper (I) iodide (300 mg) was added and the mixture was stirred for an additional 15 min. 1-Bromo-2,5-undecadiyne (7.2 g, 31.8 mmol) in THF (15 mL) was added, and the resulting mixture was stirred at room temperature for 2 h. The reaction mixture was poured into a solution of 2 N H_2SO_4 (50 mL) and ice (20 mL). The resultant solution was extracted with diethyl ether (3× 20 mL), and the diethyl ether extracts were washed with saturated aqueous NH_4Cl (20 mL) and saturated aqueous Na_2CO_3 (20 mL) and dried over $MgSO_4$. The solvent was removed and the residue was chromatographed on silica gel (ether/hexane, 1:20, v/v) to provide triyne **14** (8.2 g, yield 85%) as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.62 (dd, 1H, J = 3.1, 4.1 Hz), 3.91–3.86 (m, 1H), 3.80 (tt, 1H, J = 7.2, 7.2 Hz), 3.57–3.48 (m, 2H), 3.12 (s, 4H), 2.49–2.45 (m, 2H), 2.17–2.13 (m, 2H), 1.87–1.79 (m, 1H), 1.75–1.69 (m, 1H), 1.63–1.45 (m, 6H), 1.38–1.27 (m, 4H), 0.90 (t, 3H, J = 6.9 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 98.7, 80.8, 77.4, 74.93, 74.89, 74.4, 73.6, 65.7, 62.1, 31.0, 30.5, 28.4, 25.4, 22.1, 20.1, 19.4, 18.6, 13.9; FAB-MS(NBA-NaI) 301 $[M+H]^+$; HR-FAB-MS calcd for $[C_{20}H_{29}O_2]$ 301.2167; found 301.2144.

4.6. 2-((3*Z*,6*Z*,9*Z*)-Pentadeca-3,6,9-trienyloxy)tetrahydro-2*H*-pyran (15)

To a solution of **14** (330 mg, 1.10 mmol) in ethanol (10 mL) were added triethylamine (0.1 mL) and Lindlar catalyst (100 mg) under an argon atmosphere at room temperature. Hydrogenation was performed at atmospheric pressure at 0 to 5 °C for 5 h and then at room temperature for 24 h. The solution was filtered to remove the catalyst, and the filtrate was then evaporated at reduced pressure to give crude triene **15**, which was purified by argentation chromatography utilizing 10% silver nitrate on silica gel (diethyl ether/hexane, 1:20, v/v) to give pure **15** (208 mg, yield 62%) as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.46–5.32 (m, 6H), 4.60 (dd, 1H, J = 2.7, 4.0 Hz), 3.90–3.85 (m, 1H), 3.75 (tt, 1H, J = 7.0, 7.0 Hz), 3.53–3.48 (m, 1H), 3.42 (tt, 1H, J = 7.0, 7.0 Hz), 2.86–2.80 (m, 4H), 2.41–2.37 (m, 2H), 2.05 (dd, 2H, J = 6.7, 13.7 Hz), 1.86–1.80 (m, 1H), 1.75–1.69 (m, 1H), 1.59–1.50 (m, 4H), 1.40–1.27 (m, 6H), 0.89 (t, 3H, J = 6.9 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 130.4, 129.8, 128.5, 127.9, 127.6, 126.1, 98.7, 66.9, 62.3, 31.5, 30.7, 29.3, 28.0, 27.2, 25.7, 25.6, 25.5, 22.5, 19.6, 14.0; MS 306 $[M]^+$; HRMS calcd for $[C_{20}H_{34}O_2]$ 306.2558; found 306.2554.

4.7. 2-[(3*Z*,6*Z*,9*Z*)-Pentadeca-3,6,9-trien-1-yl] triphenylphosphonium bromide (16)

A solution of bromine (0.88 mL, 17.0 mmol) was slowly added dropwise to a solution of 1,2-bis(diphenylphosphino)ethane (3.73 g, 9.36 mmol) in CH_2Cl_2 (20 mL) under an argon atmosphere at 0 °C. A slight excess of 1,2-bis(diphenylphosphino)ethane was added back to the solution until the light yellow color disappeared. Triene **15** (2.60 g, 8.49 mmol) was added in one portion. The mixture was stirred at room temperature for 9 h. The solvent was removed in vacuo and the residue was dissolved in hexane/diethyl ether (2:1, v/v) (600 mL). The solution was filtered through a pad of silica gel to afford crude 1-bromo-3,6,9-tetradecatriene (2.22 g, yield 92%).

The bromo compound (3.60 g, 12.7 mmol) was dissolved in dry acetonitrile (100 mL), and triphenylphosphine (6.65 g, 25.3 mmol) was added. The reaction mixture was refluxed for 36 h under an argon atmosphere and then concentrated in vacuo. The phosphonium salt **16** was purified by flash column chromatography (CH_2Cl_2/MeOH, 19:1, v/v) to afford the pure phosphonium salt **16** (6.40 g, yield 92%) as a white powder: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.89–7.70 (m, 15H), 5.40–5.19 (m, 6H), 3.88–3.86 (m, 2H), 2.64 (t, 1H, J = 7.2 Hz), 2.58 (t, 1H, J = 7.4 Hz), 2.48 (m, 2H), 2.17–1.96 (m, 4H), 1.35–1.24 (m, 6H), 0.88 (t, 3H, J = 6.9 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 135.0, 133.7, 133.6, 130.6, 130.5, 130.4, 130.3, 128.9, 127.1, 118.6, 117.7, 31.4, 29.2, 27.1, 25.5, 22.4, 14.0; MS 546 $[M]^+$; HRMS calcd for $[C_{33}H_{40}PBr]$ 546.2051; found 546.2056.

4.8. 2-Phenyl-1,3-dioxan-5-yl pent-4-enoate (18a)

To a solution of 4-pentenoic acid (**17a**) (510 μL, 5.0 mmol) in toluene (10 mL) were added DCC (2.06 g, 10.0 mmol), DMAP (61 mg, 0.5 mmol), and **10** (1.16 g, 6.0 mmol) at 0 °C under an argon atmosphere. After stirring at 0 °C for 2 h and then at room temperature for 12 h, the reaction mixture was diluted with ethyl acetate (50 mL). The mixture was washed with water (30 mL) and brine (30 mL), then the organic layer was separated, dried over $MgSO_4$, and concentrated in vacuo. The residue was chromatographed on silica gel (hexane/ethyl acetate, 5:1, v/v) to give **18a** (980 mg, yield 75%) as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.52–7.50 (m, 2H), 7.40–7.34 (m, 3H), 5.90–5.80 (m, 1H), 5.55 (s, 1H), 5.09 (dd, 1H, J = 1.5, 17.1 Hz), 5.01 (dd, 1H, J = 1.5, 10.6 Hz), 4.72 (dd, 1H, J = 1.5, 3.0 Hz), 4.27 (dd, 2H, J = 1.5, 13.1 Hz), 4.16 (dd, 2H, J = 1.5, 13.1 Hz), 2.57–2.53 (m, 2H), 2.46–2.40 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.0, 137.8, 136.5, 129.0, 128.2, 126.0, 115.6, 101.2, 69.0, 65.9, 33.6, 28.8; MS 262 $[M]^+$; HRMS calcd for $[C_{15}H_{18}O_4]$ 262.1205; found 262.1209.

