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Synthesis and Biological Activities of Novel Structural Analogues of 2-Arachidonoylglycerol, An Endogenous Cannabinoid Receptor Ligand

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Abstract—Novel analogues of 2-arachidonoylglycerol (2-AG), an endogenous cannabinoid receptor ligand, were developed. Chemical synthesis of these analogues (2-AGA105 and 2-AGA109) was accomplished starting from 2-octyn-1-ol and diethyl malonate and employing Wittig coupling of triene phosphonate with an aldehyde intermediate in a convergent and stereoselective manner. These analogues should be useful lead compounds for the development of novel 2-AG mimetics.

2-Arachidonoylglycerol (2-AG) (**1**) is a unique molecular species of monoacylglycerol identified as an endogenous cannabinoid receptor ligand by us[1] and by Mechoulam et al.[2] several years ago. 2-AG binds to both central and peripheral cannabinoid receptors (CB1 and CB2)[1,2] and exhibits a variety of cannabimimetic activities in vitro and in vivo.[3] Evidence is accumulating that 2-AG is the intrinsic natural ligand for the cannabinoid receptors and that the cannabinoid receptors are primarily 2-AG receptors.[4–8]

Cannabinoid receptors are expressed in diverse mammalian tissues, and are assumed to play important regulatory roles, such as the attenuation of neurotransmission and vascular tone and the modulation of immune response;[3] it is apparent that the endogenous ligand 2-AG is an essential molecule in various mammalian tissues where the cannabinoid receptors are expressed. Nevertheless, the physiological roles of 2-AG in mammalian tissues still remain to be fully clarified, as does the significance of the cannabinoid receptor.

2-AG is a metabolically labile molecule, like acetylcholine, and therefore it is sometimes difficult to evaluate precisely its biological activities, especially in vivo. It is therefore important to develop metabolically stable

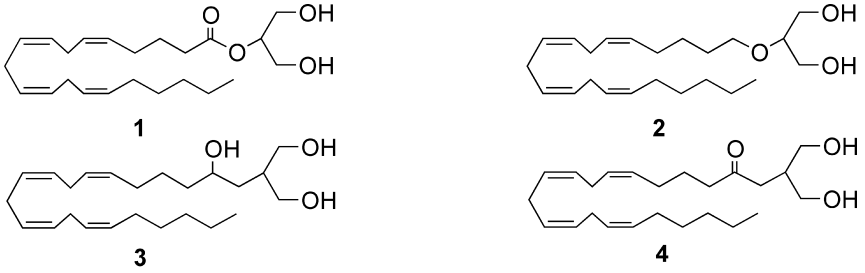

Figure 1. 2-Arachidonoylglycerol (2-AG) (**1**) and its structural analogues, 2-AG ether (**2**), 2-AGA105 (**3**), and 2-AGA109 (**4**).

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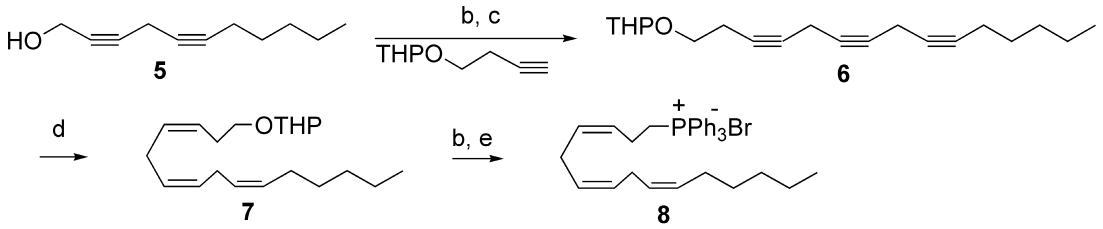


