# Synthesis and Biological Activities of 2-Arachidonoylglycerol, an Endogenous Cannabinoid Receptor Ligand, and Its Metabolically Stable Ether-linked Analogues

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**We synthesized 2-arachidonoylglycerol (1), an endogenous cannabinoid receptor ligand, and its metabolically stable ether-linked analogues. Compound 1 was synthesized from 1,3-benzylideneglycerol (6) and arachidonic acid in the presence of *N*,*N*′-dicyclohexylcarbodiimide and 4-dimethylaminopyridine followed by treatment with boric acid and trimethyl borate. An ether-linked analogue of 2-arachidonoylglycerol (2) was synthesized from 6 and 5,8,11,14-eicosatetraenyl iodide (9). The ether-linked analogues of 2-palmitoylglycerol (4) and 2-oleoyglycerol (5) were synthesized from 6 and hexadecyl iodide (12) and 9-octadecenyl iodide (14), respectively. We confirmed that 1 stimulates NG108-15 cells to induce rapid transient elevation of the intracellular free $Ca^{2+}$ concentrations through a CB1 receptor-dependent mechanism. Noticeably, 2 exhibited appreciable agonistic activity, although its activity was significantly lower than that of 1. Compound 2 would be a useful tool in exploring the physiological significance of 1, because this compound is resistant to hydrolyzing enzymes in contrast to 1. On the other hand, the ether-linked analogues of either 4 or 5 failed to act as a CB1 receptor agonist. Compounds 4 and 5 would also be valuable as control molecules in experiments where 2 is employed.**

**Key words** cannabinoid; 2-arachidonoylglycerol; anandamide; $\Delta^9$-tetrahydrocannabinol; monoacylglycerol; ether-linked analogue

$\Delta^9$-Tetrahydrocannabinol ($\Delta^9$-THC) is a psychoactive ingredient of marijuana and is known to exhibit a variety of pharmacological activities *in vitro* and *in vivo*.[1] For example, $\Delta^9$-THC induces euphoria, heightened sensory awareness, altered cognition, inhibition of memory and reduced spontaneous activity. Noticeably, $\Delta^9$-THC also exerts several beneficial effects such as analgesia, antiemesis, promotion of appetite and immunosuppression in humans and in experimental animals; cannabimimetic molecules without deleterious activities should be of potential value as possible therapeutic drugs.

The mechanism underlying various *in vitro* and *in vivo* actions of cannabinoids remained unclear until the late 1980's. It has long been assumed that the effects of $\Delta^9$-THC may be due to the membrane perturbation of target tissues and cells. Studies by several investigators in this decade, however, revealed that various cannabinoids including $\Delta^9$-THC interact with specific receptor sites,[2] termed the CB1 receptor (present primarily in the nervous system)[3] and the CB2 receptor (present mainly in the immune system),[4] thereby eliciting the responses.

The discovery of such specific receptor sites for the cannabinoids prompted a search for the endogenous ligand as in the case of opioids. To date, two types of arachidonic acid-containing molecules have been proposed as putative endogenous ligands: *N*-arachidonoylethanolamine (anandamide)[5] and 2-arachidonoylglycerol (**1**).[6—8] Recently, we provided evidence that **1** but not anandamide is the endogenous natural ligand for the cannabinoid CB1 receptor.[9,10] Compound **1** is a potent full agonist toward the CB1 receptor and exhibits various cannabimimetic activities such as the inhibition of adenylyl cyclase in mouse splenocytes, inhibition of twitch response in mouse vas deferens, modulation of proliferation of mouse lymphocytes, $Ca^{2+}$ transients in NG108-15 cells, inhibition of long-term potentiation in hippocampus and the induction of hypomotility, hypothermia and analgesia when administered to mice.[7—13]

Compound **1** is the natural ligand of the cannabinoid receptor; it seems possible that cannabimimetic compounds without undesirable deleterious properties and therapeutically valuable will be found from among the structural analogues or derivatives of **1**. One important problem concerning **1** as a pharmacological tool or a therapeutic drug is that it is very easily cleaved by hydrolyzing enzymes.[14,15] The development of metabolically stable analogues is, therefore, essential. Previously, we[10] and Mechoulam *et al.*[16] developed an ether-linked analogue of 2-arachidonoylglycerol (**2**). This compound exhibited appreciable agonistic activities *in vitro* and *in vivo*.[10,16] However, detailed information including the chemical data concerning the synthesis of this novel class of the 2-arachidonoylglycerol analogue has not yet been reported. Furthermore, not much is yet known concerning the chemical synthesis of **1** itself. It is important to provide detailed information concerning chemical synthesis of these compounds in order to develop more potent and valuable analogues of 2-arachidonoylglycerol in the future. It is also necessary to develop an analogue of **2** lacking agonistic activity as a control molecule, because high concentrations of the ether-linked analogues of the monoacylglycerols may also exert nonspecific physiocochemical effects such as detergent effects.

