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Total Synthesis of 2-Arachidonylglycerol (2-Ara-Gl)

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Abstract: *Total synthesis of 2-Arachidonylglycerol (2-Ara-Gl) was accomplished starting from arachidonic acid and glycerol. The sequence involves selective protection of the primary alcohols of glycerol, esterification, and mild hydrolysis of the protecting groups. A unique hydrolysis condition was developed to prevent isomerization of the 2-Ara-Gl to the more stable 1-monoglyceride.*

2-Arachidonylglycerol (2-Ara-Gl), an arachidonic acid derivative, was recently isolated from canine gut[1] and rat brain.[2,3] It is a putative endogenous ligand, binding to both the central and peripheral cannabinoid receptors, namely CB1 and CB2.[1] 2-Ara-Gl shares some of the pharmacological and behavioral actions typical of cannabinoids, such as inhibition of forskolin-induced adenylate cyclase in spleen cells and the twitch response in the mouse vas deferens, modulation of lymphocyte proliferation, and production of hypomotility, hypothermia and analgesia.[1-6] Furthermore it has been shown to have immunomodulatory effects in mouse splenocytes.[7]

Since isolation of 2-Ara-Gl from cells could only yield micromolar quantities of material, we developed the total synthesis of 2-Ara-Gl to provide enough compound for the structure-activity relationships (SAR) study. The major difficulty in the synthesis of pure 2-Ara-Gl is the competitive formation of the more stable 1-Ara-Gl, resulting from the migration of the arachidonyl group from the 2-oxygen to the adjacent primary hydroxyl groups. This transesterification process is catalyzed by acid, base, or heat. The equilibrium ratio of the 1-monoglycerides to 2-mono-glycerides is generally 9 to 1.[8,9] To overcome this problem, we have developed a synthesis utilizing the triisopropylsilyl (TIPS) protecting group which could be removed under very mild hydrolytic conditions without triggering the transesterification process. This was achieved by utilizing tetra-*n*-butylammoniumfluoride (TBAF) solution, buffered with acetic acid, at low temperatures.

The synthesis was carried out from commercially available arachidonic acid and glycerol (scheme 1). The primary hydroxy groups of glycerol were selectively protected by TIPS chloride with imidazole in DMF to give secondary alcohol **1**[10] in 97% yield. Esterification of arachidonic acid with alcohol **1** using 4-dimethylaminopyridine (DMAP) and 1-(3-dimethyl-aminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI) in methylene chloride at room temperature for 24 h provided ester **2**[11] in 94% yield. Hydrolysis of the TIPS groups without isomerization of the subsequent 2-Ara-Gl was accomplished by dissolving ester **2** in THF at -30 °C and adding 6 equivalents of both AcOH and TBAF solution (1 M in THF), dropwise. The reaction was

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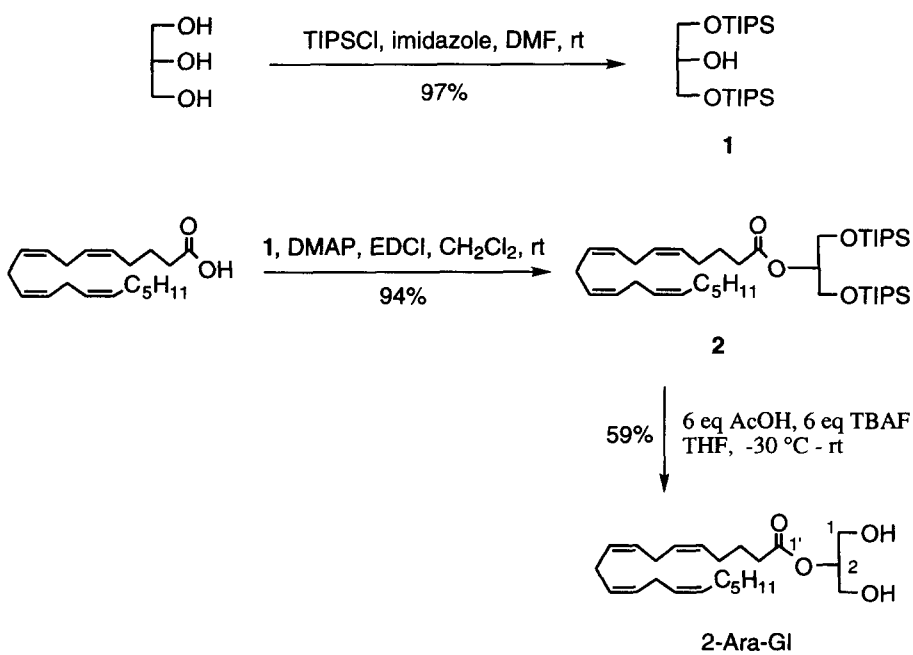
stirred at -25 °C overnight and then at room temperature for 1 h. Flash chromatography on silica gel gave pure 2-Ara-Gl[12] in 59% yield with 65% conversion. Confirmatory evidence for the purity of 2-Ara-Gl was provided by ^{13}C NMR data with peaks at δ 173.8 (C-1'), 75.0 (C-2) and 62.3 (C-1, 2C) which are in agreement with those found in 1,3-dihydroxy-2-propyl acetate and other related compounds.[13] In addition, the purity was established by HPLC using Resolve C18 reverse phase column which separated 2-Ara-Gl from 1-Ara-Gl.[14] It is noteworthy that the identification of 2-Ara-Gl in rat brain was reported using a GC/MS procedure which entailed derivitization, as bis trimethylsilyl ethers, of the isolated monoacylglycerols. The authors[3] reported the isomerization of 2-Ara-Gl to 1-Ara-Gl following this procedure. We have found similar results in the identification of 2-Ara-Gl using the GC procedure. However the problem of isomerization is circumvented in our HPLC procedure and it may prove useful in future studies with 2-Ara-Gl.