4.9. 2-Phenyl-1,3-dioxan-5-yl hept-6-enoate (18b)

Compound **18b** was prepared from 6-heptenoic acid (**17b**) (946 mg, 7.38 mmol) in a manner similar to that for **18a**. Chromatography on silica gel (hexane/ethyl acetate, 5:1, v/v) gave **18b** (1.50 g, yield 70%) as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.52–7.49 (m, 2H), 7.39–7.34 (m, 3H), 5.84–5.74 (m, 1H), 5.55 (s, 1H), 5.01 (dt, 1H, J = 1.1, 7.1 Hz), 4.95 (dt, 1H, J = 1.1, 10.0 Hz), 4.70 (s, 1H), 4.27 (d, 2H, J = 13.0 Hz), 4.15 (dd, 2H, J = 1.0, 13.0 Hz), 2.44 (t, 2H, J = 7.6 Hz), 2.08 (dd, 2H, J = 6.8, 13.9 Hz), 1.73–1.63 (m, 2H), 1.50–1.41 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.3, 138.1, 137.7, 128.9, 128.1, 125.8, 114.5, 101.1,

69.1, 65.7, 34.3, 33.4, 28.4, 24.5; MS 290 $[M]^+$; HRMS calcd for $[C_{17}H_{22}O_4]$ 290.1518; found 290.1525.

4.10. 2-Phenyl-1,3-dioxan-5-yl 3-formylpropanoate (19a)

A solution of **18a** (720 mg, 2.75 mmol) in methanol (20 mL) and methylene chloride (5 mL) under nitrogen was ozonized at −78 °C for 1 h. After the solution turned blue, the ozone was removed, and the reaction mixture was quenched with dimethyl sulfide (5 mL) and allowed to warm to 23 °C. The solvent was removed and the crude product dissolved in diethyl ether (10 mL) and washed with dilute acetic acid and water. The diethyl ether layer was dried ($MgSO_4$), the solvent was removed, and the crude residue was chromatographed on silica gel (ether/pentane, 1:3, v/v) to give **19a** (522 mg, yield 72%) as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.82 (s, 1H), 7.51–7.49 (m, 2H), 7.39–7.35 (m, 3H), 5.56 (s, 1H), 4.73 (br s 1H), 4.28 (dd, 2H, J = 1.0, 13.2 Hz), 4.16 (dd, 2H, J = 1.0, 12.8 Hz), 2.84 (t, 1H, J = 6.6 Hz), 2.76 (dt, 1H, J = 1.1, 6.1 Hz), 2.60–2.53 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 199.8, 172.2, 137.8, 129.1, 128.3, 126.0, 101.2, 69.0, 38.5, 26.8; MS 264 $[M]^+$; HRMS calcd for $[C_{14}H_{16}O_5]$ 264.0998; found 264.0997.

4.11. 2-Phenyl-1,3-dioxan-5-yl 4-formylbutanoate (19b)

Compound **19b** was prepared from **18b** (400 mg, 1.37 mmol) in the same fashion as described for **19a**. Purification with silica gel column chromatography (diethyl ether/pentane, 1:3, v/v) gave **19b** (298 mg, yield 74%) as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.72 (s, 1H), 7.50–7.49 (m, 2H), 7.39–7.33 (m, 3H), 5.06 (s, 1H), 4.69 (s, 1H), 4.24 (d, 2H, J = 12.7 Hz), 4.13 (d, 2H, J = 13.0 Hz), 2.47–2.42 (m, 2H), 2.14 (br s, 2H), 1.80–1.56 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 201.7, 173.1, 129.2, 127.8, 125.6, 100.8, 68.7, 65.7, 33.9, 30.7, 23.9; MS 292 $[M]^+$; HRMS calcd for $[C_{16}H_{20}O_5]$ 292.1311; found 292.1313.

4.12. (4*Z*,7*Z*,10*Z*,13*Z*)-2-Phenyl-1,3-dioxan-5-yl nonadeca-4,7,10,13-tetraenoate (20a)

To a cooled (−78 °C), stirred solution of the phosphonium salt **16** (200 mg, 442 μmol) in THF (5 mL) was added lithium hexamethyldisilazide (LiHMDS) (1 M THF solution; 363 μL, 363 μmol) dropwise under an argon atmosphere. After stirring at −78 °C for 2 h, hexamethylphosphoramide (HMPA) (0.5 mL) was added, the reaction mixture was stirred for 10 min, and then aldehyde **19a** (86 mg, 325 μmol) in THF (0.5 mL) was added to the resulting red solution. The reaction mixture was stirred at −78 °C for 1 h and then allowed to warm slowly to 0 °C over a period of 1 h. It was then quenched by the addition of 2 N HCl solution (1 mL) and extracted with diethyl ether (3× 10 mL). The combined extracts were washed with cold water (3× 5 mL), dried over $MgSO_4$, filtered, and concentrated under reduced pressure to afford the crude product which was purified by flash column chromatography (diethyl ether/hexane, 1:20, v/v) to give pure **20a** (81 mg, yield 55%) as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.52–7.50 (m, 2H), 7.38–7.35 (m, 3H), 5.56 (s, 1H), 5.40–5.32 (m, 8H), 4.72 (t, 1H, J = 1.5 Hz), 4.27 (dd, 2H, J = 1.5, 12.8 Hz), 4.17 (dd, 2H, J = 1.5, 12.8 Hz), 2.85–2.80 (m, 6H), 2.47–2.43 (m, 4H), 2.12–2.03 (m, 2H), 1.45–1.26 (m, 6H), 0.88 (t, 3H, J = 6.9 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.1, 137.8, 130.5, 129.6, 129.4, 129.0, 128.6, 128.4, 128.3, 128.0, 127.8, 127.7, 127.5, 126.0, 101.2, 69.1, 65.9, 34.2, 31.5, 29.3, 27.2, 25.6, 22.7, 22.5, 14.0; MS 452 $[M]^+$; HRMS calcd for $[C_{29}H_{40}O_4]$ 452.2926; found 452.2924.