Here, we report the biological activities as well as details of the chemical synthesis of **1**, its ether-linked analogue (**2**) which acts as the CB1 receptor agonist, and the newly developed ether-linked analogues of 2-arachidonoylglycerol (**3**)—(**5**) which lack agonistic activities toward the CB1 receptor.

## Results and Discussion

As shown in Chart 1, the synthesis of 2-arachidonoylglycerol (**1**) was carried out from commercially available arachi-

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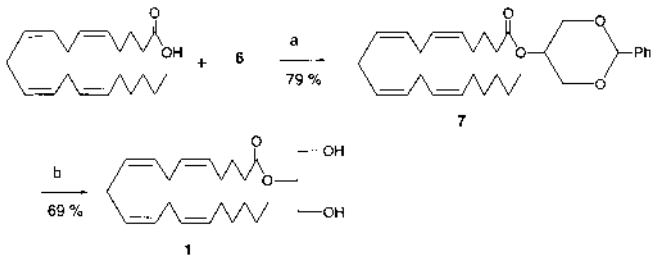


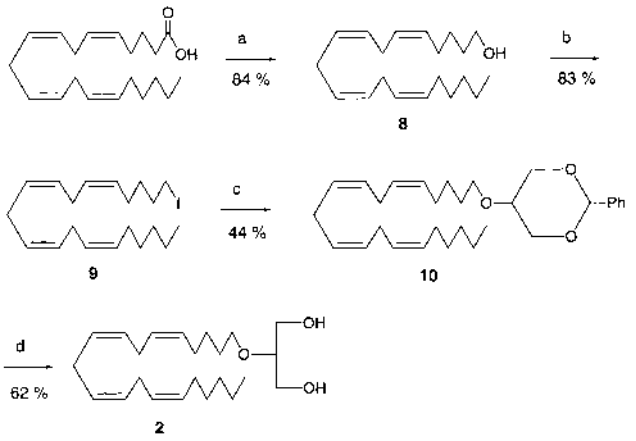

Reagents and conditions: (a) **6**:1, 3-benzylideneglycerol, DCC, DMF; (b) boric acid, trimethyl borate.

Chart 1

donic acid and glycerol. The primary hydroxy groups of glycerol were selectively protected by benzaldehyde using 5-sulfosalicilic acid dihydrate as a catalyst in benzene, and then recrystallized from benzene/hexane to give a secondary alcohol **6** in 17% yield. Esterification of the arachidonic acid with alcohol **6** using 4-dimethylaminopyridine (DMAP) and *N*,*N*-dicyclohexylcarbodiimide (DCC) in dry toluene at room temperature for 6 h provided ester **7** in 79% yield. Deprotection of the benzylidene group was accomplished by dissolving ester **7** in trimethyl borate at room temperature followed by the addition of boric acid.[17] The reaction mixture was stirred at 100 °C for 30 min, then cooled to room temperature. Chromatography on silica gel gave **1** in 69% yield with 55% conversion. In this process, about 10% of transesterification was observed in **1** by $^1$H- NMR, therefore, compound **1** was further purified by borate-impregnated TLC to remove any contaminating 1- or 3-isomer. The purity was confirmed by $^{13}$C-NMR and MS. Recently, Han and Razdan[18] also reported the chemical synthesis of 2-arachidonoylglycerol, although their method employing triisopropylsilyl chloride as a hydroxy group-protecting reagent was different from our currently described procedure. The advantage in using 1,3-benzylideneglycerol (**6**) for the synthesis of **1** and its analogues is that the coupling products **7**, **10**, **13**, and **15** are detectable under UV lamp (254 nm).

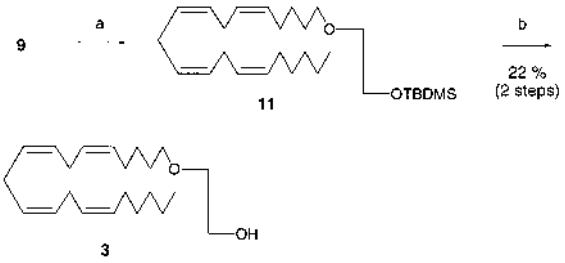
Chart 2 summarizes the synthesis of an ether-linked analog of 2-arachidonoylglycerol, 2-(5,8,11,14-eicosatetraenyl)-glycerol (**2**), which is a metabolically stable cannabinoid receptor ligand. To make an ether bond, arachidonic acid was first converted to an alcohol **8** with lithium aluminum hydride ($LiAlH_4$) in dry diethyl ether at 0 °C, and then treated with methyltriphenoxyphosphonium iodide in *N*,*N*-dimethylformamide (DMF) to give the iodo derivative **9** in good yield. 2-(5,8,11,14-Eicosatetraenyl)-1,3-benzylideneglycerol (**10**) was obtained by condensing **9** and **6** using $Ag_2O$ and tetra-*n*-butylammonium iodide (*n*-$Bu_4NI$) in DMF. After stirring at 90 °C for 6 h in the dark, the coupling product **10** was isolated in 30% yield. Finally, deprotection of the benzylidene group in **10** with boric acid and trimethyl borate gave **2** in 62% yield.

