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Chemoenzymatic synthesis of 2-arachidonoylglycerol, an endogenous ligand for cannabinoid receptors

Subramanian K. Vadivel *, Kyle M. Whitten, A. Makriyannis

Center for Drug Discovery, 116 Mugar Hall, 360 Huntington Avenue, Northeastern University, Boston, MA 02115, USA

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ABSTRACT

A simple and efficient synthesis of 2-arachidonoyl glycerol, an endogenous agonist for cannabinoid receptors was achieved using Novozym 435, immobilized lipase from *Candida antarctica*.

2-Arachidonoylglycerol (2-AG) is an endogenous cannabinergic ligand that interacts with both CB1 and CB2 receptors. Although 2-AG synthesis involves several candidate enzymes,[1] 2-AG is inactivated principally by monoacylglycerol lipase[2](MGL), although fatty acid amide hydrolase (FAAH) may contribute to its degradation.[3] 2-AG was shown to possess various biological activities, such as binding to CB1 and CB2 cannabinoid receptors, inhibition of adenylyl cyclase in mouse spleen cells, and inducing hypothermia, reducing spontaneous activity, analgesia, and immobility in mice.[4] 2-AG acts as a full cannabinergic agonist, and the structure of 2-AG is strictly recognized by the cannabinoid receptors (CB1 and CB2). Thus, 2-AG rather than anandamide may represent the true natural ligand for cannabinoid receptors.[5–7]

The major problem in the synthesis of pure 2-AG is the rapid migration of the arachidonoyl group from the secondary to the primary hydroxyl group, resulting in the formation of more stable 1-arachidonoyl glycerol. This migration is catalyzed by water, acid, base, or heat.[8] Earlier synthetic methods utilized coupling of 1,3-silyl[9] or benzylidine[10] protected glycerol with arachidonic acid and followed by deprotection and separation of the isomeric arachidonoyl glycerols. All these methods suffer from extended reaction time, acidic conditions required for the removal of the protecting groups as well as extensive work up and purification. Another interesting method also appeared in the literature, which utilizes regioselective transformation of glycidyl arachidonate into 2-arachidonoyl-1,3-bis(trifluoroacetyl)glycerol followed by the cleavage of trifluroacetyl group with pyridine.[11]

Searching for an alternative, green, and efficient methodology that would circumvent these problems during 2-arachidonoyl glycerol synthesis, we have developed an enzyme catalyzed efficient and highly regioselective synthesis. The advantage of biocatalysis is that the reactions are carried out at ambient temperature, nearly neutral pH and the reactions are often highly regio- and stereoselective.[12,13] Enzymes derived from microbial cells can be immobilized and reused for many cycles. It was known that enzymes can show selectivity during glycerolysis of fatty acids as well as transesterification of symmetrical triglycerides.[14–18] Herein, we report an improved, practical application of the above methodology in the synthesis of 2-arachidonoyl glycerol utilizing unsymmetrical triglyceride.

The synthesis is based on a two-step enzymatic process (Scheme 1). The first involves the synthesis of 1,3-diacyl glycerol using the method reported by Halldorsson et al.[19] Enzymatic acylation of glycerol was carried out in anhydrous dichloromethane using vinylbutyrate as an acyl transfer agent. The reaction proceeded smoothly at 0 °C to provide exclusively 1,3-butyroyl glycerol (**1**) with greater than 90% yield.[20] The 1,3-diacylglycerol was coupled with arachidonic acid using 4-dimethylaminopyridine (DMAP) and 1-(3-dimethyl-aminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI) in dichloromethane at room temperature for 12 h providing the required triglyceride (**2**) in 84% yield.[21] Triglyeride (**2**) was subjected to immobilized *Candida antarctica* (Novozym 435) known for its high 1,3-regioselective glycerolysis of triglycerides. The reaction was facile and afforded the required 2-arachidonoyl glycerol (**3**) exclusively (67%) and the by-product

* Corresponding author. Address: Center for Drug Discovery, Northeastern University, 360 Huntington Avenue, 116 Mugar LifeSciences Building, Boston, MA 02115, USA. Tel.: +1 617 373 7620; fax: +1 617 373 7493.
E-mail address: s.vadivel@neu.edu (S.K. Vadivel).

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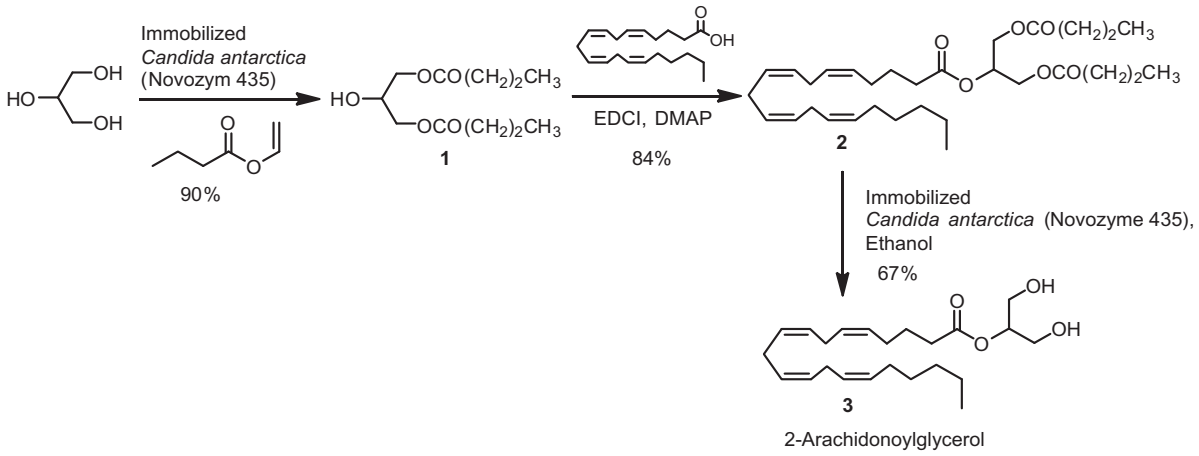