4.13. (6*Z*,9*Z*,12*Z*,15*Z*)-2-Phenyl-1,3-dioxan-5-yl henicosa-6,9,12,15-tetraenoate (20b)

Compound **20b** was prepared from **19b** (143 mg, 490 μmol) as described for **20a**. Purification with silica gel column chromatography (hexane/ethyl acetate, 5:1, v/v) provided **20b** (141 mg, yield 60%) as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.52–7.49 (m, 2H), 7.40–7.34 (m, 3H), 5.56 (s, 1H), 5.43–5.31 (m, 8H), 4.72 (t, 1H, J = 1.7 Hz), 4.27 (dd, 2H, J = 1.7, 13.1 Hz), 4.17 (dd, 2H, J = 1.7, 13.1 Hz), 2.85–2.77 (m, 6H), 2.45 (t, 2H, J = 7.5 Hz), 2.12–2.03 (m, 4H), 1.74–1.66 (m, 2H), 1.47–1.24 (m, 8H), 0.89 (t, 3H, J = 6.9 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.6, 137.8, 130.5, 129.7, 129.0, 128.5, 128.3, 128.2, 128.1, 127.9, 127.6, 126.0, 101.2, 69.1, 65.8, 34.3, 31.5, 29.3, 29.1, 27.2, 26.9, 25.6, 24.6, 22.6, 14.0; MS 480 $[M]^+$; HRMS calcd for $[C_{31}H_{44}O_4]$ 480.3239; found 480.3249.

4.14. (4*Z*,7*Z*,10*Z*,13*Z*)-1,3-Dihydroxypropan-2-yl nonadeca-4,7,10,13-tetraenoate (4)

Compound **4** was prepared from **20a** (60 mg, 133 μmol) as described for **3**. Purification with silica gel column chromatography (hexane/ethyl acetate, 5:1) and borate-impregnated TLC gave pure **4** (21 mg, yield 43% overall) as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.46–5.32 (m, 8H), 4.93 (tt, 1H, J = 5.0, 5.0 Hz), 3.83 (dd, 4H, J = 1.5, 5.0 Hz), 2.86–2.80 (m, 6H), 2.47–2.40 (m, 4H), 2.17 (br s, 2H), 2.05 (dd, 2H, J = 6.6, 13.7 Hz), 1.38–1.25 (m, 6H), 0.89 (br t, 3H, J = 7.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.3, 130.6, 129.7, 128.7, 128.5, 127.84, 127.79, 127.72, 127.5, 75.2, 62.4, 34.2, 31.5, 29.3, 27.2, 25.65, 25.62, 22.8, 22.6, 14.1; MS 364 $[M]^+$; HRMS calcd for $[C_{22}H_{36}O_4]$ 364.2613; found 364.2621.

4.15. (6*Z*,9*Z*,12*Z*,15*Z*)-1,3-Dihydroxypropan-2-yl henicosa-6,9,12,15-tetraenoate (5)

Compound **5** was prepared from **20b** (70 mg, 145 μmol) as described for **3**. Purification with silica gel column chromatography (hexane/ethyl acetate, 5:1, v/v) and borate-impregnated TLC gave **5** (25 mg, yield 44%) as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.46–5.32 (m, 8 H), 4.93 (tt, 1H, J = 4.5, 4.5 Hz), 3.84 (dd, 4H, J = 1.6, 4.5 Hz), 2.85–2.80 (m, 6H), 2.39 (t, 2H, J = 7.5 Hz), 2.11–2.00 (m, 4H), 1.67 (tt, 2H, J = 7.3, 7.3 Hz), 1.60 (br s, 2H), 1.44–1.26 (m, 8H), 0.89 (br t, 3H, J = 6.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.8, 130.5, 129.6, 128.6, 128.31, 128.26, 128.18, 127.9,

127.6, 75.1, 62.6, 34.2, 31.5, 29.3, 29.0, 27.2, 26.8, 25.7, 24.6, 22.6, 14.1; MS 392 $[M]^+$; HRMS calcd for $[C_4H_{40}O_4]$ 392.2927; found 392.2934.

4.16. 5-Hexenyloxymethylbenzene (22)

To a solution of 5-hexen-1-ol (**21**) (10 mL, 83 mmol) in DMF (50 mL) were added sodium hydride (60% in oil 4.0 g, 100 mmol), benzyl bromide (14.9 g, 125 mmol), and tetra-*n*-butylammonium iodide (3.1 g, 8.3 mmol) at 0 °C under an argon atmosphere. The reaction mixture was stirred at room temperature for 6 h, then diluted with ethyl acetate (300 mL). The mixture was washed with water (200 mL) and brine (200 mL), and the organic layer was dried over $MgSO_4$ and evaporated in vacuo. The residue was chromatographed on silica gel (hexane/ethyl acetate, 40:1, v/v) to give **22** (15 g, yield 95%) as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36–7.22 (m, 5H), 5.85–5.75 (m, 1H), 5.02–4.92 (m, 2H), 4.49 (s, 2H), 3.47 (t, 2H, J = 6.4 Hz), 2.06 (dd, 2H, J = 7.2, 14.4 Hz), 1.67–1.60 (m, 2H), 1.51–1.43 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 128.4, 127.7, 127.4, 114.5, 72.8, 72.1, 70.2, 33.5, 29.2, 25.5; MS 190 $[M]^+$; HRMS calcd for $[C_{13}H_{18}O]$ 190.1357; found 190.1358.

4.17. 6-Benzyloxyhexane-1,2-diol (23)

Compound **22** (10.1 g, 53.1 mmol) was dissolved in CH_3CN/water (2:1, v/v) (100 mL), and then a 2% OsO_4 solution in water (5 mL) and 4-methylmorpholine *N*-oxide (12.5 g, 0.11 mol) were added to the solution. After the mixture was stirred at room temperature for 12 h, Na_2SO_3 (20 mL) was added. The mixture was stirred at room temperature for 30 min, then extracted with ethyl acetate (3× 50 mL). The organic layer was combined and washed with 1 N HCl (50 mL) and brine (50 mL), dried over $MgSO_4$, and evaporated in vacuo. The crude residue was chromatographed on silica gel (hexane/ethyl acetate, 1:1 to ethyl acetate only, v/v) to afford **23** (8.57 g, yield 72%) as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34–7.25 (m, 5H), 4.49 (s, 2H), 3.68–3.62 (m, 1H), 3.57 (dd, 1H, J = 2.8, 11.2 Hz), 3.47 (t, 2H, J = 6.4 Hz), 3.37 (dd, 1H, J = 7.6, 11.3 Hz), 3.03 (br s, 2H), 1.69–1.59 (m, 2H), 1.54–1.38 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.4, 128.3, 127.6, 127.5, 72.9, 72.0, 70.2, 66.6, 32.8, 29.5, 22.2; MS 224 $[M]^+$; HRMS calcd for $[C_{13}H_{20}O_3]$ 224.1412; found 224.1415.