The synthesis of an ether-linked analogue of monoarachidonoylethyleneglycol (1-eicosatetraenyloxyethanol (**3**)) is outlined in Chart 3. The coupling of 5,8,11,14-eicosatetraenyl iodide (**9**) with 3-(*tert*-butyldimethylsilyl)oxyethanol and subsequent removal of the *tert*-butyldimethylsilyl (TBDMS) ether moiety in **11** by treatment with tetrabutylammonium fluoride (TBAF) gave **3** in 22% yield in two steps.



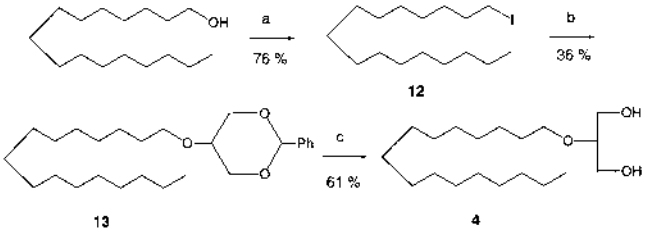

Reagents and conditions: (a) $LiAlH_4$, ether; (b) $CH_3P(OC_6H_5)_3I$, DMF; (c) $Ag_2O$, *n*-$Bu_4NI$, DMF, **6**, at 90°C; (d) boric acid, trimethyl borate.

Chart 2




Reagents and conditions: (a) $Ag_2O$, *n*-$Bu_4NI$, DMF, 2-(*tert*-butyldimethylsilyl)oxyethanol, at 90°C; (b) 1M TBAF in THF.

Chart 3



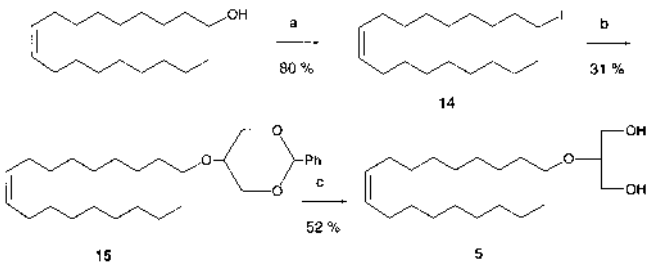

Reagents and conditions: (a) $CH_3P(OC_6H_5)_3I$, DMF; (b) $Ag_2O$, *n*-$Bu_4NI$, DMF, **6**, at 90°C; (c) boric acid, trimethyl borate

Chart 4

2-Hexadecylglycerol (**4**) was prepared from commercially available hexadecanol as shown in Chart 4. The alcohol was converted to the iodide **12** using methyltriphenoxyphosphonium iodide in DMF. The coupling of **12** with 1,3-benzylideneglycerol gave **13**, which was then hydrolyzed to give **4** as described for **2**.

2-(9-Octadecenyl)glycerol (**5**) was synthesized from commercially available *cis*-9-octadecen-1-ol as shown in Chart 5. The iodide **14** was obtained in 80% yield using methyltriphenoxyphosphonium iodide in DMF. The coupling of **14** with 1,3-benzylideneglycerol gave **15**, which was then hydrolyzed to give **5** as described for **2**.

Figure 1 shows the effects of **1**, **2** and related compounds **3**—**5** on the intracellular free $Ca^{2+}$ concentrations ($[Ca^{2+}]_i$) in NG108-15 cells which are known to express the cannabi-




Reagents and conditions: (a) $CH_3P(OC_6H_5)_3I$, DMF; (b) $Ag_2O$, $n$-$Bu_4NI$, DMF, 6, at 90°C; (c) boric acid, trimethyl borate.

Chart 5

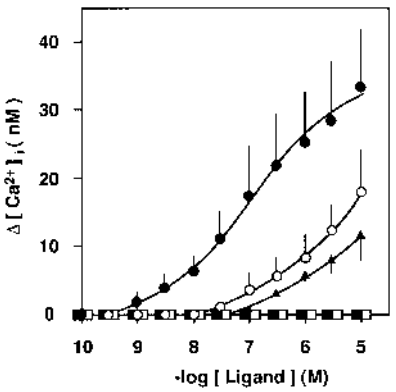



Fig. 1. Comparison of the Activities of 2-Arachidonoylglycerol and Its Ether-linked Analogues in Inducing Rapid Elevation of $[Ca^{2+}]_i$ in NG108-15 Cells

Effects of 2-arachidonoylglycerol and its ether-linked analogues on $[Ca^{2+}]_i$ were examined using Fura-2/AM and a CAF-100 $Ca^{2+}$ analyzer. (●), 2-arachidonoylglycerol (**1**) (2-AG); (○), an ether-linked analogue of 2-arachidonoylglycerol (**2**) (2-AG ether); (▲), an ether-linked analogue of monoarachidonoylethyleneglycol (**3**); (□), an ether-linked analogue of 2-palmitoylglycerol (**4**); (■), an ether-linked analogue of 2-oleoylglycerol (**5**). The mean values ±S.D. were taken from five determinations.