In summary, the total synthesis of 2-Ara-Gl was accomplished without isomerization to the more stable 1-Ara-Gl. The unique mild hydrolysis condition provides a new synthetic strategy to control the isomerization of 2-monoglycerides to 1-monoglycerides. The analytical HPLC procedure for the detection of 2-Ara-Gl may prove useful in future studies of this endogenous ligand.

Scheme 1



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Reference and Notes

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10. Satisfactory spectral data were obtained for all new compounds using chromatographically homogeneous samples. NMR data were recorded at 300 MHz in CDCl3. Chemical shifts are reported in δ and coupling constants in Hz.
    Data for **1**: ^{1}H NMR 4.09 (m, 1H), 3.73-3.68 (m, 4H), 2.58 (m, 1H, OH), 1.22-0.90 (m, 6H), 1.06 (br d, 36H, J = 7.0). ^{13}C NMR 72.2, 63.9 (2C), 17.9 (12C), 11.9 (6C).
11. Data for **2**: ^{1}H NMR 5.43-5.31 (m, 8H), 4.95 (tt, 1H, J = 5.5, 5.0), 3.88 (dd, 2H, J = 10.5, 5.0), 3.82 (dd, 2H, J = 10.5, 5.5), 2.86-2.78 (m, 6H), 2.32 (t, 2H, J = 7.7), 2.14-2.02 (m, 4H), 1.75-1.65 (m, 2H), 1.40-1.25 (m, 6H), 1.15-0.98 (m, 6H), 1.05 (br s, 36H), 0.91-0.85 (m, 3H).
    ^{13}C NMR 173.0, 130.4, 129.0, 128.6, 128.5, 128.1 (2C), 127.8, 127.5, 75.0, 61.7 (2C), 33.8, 31.5, 29.2, 27.1, 26.5, 25.5 (3C), 24.7, 22.5, 17.8 (12C), 14.0, 11.8 (6C).
12. Data for 2-Ara-Gl: ^{1}H NMR 5.47-5.28 (m, 8H), 4.93 (tt, 1H, J = 4.7, 4.7), 3.83 (d, 4H, J = 4.7), 3.05-2.78 (m, 8H), 2.39 (t, 2H, J = 7.4), 2.18-2.00 (m, 4H), 1.73 (tt, 2H, J = 7.4, 7.4), 1.41-1.22 (m, 6H), 0.89 (br t, 3H, J = 6.7).
    ^{13}C NMR 173.8, 130.5, 129.0, 128.8, 128.6, 128.3, 128.1, 127.8, 127.5, 75.0, 62.3 (2C), 33.7, 31.5, 29.3, 27.2, 26.5, 25.6 (3C), 24.8, 22.6, 14.1. Anal. Calcd for $C_{23}H_{38}O_4$•0.05 $CHCl_3$ C, 71.95; H, 9.97. Found: C, 71.68; H, 9.94.

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