4.18. 6-(Benzyloxy)-2-hydroxyhexyl 4-methylbenzenesulfonate (24)

To a solution of **23** (11.4 g, 50.9 mmol) in pyridine (50 mL) was added *p*-toluenesulfonyl chloride (TsCl) (9.70 g, 50.9 mmol) at 0 °C under an argon atmosphere. The suspension was stirred at room temperature for 12 h. The reaction mixture was diluted with CH_2Cl_2 (300 mL) and successively washed with 100 ml of 1 N HCl, water, saturated aqueous $NaHCO_3$, and brine. The organic layer was dried over $MgSO_4$ followed by filtration and evaporation in vacuo. The residue was chromatographed on silica gel (hexane/ethyl acetate, 3:1, v/v) to give **24** (14.2 g, yield 74%) as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.81–7.78 (m, 2H), 7.35–7.25 (m, 7H), 4.48 (br s, 2H), 4.01 (dd, 1H, J = 2.8, 9.5 Hz), 3.87 (dd, 1H, J = 7.0, 16.5 Hz), 3.85–3.80 (m, 1H), 3.45 (t, 2H, J = 6.4 Hz), 2.42 (br s, 3H), 2.27 (br s, 1H), 1.64–1.36 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 129.9, 128.4, 127.9, 127.6, 127.5, 73.9, 72.9, 70.0, 69.3, 32.4, 29.4, 22.0, 21.6; MS 378 $[M]^+$; HRMS calcd for $[C_{20}H_{26}O_5S]$ 378.1501; found 378.1502.

4.19. 6-Benzyloxy-2-(*tert*-butyldimethylsilanyloxy)hexyl 4-methylbenzenesulfonate (25)

To a 0°C cooled solution of **24** (5.00 g, 13.2 mmol) in DMF (50 mL) was added imidazole (1.98 g, 29.1 mmol) followed by *tert*-butyldimethylsilyl chloride (TBSCl) (3.99 g, 26.4 mmol) and stirred at room temperature for 1 h. The reaction mixture was poured into water (200 mL) and extracted with ethyl acetate (3× 100 mL). The ethyl acetate layer was combined and washed with brine (100 mL), dried over $MgSO_4$, and filtered. The solvent was evaporated under reduced pressure, and the product was purified by flash column chromatography (hexane/ethyl acetate, 20:1, v/v) to afford **25** (5.92 g, yield 91%) as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.79–7.75 (m, 2H), 7.34–7.24 (m, 7H), 4.46 (br s, 2H), 3.88–3.80 (m, 2H), 3.69–3.61 (m, 1H), 3.45–3.36 (m, 2H), 2.42 (br s, 3H), 1.65–1.24 (m, 6H), 0.84 (s, 9H), 0.00 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 129.9, 129.8, 129.6, 128.3, 127.9, 127.6, 83.1, 72.9, 70.0, 33.8, 32.4, 29.4, 22.0, 21.4, −3.0, −3.6; MS 492 $[M]^+$; HRMS calcd for $[C_{26}H_{40}O_5SiS]$ 492.2366; found 492.2369.

4.20. (6-Benzyloxy-1-iodohexan-2-yloxy) *tert*-butyldimethylsilane (26)

To a solution of **25** (3.20 g, 6.50 mmol) in dry acetone (300 mL) was added lithium iodide (9.70 g, 64.7 mmol), and the solution was refluxed for 14 h. The reaction mixture was concentrated under reduced pressure, then the residue was dissolved in ethyl acetate (300 mL). The ethyl acetate solution was washed with water (100 mL) and brine (100 mL), dried over $MgSO_4$, filtered, and concentrated under reduced pressure to afford the crude product, which was purified by flash column chromatography (hexane/ethyl acetate, 20:1, v/v) to give pure **26** (2.56 g, yield 88%) as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.29–7.19 (m, 5H), 4.40 (s, 2H), 3.50–3.45 (m, 1H), 3.41 (t, 2H, J = 6.4 Hz), 3.12 (d, 2H, J = 5.2 Hz), 1.62–1.55 (m, 6H), 0.84 (s, 9H), 0.03 (s, 3H), 0.00 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 128.3, 127.6, 127.5, 72.9, 71.3, 70.2, 36.7, 29.7, 25.8, 21.7, 13.9, −4.4, −4.6; MS 448 $[M]^+$; HRMS calcd for $[C_{19}H_{33}O_2SiI]$ 448.1295; found 448.1299.

4.21. Diethyl 2-[6-benzyloxy-2-(*tert*-butyldimethylsilanyloxy)hexyl] malonate (27)

To a suspension of NaH (60% in oil; 0.99 g, 24.8 mmol) in a mixture of dry THF and dry DMF (1:1, v/v, 100 mL), diethyl malonate (6.8 mL, 44.8 mmol) was added dropwise at 0 °C under an argon atmosphere. After stirring at room temperature for 15 min, the iodide

26 (10.1 g, 22.5 mmol) in THF (10 mL) was added dropwise at 0 °C under an argon atmosphere and the mixture was refluxed for 4 h. The reaction mixture was cooled to room temperature, then diluted with saturated aqueous NH_4Cl (300 mL) and extracted with ethyl acetate (3× 100 mL). The organic layer was combined, dried over $MgSO_4$, filtered, and concentrated under reduced pressure to give the crude product, which was purified by flash column chromatography over silica gel (hexane/ethyl acetate, 10:1, v/v) to give the pure product **27** (9.50 g, yield 88%) as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31–7.22 (m, 5H), 4.46 (s, 2H), 4.20–4.10 (m, 4H), 3.68–3.65 (m, 1H), 3.51 (dd, 1H, J = 5.3, 9.0 Hz), 3.43 (t, 2H, J = 6.5 Hz), 2.11–2.04 (m, 1H), 1.94–1.87 (m, 1H), 1.57 (tt, 2H, J = 6.4, 6.7 Hz), 1.48–1.35 (m, 4H), 1.231 (t, 3H, J = 7.2 Hz), 1.225 (t, 3H, J = 7.2 Hz), 0.84 (s, 9H), 0.00 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.9, 169.5, 138.6, 128.3, 127.6, 127.4, 72.9, 70.3, 69.7, 61.3, 61.2, 48.3, 37.1, 35.6, 29.9, 25.8, 21.5, 18.0, 14.0, −4.35, −4.82; MS 480 $[M]^+$; HRMS calcd for $[C_{26}H_{44}O_6Si]$ 480.2908; found 480.2906.