noid CB1 receptor. Low concentrations of **1** induce rapid transient increases in $[Ca^{2+}]_i$ in the NG108-15 cells; the response was detectable from as low as 1 nM, reaching a maximum around 10 $\mu$M. Such a response induced by **1** was blocked by pretreatment of the cells with SR141716A, a cannabinoid CB1 receptor-specific antagonist (data not shown), suggesting that rapid transient increases in $[Ca^{2+}]_i$ occurred through a CB1 receptor-dependent mechanism. Noticeably, **2** also exhibited appreciable agonistic activity, yet its activity was significantly lower than that of **1**. These observations are in general agreement with the result reported earlier,[10] but appear to be inconsistent with the result of the *in vivo* experiment where **2** exhibited much more potent biological activity than **1**.[16] We also found that an ether-linked analogue of monoarachidonoylethyleneglycol (**3**) possesses some weak agonistic activity.

We next examined the activities of the structural analogues of **2** having saturated or monoenoic fatty chains. We found that neither the ether-linked analogue of 2-palmitoylglycerol (**4**) nor the ether-linked analogue of 2-oleoylglycerol (**5**) elicits rapid, transient increases in $[Ca^{2+}]_i$. It is apparent, therefore, that both **4** and **5** are devoid of appreciable agonistic activity toward the cannabinoid CB1 receptor. Compound **2** is resistant to hydrolyzing enzymes and may exist for a long period *in vitro* or *in vivo* without receiving hydrolysis; thus, in some cases, it is not clear whether the effects of **2** are mediated only through the cannabinoid receptors or also through other sites of action. To clarify this issue, compounds **4** and **5**, which lack cannabimimetic activity, should be valuable tools.

Despite the increasing attention paid to **1** these days,[19,20] information concerning it and its structural analogues is still quite limited. Thus, the synthesis of a number of 2-arachidonoylglycerol analogues and evaluation of their biological and pharmacological activities are essential to better understand the physiological significance of **1** and to develop a new class of therapeutic drugs that acts on the cannabinoid receptors in the future.

## Experimental

**General Methods** Melting points were determined using a Yanagimoto hot-stage melting point apparatus and are uncorrected. $^1$H-NMR and $^{13}$C-NMR spectra were recorded in $CDCl_3$ on a GSX 400 spectrometer. Chemical shifts are given in ppm ($\delta$) using tetramethylsilane (TMS) as the internal standard. Mass spectra were registered on a JMS SX-102A instrument. Elemental analysis was performed with a Perkin-Elmer 2400 II instrument. Column chromatography was carried out on silica gel 60 (70—230 mesh, Merck) and preparative TLC was run on silica gel 60 $F_{254}$ (Merck). Unless otherwise noted, all reagents were purchased from commercial suppliers and used as received.

**1,3-Benzylideneglycerol (6)** Glycerol (120 g, 1.30 mol), benzaldehyde (120 g, 1.13 mol), and 5-sulfosalicylic acid dihydrate (1.20 g, 55.0 $\mu$mol) were dissolved in benzene (120 ml), and the mixture was then refluxed at 120 °C overnight with a Dean–Stark apparatus to remove the water. After having cooled to room temperature, the reaction mixture was diluted with diethyl ether (500 ml) and washed with saturated aqueous $NaHCO_3$ and brine. The organic layer was dried over $MgSO_4$ followed by filtration and evaporation. The residue was recrystallized from benzene/hexane (20 : 1) (2 l) to give **6** (41 g, yield 17%) as white needles: mp 79—81 °C. $^1$H-NMR ($CDCl_3$) $\delta$: 7.52—7.35 (m, 5H), 5.55 (s, 1H), 4.15 (dd, 4H, $J$=11.2, 19.5 Hz), 3.62 (t, 1H), 3.11 (br s, 1H). $^{13}$C-NMR ($CDCl_3$) $\delta$: 137.8, 129.1, 128.3, 125.8, 101.7, 72.3, 64.0. High resolution (HR)-EI-MS *m/z*: 180.0785 (Calcd for $C_{10}H_{12}O_3$: 180.0787). *Anal*. Calcd for $C_{10}H_{12}O_3$: C, 66.65; H, 6.71. Found: C, 66.71; H, 6.58.