Scheme 1.

butylacetate was easily removed under vacuum. We also noted the formation of ethyl arachidonate (27%) as a side product. It is noteworthy that there was no formation of 1-arachidonoylglycerol observed in the reaction. The crude product was purified by a small filter column of boric acid impregnated silica.[22]

In summary, the improved and practical synthesis of 2-AG was successfully carried out without any isomerization of the more stable 1-arachidonoyl glycerol. The mild neutral conditions, easy scalability, and removal of the volatile byproduct provide added advantages to the current method for the synthesis of 2-monoacylglycerides.

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19. Halldorsson, A.; Magnusson, C. D.; Haraldsson, G. G. *Tetrahedron* **2003**, *59*, 9101. NMR data for compound **1**: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 4.12–4.27 (m, 4H), 4.03–4.12 (m, 1H), 2.20–2.41 (t, J = 7.4 Hz, 4H), 1.67 (sxt, J = 7.42 Hz, 4H), 0.96 (t, J = 7.32 Hz, 6H).
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21. Preparation of **2**: To a solution of 1,3-dibutanoylglyerol (**1**) (0.500 g, 2.45 mmol) and arachidonic acid (0.74 g, 2.45 mmol) in anhydrous dichloromethane (10 mL) was added DMAP (2.4 g, 19.6 mmol) and EDCI (1.88 g, 9.8 mmol). The resulting solution was stirred for 24 h. Cold water was added to the reaction mixture and washed with 5% HCl. The aqueous layer was extracted with dichloromethane and the combined organic extracts were washed with water, brine, and dried over sodium sulfate. The reaction mixture was subjected to flash chromatography (hexanes/acetone; 7:3) to afford pure triglyceride as colorless oil (1.06 g, 88%). IR (neat) 3012, 2931, 1741, 1167, 1094 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 5.30–5.45 (m, 8H), 5.23–5.30 (m, 1H), 4.10–4.37 (m, 4H), 2.76–2.91 (m, 6H), 2.34 (t, J = 7.57 Hz, 2H), 2.30 (t, J = 7.57 Hz, 4H), 2.12 (q, J = 7.16 Hz, 2H), 2.06 (q, J = 7.32 Hz, 2H), 1.68–1.75 (m, 2H), 1.60–1.68 (m, 4H), 1.23–1.42 (m, 6H), 0.91–0.99 (m, 6H), 0.84–0.91 (m, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.3, 172.8 (2C), 130.7, 129.2, 128.9, 128.8, 128.5, 128.3, 128.0, 127.7, 69.2, 62.3 (2C), 36.1 (2C), 33.8, 31.7, 29.5, 27.4, 26.7, 25.8 (3C), 24.9, 22.8, 18.5 (2C), 14.3, 13.8 (2C).
22. Preparation of 2-arachidonoyl glycerol (**3**): Triglyceride **2** (0.050 g, 0.102 mmol) and anhydrous ethanol (0.7 mL) were stirred at room temperature and the reaction was started by the addition of Novozym 435 (75 mg) and the reaction mixture was stirred for 1 h and the starting material was completely consumed. An additional 50 mg was added and the reaction mixture was stirred for another 1 h. After completion of the reaction the enzyme was filtered off and washed with ether and the solvent was evaporated and the crude product was purified by plug of boric acid impregnated silica gel (hexanes/acetone; 9:1–3:2) to afford analytically pure 2-AG (0.026 g, 67%) as a colorless oil. IR (neat) 3420, 3012, 2927, 1736, 1456, 1378, 1277, 1152 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.37–5.49 (m, 8H), 4.93 (p, 5.0 Hz, 1H), 3.84 (t, J = 5.0 Hz, 4H), 2.79–2.88 (m, 6H), 2.39 (t, J = 7.5 Hz, 2H), 2.13 (q, J = 7.0 Hz, 2H), 2.06 (q, J = 7.0 Hz, 2H), 2.00 (br t, J = 5.5 Hz, 2H), 1.72 (p, J = 7.2 Hz, 2H), 1.30–1.40 (m, 6H), 0.92 (t, J = 7.2 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 174.1, 130.7, 129.3, 128.9, 128.8, 128.5, 128.3, 128.1, 127.8, 75.2, 62.5 (2C), 33.8, 31.7, 29.6, 27.4, 26.7, 25.8, 25.8, 25.8, 24.9, 22.8, 14.3.