4.22. Diethyl 2-[2-(*tert*-butyldimethylsilanyloxy)-6-hydroxyhexyl] malonate (28)

To a solution of **27** (9.5 g, 19.8 mmol) in ethanol (100 mL) was added 10% Pd/C (2 g) under an argon atmosphere at room temperature. Hydrogenation was performed at atmospheric pressure at room temperature for 12 h. The solution was filtered to remove the catalyst, and the filtrate was then evaporated at reduced pressure. Silica gel column chromatography (hexane/ethyl acetate, 10:1, v/v) gave **28** (7.49 g, yield 97%) as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.19–4.11 (m, 4H), 3.70–3.64 (m, 1H), 3.60 (t, 2H, J = 6.5 Hz), 3.52 (dd, 1H, J = 5.2, 9.1 Hz), 2.11–2.04 (m, 1H), 1.94–1.87 (m, 1H), 1.67 (br s, 1H), 1.56–1.31 (m, 6H), 1.230 (t, 3H, J = 7.2 Hz), 1.228 (t, 3H, J = 7.3 Hz), 0.85 (s, 9H), 0.00 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.9, 169.5, 69.7, 62.6, 61.3, 61.2, 48.3, 37.0, 35.4, 32.7, 25.8, 20.9, 18.0, 14.0, −4.4, −4.9; MS 333 $[M-t\text{-Bu}]^+$; HRMS calcd for $[C_{15}H_{29}O_6Si]$ 333.1734; found 333.1741.

4.23. Diethyl 2-[2-(*tert*-butyldimethylsilanyloxy)-6-oxohexyl] malonate (29)

To a cooled (−78 °C) solution of oxalyl chloride (984 μL, 11.3 mmol) in dry CH_2Cl_2 (100 mL) was added dimethylsulfoxide (1.20 mL, 16.9 mmol) in dry CH_2Cl_2 (10 mL) and stirred at this temperature for 10 min. A solution of **28** (2.20 g, 5.64 mmol) in CH_2Cl_2 (10 mL) was slowly added to the cooled solution for 5 min. After the addition was completed, the reaction mixture was stirred at −78 °C for 15 min and then at −48 °C for 45 min. Triethylamine (5.50 mL, 39.5 mmol) was added and the mixture was stirred at 0 °C for 20 min. The resulting solution was quenched by being poured into saturated aqueous NH_4Cl (100 mL) and extracted with ethyl acetate (3× 100 mL). The combined extracts were washed with brine (100 mL), dried over $MgSO_4$, filtered, and concentrated under reduced pressure to afford the crude product, which was purified by flash column chromatography (hexane/ethyl acetate, 10:1, v/v) to give pure **29** (1.95 g, yield 89%) as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.72 (s, 1H), 4.17–4.11 (m, 4H), 3.70–3.66 (m, 1H), 3.49 (dd, 1H, J = 5.5, 8.8 Hz), 2.392 (t, 1H, J = 7.3 Hz), 2.387 (t, 1H, J = 7.3 Hz), 2.10–2.03 (m, 1H), 1.96–1.90 (m, 1H), 1.68–1.60 (m, 2H), 1.47–1.42 (m, 2H), 1.23 (t, 6H, J = 7.3 Hz), 0.84 (s, 9H), 0.00 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 202.1, 169.8, 169.4, 69.3, 61.4, 61.3, 48.2, 43.7, 36.5, 35.4, 25.8, 17.3, 14.0, −4.4, −4.9; MS 388 $[M]^+$; HRMS calcd for $[C_{19}H_{36}O_6Si]$ 388.2281; found 388.2276.

4.24. Diethyl (6*Z*,9*Z*,12*Z*,15*Z*)-2-[2-(*tert*-butyldimethylsilanyloxy)henicosa-6,9,12,15-tetraenyl] malonate (30)

To a cooled (−78 °C), stirred solution of the phosphonium salt **16** (3.0 g, 5.48 mmol) in THF (30 mL) was added lithium hexamethyldisilazide (1 M THF solution; 5.46 mL, 5.46 mmol) dropwise under an argon atmosphere. After stirring at −78 °C for 2 h, HMPA (3 mL) was added, the reaction mixture was stirred for 10 min, and then aldehyde **29** (1.90 g, 4.89 mmol) in THF (6 mL) was added to the resulting red solution. The reaction mixture was stirred at −78 °C for 1 h and then allowed to warm slowly to 0 °C over a period of 1 h. It was then quenched by the addition of a 2 N HCl solution (2 mL) and extracted with diethyl ether (3× 50 mL). The combined extracts were washed with cold water (3× 25 mL), dried over $MgSO_4$, filtered, and concentrated under reduced pressure to afford the crude product, which was purified by flash column chromatography (diethyl ether/hexane, 1:20, v/v) to give pure **30** (1.95 g, yield 69%) as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.38–5.29 (m, 8H), 4.17–4.12 (m, 4H), 3.66 (tt, 1H, J = 3.9, 4.2 Hz), 3.51 (dd, 1H, J = 5.1, 8.8 Hz), 2.84–2.76 (m, 6H), 2.10–1.87 (m, 6H), 1.47–1.17 (m, 16H), 0.85 (br s, 12H), 0.00 (br s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.9, 169.5, 130.4, 129.9, 128.5, 128.3, 128.1, 127.9, 127.5, 69.7, 61.3, 61.2, 48.3, 36.9, 35.6, 31.5, 29.3, 27.2, 25.8, 25.6, 24.7, 22.5, 18.0, 14.0, −4.3, −4.8; MS 576 $[M]^+$; HRMS calcd for $[C_{34}H_{60}O_5Si]$ 576.4210; found 576.4202.