**2-Arachidonoyl-1,3-benzylideneglycerol (7)** To a solution of **6** (60 mg, 330 $\mu$mol) and arachidonic acid (all-*cis*-5,8,11,14-eicosatetraenoic acid) (100 mg, 328 $\mu$mol) in dry toluene (30 ml) was added DCC (136 mg, 656 $\mu$mol) and DMAP (12 mg, 99 $\mu$mol). The reaction mixture was stirred at room temperature for 6 h, then diluted with ethyl acetate (EtOAc) (50 ml). The mixture was washed with water (50 ml) and brine (50 ml), and the organic layer was dried over $MgSO_4$ and removed. The residue was chromatographed on silica gel (hexane/EtOAc, 3 : 1, v/v) to give **7** (121 mg, yield 79%) as a colorless oil: $^1$H-NMR ($CDCl_3$) $\delta$: 7.52—7.49 (m, 2H), 7.37—7.26 (m, 3H), 5.56 (s, 1H), 5.40—5.35 (m, 8H), 4.72 (t, 1H, $J$=1.6 Hz), 4.27 (dd, 2H, $J$=1.6, 12.8 Hz), 4.16 (dd, 2H, $J$=1.6, 12.8 Hz), 2.83—2.80 (m, 6H), 2.44 (tt, 2H, $J$=5.2, 7.6 Hz), 2.14 (tt, 2H, $J$=6.7, 7.0 Hz), 2.05 (dd, 2H, $J$= 6.7, 13.7 Hz), 1.78—1.74 (m, 2H), 1.36—1.22 (m, 6H), 0.89 (br t, 3H, $J$=6.7 Hz). $^{13}$C-NMR ($CDCl_3$) $\delta$: 173.6, 137.8, 130.5, 129.0, 128.9, 128.6, 128.3, 128.21, 128.15, 127.9, 127.5, 126.0, 101.2, 69.1, 65.8, 33.7, 31.5, 29.3, 27.2, 26.5, 25.6, 24.8, 22.5, 14.0. HR-EI-MS *m/z*: 466.3096 (Calcd for $C_{30}H_{42}O_4$: 466.3083).

**2-Arachidonoylglycerol (1)** A suspension of **7** (500 mg, 1.07 mmol) and boric acid (132 mg, 2.13 mmol) in trimethyl borate (5 ml) was refluxed at 100 °C for 20 min. After having cooled to room temperature, the reaction mixture was diluted with EtOAc (100 ml) and successively washed with 50 ml of water and brine. The organic layer was dried ($MgSO_4$), and the solvent was removed. Silica gel column chromatography (hexane/EtOAc, the ratio ranging from 1 : 1 to 0 : 1, v/v) of the crude residue provided the product **1** (280 mg, 69%) as a colorless oil. Further, compound **1** (12.0 mg) was purified by borate-impregnated TLC to remove contaminating 1- or 3-isomer. Then, pure **1** (10.7 mg) was obtained in 89% yield as a colorless oil: $^1$H-NMR ($CDCl_3$) $\delta$: (m, 8H), 4.93 (tt, 1H, $J$=4.7, 4.7 Hz), 3.83 (d, 4H, $J$=4.6 Hz), 2.85—2.78 (m, 8H), 2.38 (t, 2H, $J$=7.6 Hz), 2.18—2.03 (m, 4H), 1.73 (tt, 2H, $J$=7.3, 7.3 Hz), 1.40—1.23 (m, 6H), 0.89 (br t, 3H, $J$=6.7 Hz). $^{13}$C-NMR ($CDCl_3$) $\delta$: 173.8, 130.5, 129.0, 128.8, 128.6, 128.3, 128.1, 127.8, 127.5, 75.1, 62.5, 33.7, 31.5, 29.3, 27.2, 26.5, 25.6, 24.8, 22.5, 14.0. HR-EI-

MS *m/z*: 378.2779 (Calcd for $C_{23}H_{38}O_4$: 378.2770).

**5,8,11,14-Eicosatetraenol (8)** A solution of arachidonic acid (25 mg, 82 $\mu$mol) in dry diethyl ether (5 ml) was added dropwise to $LiAlH_4$ (30 mg, 791 $\mu$mol) in dry diethyl ether at 0 °C under argon. The reaction mixture was stirred at room temperature for 1 h. EtOAc (0.5 ml) was then added dropwise to the mixture to remove excess $LiAlH_4$ at 0 °C. The mixture was diluted with EtOAc (30 ml), then filtered through Celite. The filtrate was successively washed with 20 ml of water and brine. The organic layer was dried ($MgSO_4$) and removed *in vacuo*. The crude residue was chromatographed on silica gel (hexane : EtOAc, 5 : 1, v/v) to give **8** (20 mg, 84%) as a colorless oil: $^1$H-NMR ($CDCl_3$) $\delta$: 5.42—5.33 (m, 8H), 3.65 (t, 2H, $J$=6.5 Hz), 2.86—2.78 (m, 6H), 2.13—2.03 (m, 4H), 1.63—1.56 (m, 2H), 1.48—1.26 (m, 9H), 0.89 (br t, 3H, $J$=6.8 Hz). $^{13}$C-NMR ($CDCl_3$) $\delta$: 130.5, 129.9, 128.5, 128.3, 128.1, 127.9, 127.5, 62.8, 32.3, 31.5, 29.3, 27.2, 26.9, 25.7, 25.6, 22.5, 14.0. HR-EI-MS *m/z*: 290.2621 (Calcd for $C_{20}H_{34}O$: 290.2610).