Scheme 1. (a) EtMgBr, CuI; (b) $Ph_2PCH_2CH_2PPh_2 \cdot 2Br_2$; (c) EtMgBr, CuI, $THPOCH_2CH_2C{\equiv}CH$, 85%; (d) H_2/Lindlar catalyst, 62%; (e) Ph_3P, reflux 36 h, 92%.

analogues of 2-AG as novel experimental tools or therapeutic agents. Previously, we synthesized an ether analogue of 2-AG, 2-(5(*Z*),8(*Z*),11(*Z*),14(*Z*)-eicosatetraenyl) glycerol (2-AG ether) (**2**).[6,8] This compound was found to exhibit appreciable agonistic activities at the cannabinoid receptors,[6–8] though its activity was weak compared with that of 2-AG. Independently and concurrently, Mechoulam et al.[9] also reported the development of **2** (HU-310). However, no other stable analogues have been reported so far. Here, we report the synthesis and biological activities of novel stable analogues of 2-AG, 2-AGA105 (**3**) and 2-AGA109 (**4**).

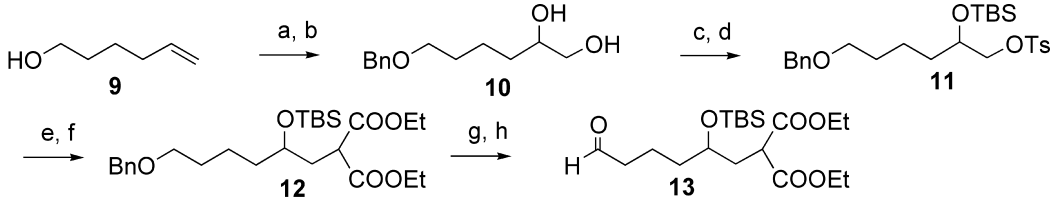
Figure 1 illustrates the chemical structures of **1**, **2** and the newly developed structurally related stable analogues of 2-AG, 2-hydroxymethyl-tricosa-8(*Z*),11(*Z*),14(*Z*), 17(*Z*)-tetraene-1,4-diol (**3**) and 1-hydroxy-2-hydroxymethyl-tricosa-8(*Z*),11(*Z*),14(*Z*),17(*Z*)-tetraen-4-one (**4**). Compound **3** is a hydroxyl group-containing and an ester linkage-lacking analogue of **1**, and **4** is a ketone analogue of **1**, which also lacks an ester linkage. These compounds are resistant to hydrolyzing enzymes such as lipase.

The outline of our synthetic plan for **3** and **4** is as follows. First, we prepared phosphonium salt (**8**) and aldehyde synthon (**13**). Then these components were combined in a convergent and stereoselective manner to obtain **14**, a key intermediate. Step-wise chemical modification of **14** gave the desired compounds **3** and **4**.