4.25. (6*Z*,9*Z*,12*Z*,15*Z*)-2-[2-(*tert*-Butyldimethylsilanyloxy)henicosa-6,9,12,15-tetraenyl]propane-1,3-diol (31)

To a solution of compound **30** (1.65 g, 493 mmol) in dry diethyl ether (50 mL) was added dropwise $LiAlH_4$ (435 mg) in dry diethyl ether at 0 °C under an argon atmosphere. The reaction mixture was stirred at room temperature for 1 h. Ethyl acetate (0.5 mL) was then added dropwise to the mixture to quench excess $LiAlH_4$ at 0 °C. The mixture was diluted with ethyl acetate (100 mL), then filtered through a pad of silica gel. The filtrate was successively washed with 50 mL of water and brine. The organic layer was dried ($MgSO_4$) and concentrated in vacuo. The crude residue was chromatographed on silica gel (hexane/ethyl acetate, 5:1, v/v) to give **31** (1.14 g, yield 81%) as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.36–5.23 (m, 8H), 3.73 (tt, 1H, J = 4.6, 6.1 Hz), 3.61–3.51 (m, 4H), 2.78–2.72 (m, 6H), 2.00–1.95 (m, 5H), 1.91–1.83 (m, 1H), 1.52–1.18 (m,

13H), 0.82 (br s, 12H), 0.00 (br s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 130.5, 129.9, 128.6, 128.3, 128.1, 127.9, 127.6, 71.0, 66.7, 65.9, 38.9, 36.5, 35.4, 31.5, 29.3, 27.3, 27.2, 25.9, 25.6, 25.4, 22.6, 18.1, 14.1, −4.4, −4.5; MS 492 $[M]^+$; HRMS calcd for $[C_{30}H_{56}O_3Si]$ 492.3999; found 492.3997.

4.26. (8*Z*,11*Z*,14*Z*,17*Z*)-2-(Hydroxymethyl)tricosa-8,11,14,17-tetraene-1,4-diol (6)

TBAF (1 M THF solution; 4.30 mL) was added dropwise to the solution of ethyl ester **31** (1.06 g, 2.15 mmol) in THF (20 mL) at 0 °C under an argon atmosphere. The reaction mixture was warmed to room temperature and stirred for 1 h. Removal of the solvent and chromatography on silica gel (hexane/ethyl acetate, 1:1, v/v) of the residue gave **6** (586 mg, yield 72%) as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$–D_2O) δ 5.41–5.33 (m, 8 H), 3.77–3.63 (m, 5H), 2.85–2.78 (m, 6H), 2.07 (dt, 4H, J = 7.0, 7.0 Hz), 1.95 (dt, 1H, J = 5.8, 5.8 Hz), 1.74–1.67 (m, 2H), 1.60–1.22 (m, 10H), 0.89 (t, 3H, J = 6.7 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 130.5, 129.8, 128.6, 128.3, 128.2, 128.1, 127.9, 127.5, 70.2, 65.6, 65.3, 40.5, 37.8, 36.7, 31.5, 29.3, 27.2, 27.1, 25.7, 25.6, 25.5, 22.5, 14.0; MS 378 $[M]^+$; HREIMS calcd for $[C_{24}H_{42}O_3]$ 378.3134; found 378.3134.

4.27. (8*Z*,11*Z*,14*Z*,17*Z*)-1-(Triisopropylsilanyloxy)-2-(triisopropylsilanyloxymethyl)tricosa-8,11,14,17-tetraen-4-ol (32)

The triol **6** (500 mg, 1.32 mmol) was dissolved in DMF (10 mL) containing imidazole (297 mg, 4.36 mmol) and cooled to 0 °C under an argon atmosphere. Triisopropylsilyl chloride (TIPSCl) (764 mg, 3.96 mmol) in DMF (5 mL) was added and the reaction mixture was stirred at room temperature for 1 h. The mixture was diluted with ethyl acetate (50 mL), then washed with water (30 mL) and brine (30 mL). The organic layer was dried over $MgSO_4$, filtered, and concentrated in vacuo. Chromatography on silica gel (hexane/ethyl acetate, 40:1, v/v) gave **32** (548 mg, yield 60%) as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.41–5.32 (m, 8H), 3.80 (dd, 2H, J = 5.8, 9.8 Hz), 3.72 (dd, 2H, J = 5.8, 9.8 Hz), 3.60 (tt, 1H, J = 6.1, 6.4 Hz), 2.85–2.80 (m, 6H), 2.11–2.03 (m, 4H), 1.96 (dt, 1H, J = 6.1, 6.4 Hz), 1.57–1.26 (m, 13H), 1.13–1.01 (m, 6H), 1.07 (br s, 36H), 0.89 (t, 3H, J = 6.9 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 130.5, 130.3, 128.52, 128.48, 128.01, 127.96, 127.7, 127.6, 69.98, 65.7, 65.3, 42.5, 38.8, 37.4, 31.5, 29.3, 27.3, 27.2, 25.9, 25.6, 22.6, 18.0, 14.1, 11.9; MS 691 $[M]^+$; HRMS calcd for $[C_{42}H_{82}O_3Si_2]$ 690.5803; found 690.5803.

4.28. (8*Z*,11*Z*,14*Z*,17*Z*)-1-(Triisopropylsilanyloxy)-2-(triisopropylsilanyloxymethyl)tricosa-8,11,14,17-tetraen-4-one (33)

PDC (503 mg, 1.34 mmol) in CH_2Cl_2 (10 mL) was added to alcohol **32** (433 mg, 627 μmol) containing molecular sieves 4A in CH_2Cl_2 (30 mL) at 0 °C under an argon atmosphere. The reaction mixture was stirred at room temperature for 20 h and then concentrated with silica gel (10 g). The residue was chromatographed on silica gel (hexane/ethyl acetate, 40:1, v/v) to give **33** (319 mg, yield 74%) as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.43–5.31 (m, 8H), 3.71 (dd, 2H, J = 5.8, 9.8 Hz), 3.65 (dd, 2H, J = 5.5, 9.8 Hz), 2.85–2.76 (m, 6H), 2.49 (d, 2H, J = 6.4 Hz), 2.43 (t, 2H, J = 7.6 Hz), 2.28 (dt, 1H, J = 5.8, 6.4 Hz), 2.10–2.01 (m, 4H), 1.64 (dt, 2H, J = 7.6, 7.6 Hz), 1.40–1.26 (m, 6H), 1.12–0.96 (m, 6H), 1.04 (br s, 36H), 0.89 (t, 3H, J = 6.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 130.5, 129.3, 128.6, 128.23, 128.17, 127.9, 127.5, 63.2, 42.7, 41.0, 40.0, 31.5, 30.9, 29.3, 27.2, 26.6, 25.6, 23.7, 22.6, 18.0, 14.1, 12.0; MS 688 $[M]^+$; HRMS calcd for $[C_{42}H_{80}O_3Si_2]$ 688.5646; found 688.5646.