**5,8,11,14-Eicosatetraenyl Iodide (9)** To a solution of **8** (100 mg, 328 $\mu$mol) in dry DMF (10 ml) was added methyltriphenoxyphosphonium iodide (280 mg, 619 $\mu$mol). The mixture was stirred at room temperature for 20 min under argon. The crude product was purified by flash column chromatography (hexane/EtOAc, 20 : 1, v/v) to afford **9** (120 mg, 83%) as a colorless oil: $^1$H-NMR ($CDCl_3$) $\delta$: 5.42—5.33 (m, 8H), 3.19 (t, 2H, $J$=6.9 Hz), 2.86—2.80 (m, 6H), 2.12—2.03 (m, 4H), 1.84 (tt, $J$=7.0, 7.4 Hz, 2H), 1.54—1.27 (m, 8H), 0.89 (br t, 3H, $J$=7.1 Hz). $^{13}$C-NMR ($CDCl_3$) $\delta$: 130.5, 129.3, 128.6, 128.4, 128.2, 127.8, 127.5, 33.0, 31.6, 31.5, 30.4, 29.3, 27.2, 26.1, 25.6, 22.6, 14.1. HR-EI-MS *m/z*: 400.1628 (Calcd for $C_{20}H_{33}I$: 400.1628).

**2-(5,8,11,14-Eicosatetraenyl)-1,3-benzylideneglycerol (10)** 1,3-Benzylideneglycerol (**6**) (200 mg, 1.11 mmol), $Ag_2O$ (191 mg, 824 $\mu$mol) and *n*-$Bu_4NI$ (20 mg, 55 $\mu$mol) were added to a stirred solution of iodide **9** (220 mg, 550 $\mu$mol) in DMF (10 ml) at room temperature under argon. The suspension was stirred at 90 °C for 6 h in the dark. After having cooled to room temperature, the mixture was diluted with EtOAc (100 ml) and water (100 ml). The generated silver was removed by filtering through Celite, and the filtrate was successively washed with 100 ml of water and brine. The organic layer was dried over $MgSO_4$ and evaporated to dryness. Column chromatography on silica gel (hexane/EtOAc, 5 : 1, v/v) of the crude residue gave **10** (109 mg, 44%) as a colorless oil: $^1$H-NMR ($CDCl_3$) $\delta$: 7.52—7.50 (m, 2H), 7.35—7.32 (m, 3H), 5.55 (s, 1H), 5.41—5.36 (m, 8H), 4.33 (dd, 2H, $J$=1.5, 12.5 Hz), 4.04 (dd, 2H, $J$=1.8, 12.5 Hz), 3.56 (t, 2H, $J$=16.7 Hz), 3.26 (t, 1H, $J$=1.5 Hz), 2.84—2.80 (m, 6H), 2.13—2.03 (m, 4H), 1.70—1.64 (m, 2H), 1.52—1.44 (m, 2H), 1.36—1.26 (m, 6H), 0.89 (br t, 3H, $J$=7.0 Hz). $^{13}$C-NMR ($CDCl_3$) $\delta$: 138.2, 130.5, 130.0, 128.8, 128.5, 128.4, 128.1, 128.0, 127.9, 127.6, 126.2, 101.3, 70.7, 69.0, 68.8, 65.8, 31.5, 29.4, 29.3, 27.2, 27.0, 26.2, 25.6, 22.6, 14.0. HR-EI-MS *m/z*: 452.3293 (Calcd for $C_{30}H_{44}O_3$: 452.3290).

**2-(5,8,11,14-Eicosatetraenyl)glycerol (2)** Compound **2** was prepared from **10** (420 mg, 929 $\mu$mol) in a manner similar to that for **1**. Column chromatography on silica gel (hexane/EtOAc, 1 : 2, v/v) gave **2** (210 mg, 62%) as a colorless oil: $^1$H-NMR ($CDCl_3$) $\delta$: 5.40—5.33 (m, 8H), 3.77 (dt, 2H, $J$=4.6, 5.8 Hz), 3.68 (dt, 2H, $J$=4.8, 5.5 Hz), 3.58 (t, 2H, $J$=6.6 Hz), 3.46 (dt, 1H, $J$=4.6, 4.9 Hz), 2.86—2.80 (m, 6H), 2.13—2.03 (m, 4H), 1.91 (br s, 2H), 1.67-1.58 (m, 2H), 1.48-1.43 (m, 2H), 1.38—1.26 (m, 6H), 0.89 (br t, 3H, $J$=6.8 Hz). $^{13}$C-NMR ($CDCl_3$) $\delta$: 130.5, 129.8, 128.6, 128.3, 128.21, 128.15, 127.9, 127.5, 79.6, 70.0, 62.3, 31.5, 29.7, 29.3, 27.2, 27.0, 26.2, 25.6, 22.6, 14.1. HR-EI-MS *m/z*: 364.2980 (Calcd for $C_{23}H_{40}O_3$: 364.2979).