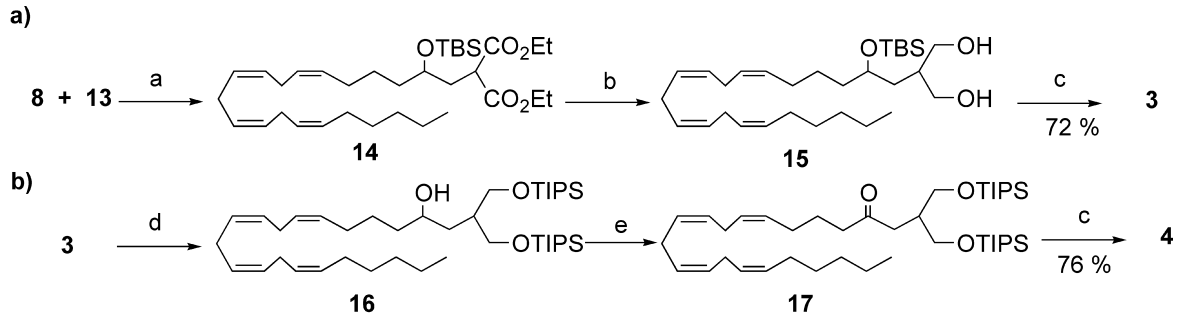
3(*Z*),6(*Z*),9(*Z*)-Tetradecatrienyl phosphonate (**8**), an intermediate in the total synthesis of the above 2-AG analogues, was prepared as illustrated in Scheme 1. Commercially available 2-octyn-1-ol was brominated with phosphorus tribromide. The resultant halide was then coupled with propargyl alcohol to give 2,4-undecadiyl-1-ol (**5**) according to the method of Kerdesky et al.[10] The diyne **5** was subsequently halogenated with 1,2-bis(diphenylphosphophino) ethane tetrabromide and the resultant bromide was coupled with the tetrahydropyranyl ether of butynyl alcohol to yield the triyne **6** in 85% yield. The triyne **6** was then subjected to controlled hydrogenation employing the Lindlar catalyst to afford the crude triene **7**. Traces of overreduced or underreduced products were removed by argentation chromatography utilizing 10% silver nitrate on silica gel, to give pure **7** in 62% yield.[10,11] The resultant pure *cis*-triene **7** was deprotected and brominated in one step with 1,2-bis(diphenylphosphino) ethane tetrabromide and finally treated with triphenyl phosphine to afford **8** in 92% yield as a colorless oil.[11]

The key aldehyde synthon **13** was prepared from commercially available 5-hexen-1-ol (**9**) as illustrated in Scheme 2. Protection of the alcohol **9** with benzyl bromide using NaH as a base followed by oxidation with osmium tetroxide gave the diol **10** in an overall yield of 72%. Introduction of tosylate at the primary alcohol of **10** (74% yield), and subsequent protection of the secondary hydroxyl group with a *tert*-butyldimethylsilyl (TBS) group afforded **11** in good yield. Then conversion of the tosylate **11** to iodide with lithium iodide in dry acetone, followed by alkylation with diethyl malonate employing NaH gave the dicarboxylate **12** (88% yield in each case). The benzyl group of **12** was hydrogenated using Pd/C in ethanol, and then Swern oxidation afforded the desired aldehyde **13** in 89% yield after purification by column chromatography.

Synthesis of **3** and **4** from **8** and **13** was carried out as follows (Scheme 3). Wittig coupling of the anion derived from the phosphonate **8** with the aldehyde **13** gave a 69% yield of adduct **14**. No trace of the *trans* isomer was found by 400 MHz NMR spectroscopy. Reduction of the diethyl ester of **14** with lithium aluminum hydride afforded the diol **15** in 81% yield. The TBS protecting group of **15** was removed by treatment with TBAF to produce the triol **3** in 72% yield.[12] Selective protection of the primary alcohol of **3** with a TIPS group, followed by oxidation of secondary alcohol **16** gave **17** in 74%



Scheme 2. (a) NaH, BnBr; (b) OsO_4, NMO, 72% (two steps); (c) TsCl, pyridine, 74%; (d) TBSCl, imidazole, 91%; (e) LiI, acetone, 88%; (f) NaH $(CO_2Et)_2$, 88%; (g) H_2, Pd/C, 97%; (h) Swern oxidation, 89%.



Scheme 3. (a) LiHMDS, HMPA, −98 to 0 °C, 69%; (b) $LiAlH_4$, 81%; (c) TBAF; (d) TIPSCl, imidazole, DMF, 60%; (e) PDC, 74%.

yield. Finally, deprotection of the TIPS group of **17** gave the ketone analogue **4** in 75% yield after the removal of small amounts of by-products.[13]