4.29. (8*Z*,11*Z*,14*Z*,17*Z*)-1-Hydroxy-2-(hydroxymethyl)tricosa-8,11, 14,17-tetraen-4-one (7)

TBAF (1 M THF solution; 777 μL, 777 μmol) was added dropwise to a solution of silyl ether **33** (228 mg, 331 μmol) in THF (10 mL) at 0 °C under an argon atmosphere. The reaction mixture was warmed to room temperature and stirred for 1 h. Removal of the solvent and chromatography on silica gel (hexane/ethyl acetate, 1:1, v/v) of the residue gave **7** (90 mg, yield 76%) as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$–D_2O) δ 5.42–5.32 (m, 8H), 3.74 (dd, 2H, J = 4.8, 10.8 Hz), 3.68 (dd, 2H, J = 5.7, 10.8 Hz), 2.85–2.79 (m, 6H), 2.57 (d, 2H, J = 6.7 Hz), 2.47 (t, 2H, J = 7.3 Hz), 2.29 (ddd, 1H, J = 4.8, 5.8, 6.7 Hz), 2.12–1.99 (m, 4H), 1.66 (dt, 2H, J = 7.3, 7.3 Hz), 1.40–1.25 (m, 6H), 0.89 (t, 3H, J = 6.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 210.9, 130.5, 129.1, 128.8, 128.6, 128.3, 128.2, 127.9, 127.6, 65.1, 42.7, 41.5, 38.3, 31.5, 29.3, 27.2, 26.5, 25.7, 23.6, 22.6, 14.1; MS 358 $[M-H_2O]^+$; HREIMS calcd for $[C_{24}H_{38}O_2]$ 358.2872; found 358.2867.

4.30. 6-(*tert*-Butyldimethylsilanyloxy)hexan-1-ol (34)

Compound **34** was prepared from 1,6-hexanediol by a reported method.[26]

4.31. *tert*-Butyl(6-iodohexyloxy)dimethylsilane (35)

To a solution of **34** (119 mg, 513 μmol) in dry DMF (10 mL) was added methyltriphenoxyphosphonium iodide (387 mg, 956 μmol). The mixture was stirred at room temperature for 20 min under an argon atmosphere. The crude product was purified by flash column chromatography (hexane/ethyl acetate, 20:1, v/v) to afford **35** (120 mg, yield 82%) as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.55 (t, 2H, J = 6.8 Hz), 3.15 (t, 2H, J = 7.2 Hz), 1.82–1.75 (m, 2H), 1.49–1.47 (m, 2H), 1.40–1.30 (m, 4H), 0.85 (s, 9H), 0.01 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 63.2, 32.7, 28.9, 25.8, 25.5, 18.3, 6.5, −5.4; MS 285 $[M-t\text{-Bu}]^+$; HREIMS calcd for $[C_8H_{18}OISi]$ 285.0172; found 285.0172.

4.32. Diethyl 2-[6-(*tert*-butyldimethylsilanyloxy)hexyl] malonate (36)

The compound **35** (70 mg, 246 μmol) was converted to dicarboxylate **36** (69 mg) according to the procedure

described above for obtaining **27** from **26** in 75% yield as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.15 (dd, 4H, J = 7.2, 14.4 Hz), 3.54 (t, 2H, J = 6.4 Hz), 3.27 (t, 1H, J= 7.6 Hz), 1.84 (dd, 2H, J = 7.6, 14.8 Hz), 1.46 (t, 2H, J = 6.4 Hz), 1.26–1.25 (m, 6H), 1.22 (t, 6H, J = 7.2 Hz), 0.81 (s, 9H), 0.01 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.5, 63.1, 61.2, 52.0, 32.7, 29.0, 28.6, 27.3, 25.9, 25.5, 18.3, 14.0, −5.4; MS 374 $[M]^+$; HREIMS calcd for $[C_{19}H_{38}O_5Si]$ 374.2489; found 374.2468.

4.33. Diethyl 2-(6-hydroxyhexyl)malonate (37)

To a solution of **36** (494 mg, 1.32 mmol) in THF was added 1.0 M TBAF in THF. The mixture was stirred at room temperature for 1 h. It was then concentrated in vacuo and the crude product was purified by flash column chromatography (hexane/ethyl acetate, 2:1, v/v) to afford **37** (120 mg, yield 35%) as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.19 (dd, 4H, J = 7.2, 14.4 Hz), 3.62 (t, 2H, J = 6.8 Hz), 3.31 (t, 1H, J = 7.6 Hz), 2.18 (s, 1H), 1.90 (dd, 2H, J = 7.6, 14.8 Hz), 1.56 (t, 2H, J = 6.8 Hz), 1.38–1.31 (m, 6H), 1.28 (t, 6H, J = 7.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.5, 62.7, 61.2, 53.8, 52.0, 32.5, 31.6, 29.2, 28.9, 28.6, 27.2, 25.4, 14.0; MS 260 $[M]^+$; HREIMS calcd for $[C_{13}H_{24}O_5]$ 260.1624; found 260.1622.

4.34. 2-((6*Z*,9*Z*,12*Z*,15*Z*)-Henicosa-6,9,12,15-tetraenyl)propane-1,3-diol (8)

The alcohol **37** (70 mg, 269 μmol) was converted to an aldehyde according to the procedure described above for obtaining **29** from **28**. The crude aldehyde was treated with **16** according to the same procedure used for **20a** and **20b**. Then, reduction of the ester to alcohol gave **8** (12 mg) in 12% overall yield in 3 steps as a colorless oil. : ^{1}H NMR (400 MHz, $CDCl_3$–D_2O) δ 5.42–5.33 (m, 8H), 3.54 (dd, 4H, J = 5.7, 10.8 Hz), 2.86–2.78 (m, 6H), 2.14–1.98 (m, 4H), 1.66 (m, 1H), 1.40–1.25 (m, 14H), 0.89 (t, 3H, J = 6.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 130.7, 129.3, 128.8, 128.6, 128.3, 128.2, 127.9, 127.7, 65.2, 44.7, 31.7, 29.4, 27.3, 26.6, 25.7, 23.6, 22.6, 14.1; MS 362 $[M]^+$; HREIMS calcd for $[C_{24}H_{42}O_2]$ 362.3185; found 362.3180.

4.35. Estimation of $[Ca^{2+}]_i$ in NG108-15 cells and HL-60 cells

Subconfluent NG108-15 cells or HL-60 cells were further incubated in fresh medium without fetal bovine serum for 24 h. The cells were next suspended by gentle pipetting in Hepes–Tyrode's solution (–Ca^{2+}) containing 3 μM Fura-2/AM and further incubated at 37 °C for 45 min. The cells were then centrifuged (180*g* for 5 min), washed twice with Hepes–Tyrode's solution (–Ca^{2+}), and resuspended in Hepes–Tyrode's solution (–Ca^{2+}) containing 0.1% bovine serum albumin. $[Ca^{2+}]_i$ was estimated using a CAF-100 Ca^{2+} analyzer (JASCO, Tokyo, Japan) as described previously.[3–5] $CaCl_2$ was added 4–5 min before the measurement (final Ca^{2+} concentration in the cuvette:1 mM). 2-AG and its structural analogs were dissolved in dimethylsulfoxide, and aliquots (1 μL each) were added to the cuvette (final concentration of dimethylsulfoxide: 0.2%).