**1-(5,8,11,14-Eicosatetraenyl)oxyethanol (3)** To a solution of iodide **9** (180 mg, 435 $\mu$mol) in DMF (5 ml) was added 1-(*tert*-butyldimethylsilyl)oxyethanol (77 mg, 435 $\mu$mol), $Ag_2O$ (151 mg, 652 $\mu$mol) and *n*-$Bu_4NI$ (16 mg, 43 $\mu$mol) at room temperature. The suspension was stirred at 90 °C for 6 h under argon in the dark. After having cooled to room temperature, the reaction mixture was diluted with EtOAc (30 ml) and water (20 ml) followed by filtration through Celite to remove the generated silver. The filtrate was successively washed with 100 ml of water and brine. The organic layer was dried over $MgSO_4$ then removed *in vacuo*. The crude residue **11** was used in the next step without further purification. Namely, a 1.0 M solution of TBAF in THF (870 $\mu$mol/870 $\mu$l) was added dropwise to crude silyl ether **11** in THF (5 ml) at 0 °C under argon. The reaction mixture was warmed to room temperature and stirred for 1 h. Removal of the solvent and subsequent chromatography on silica gel (hexane/EtOAc, 5 : 1 to 3 : 1, v/v) of the residue gave pure **3** (33 mg, 22% in two steps) as a colorless oil: $^1$H-NMR ($CDCl_3$) $\delta$: 5.42—5.33 (m, 8H), 3.73 (d, 2H, $J$=4.0 Hz), 3.53 (t, 2H, $J$=4.0 Hz), 3.48 (t, 2H, $J$=6.5 Hz), 2.86—2.78 (m, 6H), 2.08 (ddd, 4H, $J$=6.7, 14.3 Hz), 1.97 (br s, 1H), 1.65—1.57 (m, 2H), 1.47—1.26 (m, 8H), 0.89 (br t, 3H, $J$=6.9 Hz). $^{13}$C-NMR ($CDCl_3$) $\delta$: 130.5, 129.9, 128.6, 128.4, 128.1, 127.9, 127.6, 71.7, 71.2, 61.9, 31.5, 29.32, 29.29, 27.2, 27.0, 26.1, 25.6, 22.6, 14.1. HR-EI-MS *m/z*: 334.2864 (Calcd for $C_{22}H_{38}O_2$: 334.2872).

**Hexadecyl Iodide (12)** Hexadecyl iodide (**12**) was prepared from commercially available hexadecanol (100 mg, 413 $\mu$mol) as described for **9**. Column chromatography on silica gel (hexane/EtOAc, 1 : 2, v/v) gave **12** (110 mg, 76%) as a colorless oil: $^1$H-NMR ($CDCl_3$) $\delta$: 3.17 (t, 2H, $J$=7.2 Hz), 1.82 (tt, 2H, $J$=7.0, 7.3 Hz), 1.26 (br s, 26H), 0.88 (br t, 3H, $J$=6.8 Hz). $^{13}$C-NMR ($CDCl_3$) $\delta$: 33.6, 31.9, 30.5, 29.69. 29.65, 29.56, 29.43, 29.36, 28.6, 22.7, 14.1. HR-EI-MS *m/z*: 352.1635 (Calcd for $C_{16}H_{33}I$: 352.1628).

**2-Hexadecyl-1,3-benzylideneglycerol (13)** 2-Hexadecyl-1,3-benzylidene glycerol (**13**) was prepared from **12** (100 mg, 284 $\mu$mol) as described for **10**. Silica gel column chromatography (hexane/EtOAc, 1 : 2, v/v) gave **13** (41 mg, 36%) as a white solid: mp 49—51 °C. $^1$H-NMR ($CDCl_3$) $\delta$: 7.52—7.50 (m, 2H), 7.37—7.26 (m, 3H), 5.54 (s, 1H), 4.33 (d, 2H, $J$=11.9 Hz), 4.04 (d, 2H, $J$=11.6 Hz), 3.54 (t, 2H, J=6.8 Hz), 3.25 (br s, 1H), 1.68—1.61 (m, 2H), 1.26 (br s, 26H), 0.89 (br t, 3H, $J$=7.0 Hz). $^{13}$C-NMR ($CDCl_3$) $\delta$: 138.2, 128.8, 128.1, 126.2, 101.3, 70.6, 69.05, 68.97, 31.9, 29.8, 29.7, 29.6, 29.5, 29.3, 26.1, 22.7, 14.1. HR-EI-MS *m/z*: 404.3293 (Calcd for $C_{26}H_{44}O_3$: 404.3291). *Anal*. Calcd for $C_{26}H_{44}O_3$: C, 77.18; H, 10.96. Found: C, 77.27; H, 11.23.

**2-Hexadecylglycerol (4)** 2-Hexadecylglycerol (**4**) was prepared from **13** (40 mg, 99 $\mu$mol) in the manner described for **1**. Silica gel column chromatography (hexane/EtOAc, 1 : 2, v/v) provided **4** (19 mg, 61%) as a white solid: mp 56—58 °C. $^1$H-NMR ($CDCl_3$) $\delta$: 3.77 (tt, 2H, $J$=5.5, 5.5 Hz), 3.68 (tt, 2H, $J$=5.5, 5.5 Hz), 3.57 (t, 2H, $J$=6.7 Hz), 3.46 (t, 1H, $J$=4.8 Hz), 1.93 (brs, 2H), 1.64—1.54 (m, 2H), 1.26 (br s, 26H), 0.88 (br t, 3H, $J$=7.3 Hz). $^{13}$C-NMR ($CDCl_3$) $\delta$: 79.5, 70.2, 62.2, 31.9, 30.0, 29.6, 29.5, 29.4, 29.3, 26.1, 22.6, 14.1. HR-EI-MS *m/z*: 316.2980 (Calcd for $C_{19}H_{40}O_3$: 316.2979). *Anal*. Calcd for $C_{19}H_{40}O_3$ C, 72.10; H, 12.74. Found: C 72.66; H, 12.88.