The biological activities of **3** and **4** as cannabinoid receptor agonists were evaluated by estimating their capacities to induce Ca^{2+} transients in NG108-15 cells, which express the CB1 receptor, and in HL-60 cells, which express the CB2 receptor, according to the method described previously.[4–8] As shown in Figures 2 and 3, **3** and **4** exhibited appreciable agonistic activities. The agonistic activity of **4** toward NG 108-15 cells was comparable to that of 2-AG ether (**2**). Also, the agonistic activity of **3** toward HL-60 cells was nearly comparable to that of 2-AG ether (**2**), yet it will be necessary to determine in the future whether two enantiomers of compound **3** possess similar biological activities. The agonistic activities of these novel 2-AG analogues (**2**, **3** and **4**) were, however, approximately 100 times lower than that of 2-AG (**1**).[6,7]

In summary, we have developed two novel structural analogues of 2-AG, 2-AGA105 (**3**) and 2-AGA109 (**4**). These compound, as well as 2-AG ether (**2**), should be useful lead compounds in the development of novel stable analogues of 2-AG (**1**).

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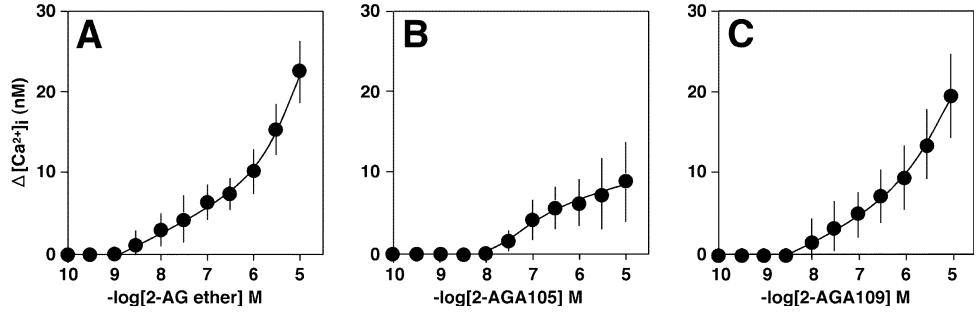

Figure 2. Effects of 2-AG ether (**2**)(A), 2-AGA105 (**3**)(B) and 2-AGA109 (**4**)(C) on the intracellular free Ca^{2+} concentrations in NG108-15 cells which express the CB1 receptor. The mean values ± SD were taken from six determinations.

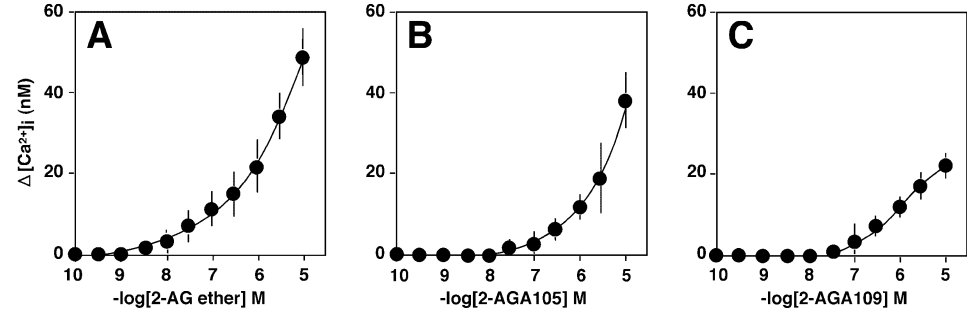

Figure 3. Effects of 2-AG ether (**2**)(A), 2-AGA105 (**3**)(B) and 2-AGA109 (**4**)(C) on the intracellular free Ca^{2+} concentrations in HL-60 cells which express the CB2 receptor. The mean values ± SD were taken from six determinations.

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12. Data for **3**: ^{1}H NMR (400 MHz, $CDCl_3$–D_2O) δ 5.41–5.33 (m, 8H), 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 $[M]^+$; HR-EIMS calcd for $[C_{24}H_{42}O_3]$ 378.3134, found 378.3134.
13. Data for **4**: ^{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), 1.59–1.52 (m, 2H), 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 $[M-H_2O]^+$; HR-EIMS calcd for $[C_{24}H_{38}O_2]$ 358.2872, found 358.2867.