Acknowledgments

We are grateful to Junko Shimode and Maroka Kitsukawa for the spectroscopic measurements. This study was supported in part by a Grant-in-Aid from the Ministry of Education, Culture, Sports, Science and Technology, the Japanese Government.

References and notes

1. Sugiura, T.; Kondo, S.; Sukagawa, A.; Nakane, S.; Shinoda, A.; Itoh, K.; Yamashita, A.; Waku, K. *Biochem. Biophys. Res. Commun.* **1995**, *215*, 89–97.
2. Mechoulam, R.; Ben-Shabat, S.; Hanus, L.; Ligumsky, M.; Kaminski, N. E.; Schatz, A. R.; Gopher, A.; Almong, S.; Martin, B. R.; Compton, D. R.; Pertwee, R. G.; Griffin, G.; Bayewitch, M.; Barg, J.; Vogel, Z. *Biochem. Pharmacol.* **1995**, *50*, 83–90.
3. Sugiura, T.; Kodaka, T.; Kondo, S.; Nakane, S.; Kondo, H.; Waku, K.; Ishima, Y.; Watanabe, K.; Yamamoto, I. *J. Biochem.* **1997**, *122*, 890–895.
4. Sugiura, T.; Kodaka, T.; Nakane, S.; Miyashita, T.; Kondo, S.; Suhara, Y.; Takayama, H.; Waku, K.; Seki, C.; Baba, N.; Ishima, Y. *J. Biol. Chem.* **1999**, *274*, 2794–2801.
5. Sugiura, T.; Kondo, S.; Kishimoto, S.; Miyashita, T.; Nakane, S.; Kodaka, T.; Suhara, Y.; Takayama, H.; Waku, K. *J. Biol. Chem.* **2000**, *275*, 605–612.
6. Sugiura, T.; Kodaka, T.; Kondo, S.; Tonegawa, T.; Nakane, S.; Kishimoto, S.; Yamashita, A.; Waku, K. *Biochem. Biophys. Res. Commun.* **1997**, *233*, 207–210.
7. Stella, N.; Schweitzer, P.; Piomelli, D. *Nature* **1997**, *388*, 773–778.
8. Ameri, A.; Simmet, T. *Naunyn Schmiedebergs Arch. Pharmacol.* **2000**, *361*, 265–272.
9. Lee, M.; Yang, K. H.; Kaminski, N. E. *J. Pharmacol. Exp. Ther.* **1995**, *275*, 529–536.
10. Mechoulam, R.; Fride, E.; Ben-Shabat, S.; Meiri, U.; Horowitz, M. *Eur. J. Pharmacol.* **1998**, *362*, R1–R3.
11. Jorda, M. A.; Verbakel, S. E.; Valk, P. J.; Vankan-Berkhoudt, Y. V.; Maccarrone, M.; Finazzi-Agro, A.; Lowenberg, B.; Delwel, R. *Blood* **2002**, *99*, 2786–2793.
12. Walter, L.; Franklin, A.; Witting, A.; Wade, C.; Xie, Y.; Kunos, G.; Mackie, K.; Stella, N. *J. Neurosci.* **2003**, *23*, 1398–1405.
13. Kishimoto, S.; Gokoh, M.; Oka, S.; Muramatsu, M.; Kajiwara, T.; Waku, K.; Sugiura, T. *J. Biol. Chem.* **2003**, *278*, 24469–24475.
14. Sugiura, T.; Waku, K. *Chem. Phys. Lipids* **2000**, *108*, 89–106.
15. Sugiura, T.; Kobayashi, Y.; Oka, S.; Waku, K. *Prostaglandins Leukot. Essent. Fatty Acids* **2002**, *66*, 173–192.
16. Suhara, Y.; Takayama, H.; Nakane, S.; Miyashita, T.; Waku, K.; Sugiura, T. *Chem. Pharm. Bull.* **2000**, *48*, 903–907.
17. Khanapure, P. S.; Shi, X.-X.; Powell, S. W.; Rokach, J. *J. Org. Chem.* **1998**, *63*, 337–427.
18. Kerdesky, A. J. F.; Schmidt, P. S.; Holms, H. J.; Dyer, D. R.; Carter, W. G.; Brooks, W. D. *J. Med. Chem.* **1987**, *30*, 1177–1186.
19. Parkkari, T.; Salo, O. M.; Huttunen, K. M.; Savinainen, J. R.; Laitinen, J. T.; Poso, A.; Nevalainen, T.; Jarvinen, T. *Bioorg. Med. Chem.* **2006**, *14*, 2850–2858.

20. Bobrov, M.; Gretskaya, N.; Payet, O.; Bezuglov, V.; Durand, T.; Maurin, L.; Tourrel, F.; Adjali, O.; Rinaldi-Carmona, M.; Muller, A. *Life Sci.* **2005**, *77*, 1425–1440.
21. Parkkari, T.; Myllymaki, M.; Savinainen, J. R.; Saario, S. M.; Castillo-Melendez, J. A.; Laitinen, J. T.; Nevalainen, T.; Koskinen, A. M.; Jarvinen, T. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 2437–2440.
22. Vandevoorde, S.; Saha, B.; Mahadevan, A.; Razdan, R. K.; Pertwee, R. G.; Martin, B. R.; Fowler, C. J. *Biochem. Biophys. Res. Commun.* **2005**, *337*, 104–109.
23. Ghafouri, N.; Tiger, G.; Razdan, R. K.; Mahadevan, A.; Pertwee, R. G.; Martin, B. R.; Fowler, C. J. *Br. J. Pharmacol.* **2004**, *143*, 774–784.
24. Hanus, L.; Abu-Lafi, S.; Fride, E.; Breuer, A.; Vogel, Z.; Shalev, D. E.; Kustanovich, I.; Mechoulam, R. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 3662–3665.
25. Kishimoto, S.; Kobayashi, Y.; Oka, S.; Gokoh, M.; Waku, K.; Sugiura, T. *J. Biochem.* **2004**, *135*, 517–524.
26. Brown, J. M.; Leppard, S. J.; Oakes, J.; Thornthwaite, D. *Chirality* **2000**, *12*, 496–504.