**9-Octadecenyl Iodide (14)** 9-Octadecenyl iodide (**14**) was prepared from commercially available *cis*-9-octadecen-1-ol (940 mg, 3.50 mmol) as described for **8**. Column chromatography on silica gel (hexane/EtOAc, 1 : 2, v/v) gave **14** (1060 mg, 80%) as a colorless oil: $^1$H-NMR ($CDCl_3$) $\delta$: 5.39—5.32 (m, 2H), 3.18 (t, 2H, $J$=7.0 Hz), 2.06—1.99 (m, 4H), 1.82 (dt, 2H, $J$=7.1, 7.1 Hz), 1.40—1.22 (m, 22H), 0.88 (br t, 3H, $J$=6.7 Hz). $^{13}$C-NMR ($CDC_{l3}$) $\delta$: 130.0, 129.7, 33.6, 31.9, 30.5, 29.76, 29.69, 29.5, 29.4, 29.3, 29.2, 28.5, 27.22, 27.16, 22.7, 14.1. HR-EI-MS *m/z*: 378.1791 (Calcd for $C_{18}H_{35}I$: 378.1783).

**2-(9-Octadecenyl)-1,3-benzylideneglycerol (15)** 2-(9-Octadecenyl)-1,3-benzylideneglycerol (**15**) was prepared from **14** (910 mg, 2.41 mmol) as described for **10**. Silica gel column chromatography (hexane/EtOAc, 1 : 2, v/v) gave **15** (321 mg, 31%) as a colorless oil: $^1$H-NMR ($CDCl_3$) $\delta$: 7.52—7.50 (m, 2H), 7.37—7.29 (m, 3H), 5.55 (s, 1H), 5.38—5.31 (m, 2H), 4.33 (dd, 2H, $J$=1.5, 12.5 Hz), 4.03 (dd, 2H, $J$=1.8, 12.5 Hz), 3.54 (t, 2H, $J$=6.7 Hz), 3.26 (t, 1H, $J$=1.8 Hz), 2.02—1.99 (m, 4H), 1.68—1.60 (m, 2H), 1.30—1.26 (m, 22H), 0.88 (br t, 3H, $J$=6.7 Hz). $^{13}$C-NMR ($CDCl_3$) $\delta$: 138.2, 129.9, 129.83, 129.77, 128.8, 128.1, 126.2, 101.3, 70.6, 69.03, 68.95, 65.5, 31.9, 29.8, 29.74, 29.65, 29.59, 29.48, 29.43, 29.29, 29.25, 29.20, 29.1, 27.2, 26.1, 25.9, 22.6, 14.1. HR-EI-MS *m/z*: 430.3444 (Calcd for $C_{28}H_{46}O_3$: 430.3447).

**2-(9-Octadecenyl)glycerol (5)** 2-(9-Octadecenyl)glycerol (**5**) was prepared from **15** (270 mg, 626 $\mu$mol) in the same manner described for **1**. Silica gel column chromatography (hexane/EtOAc, 1 : 2, v/v) provided **5** (112 mg, 52%) as a colorless oil: $^1$H-NMR ($CDCl_3$) $\delta$: 5.36—5.33 (m, 2H), 3.77 (dt, 2H, $J$=5.5, 5.5 Hz), 3.68 (dt, 2H, $J$=5.5, 5.5 Hz), 3.57 (t, 2H, $J$=6.7 Hz), 3.46 (t, 1H, $J$=4.8 Hz), 2.04—1.99 (m, 4H), 1.93 (br s, 2H), 1.62—1.55 (m, 2H), 1.38—1.24 (m, 22H), 0.88 (br t, 3H, $J$=6.9 Hz). $^{13}$C-NMR ($CDCl_3$) $\delta$: 130.0, 129.8, 79.5, 70.2, 62.2, 31.9, 30.1, 29.7, 29.52, 29.47, 29.43, 29.32, 29.25, 27.2, 26.1, 22.7, 14.1. HR-EI-MS *m/z*: 342.3142 (Calcd for $C_{21}H_{42}O_3$: 342.3134).

**Determination of $[Ca^{2+}]_i$ in NG108-15 Cells** $[Ca^{2+}]_i$ in neuroblastoma×glioma hybrid NG108-15 cells was determined using Fura-2/AM in a CAF-100 $Ca^{2+}$ analyzer as previously described.[9—11]

**Acknowledgments** We are grateful to Junko Shimode, Jinko Nonobe and Maroka Kitsukawa for the spectroscopic measurements. This study was supported in part by Grants-in-Aid from the Ministry of Education, Science, Sports and Culture of Japan.

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