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Novel, regioselective transformation of an oxirane system. An efficient approach to the synthesis of endocannabinoid 2-arachidonoylglycerol

Stephan D. Stamatov[a,*] and Jacek Stawinski[b,c,*]

[a]*Department of Chemical Technology, University of Plovdiv, 24 Tsar Assen St., Plovdiv 4000, Bulgaria*
[b]*Department of Organic Chemistry, Arrhenius Laboratory, Stockholm University, S-106 91 Stockholm, Sweden*
[c]*Institute of Bioorganic Chemistry, Polish Academy of Sciences, Noskowskiego 12/14, 61-704 Poznan, Poland*

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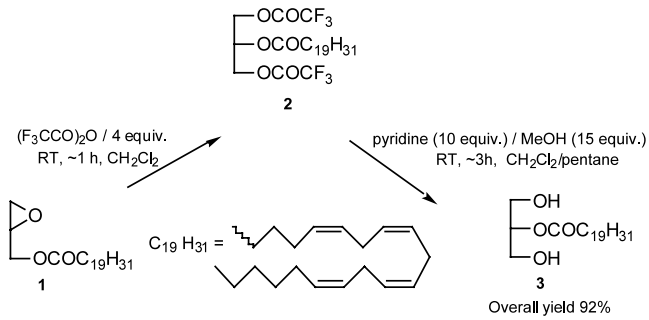
Abstract—A trifluoroacetic anhydride-catalysed opening of the oxirane system of glycidyl arachidonate with a simultaneous migration of the acyl group provides a new, efficient entry to 2-arachidonoylglycerol. © 2002 Published by Elsevier Science Ltd.

It has only recently been discovered[1] that 2-arachidonoylglycerol (2-AG) might be an intrinsic, natural ligand for central and peripheral cannabinoid receptors (CB1 and CB2), that had previously been identified as specific targets for a major psychoactive ingredient of marijuana, Δ^9-tetrahydrocannabinol. There is a growing line of evidence that 2-AG is a lipid mediator governing a variety of protective or physiopathological events in the central nervous,[2] cardiovascular,[3] and immune systems,[4] including hormone regulation,[5] inflammation control,[6,7] and also inhibiting proliferation of human breast and prostate cancer cells.[7,8]

This diverse array of biological activities exhibited by 2-AG caused a high demand for isomerically pure 2-arachidonoylglycerol for biochemical and structure-activity relation studies. Despite the apparent simplicity of the chemical structure of 2-AG, access to this ligand is still limited and in most instances the compound is isolated from natural sources,[7] as chemical[9] and enzymatic methods[10] for its preparation are rather inefficient.

There are two problems that make synthesis of 2-AG most difficult. Firstly, due to the presence of two adjacent primary hydroxyl functions, 2-acyl glycerols show high propensity towards isomerisation[11] (acid, base and heat promoted migration of an acyl group) and this poses severe complications in their synthesis, isolation, storage, etc. Secondly, a problem specific to 2-AG is that the arachidonoyl moiety exhibits pronounced susceptibility to autoxidation affecting integrity of the native olefinic system and thus limiting the number of available procedures for its preparation.

Two chemical methods described in the literature for the synthesis of 2-AG are based on the same chemistry: acylation of suitable 1,3-protected glycerol precursors with an arachidonic acid derivative, followed by deprotection and separation of the isomeric arachidonoylglycerols. In the original method developed by Martin[12] and its two most recent modifications,[9,13] 1,3-benzylideneglycerol is used as a substrate and, after introduction of the arachidonoyl moiety, the acetal group is removed using boric acid derivatives. In the other approach,[14] triisopropylsilyl (TIPS) groups are used for



Scheme 1.

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* Corresponding authors.

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the protection of 1- and 3-hydroxyl functions of glycerol and their removal from 1,3-bissilyl-2-arachidonoyl intermediate is effected by a prolonged treatment with tetra-*n*-butylammonium fluoride (TBAF) and acetic acid at low temperature.

Although useful in a general sense, these methods provide only a partial solution to synthetic problems. Difficulties include extended reaction time, acidic conditions required for the removal of protecting groups, necessity for workup after each synthetic step or separation of the intermediates from the accompanying by-products, etc. These have frequently been reported to contribute to isomerisation and oxidative or hydrolytic side-reactions during the synthesis of 2-acylglycerols. To lessen the problem of acyl migrations, in these synthetic procedures the deprotection steps were either not taken to completion,[14] or the produced isomeric compounds were separated by various chromatographic techniques.[9,14]

Searching for an alternative methodology that would circumvent these shortcomings during 2-acylglycerol synthesis, we have developed an efficient and highly stereoselective transformation of glycidyl arachidonate **1** into 2-arachidonoylglycerol derivative **2**, promoted by trifluoroacetic anhydride (TFAA) (Scheme 1). Mechanistic details of this transformation remain to be clarified, but the observed high regioselectivity suggested intermediacy of the cyclic 1-trifluoroacetyl-2,3-acyliumglycerol cation (formed via an intramolecular attack of the adjacent carbonyl group on a trifluoroacetic anhydride-activated epoxide function in **1**) that in the presence of trifluoroacetate ion collapses into 1,3-bis(trifluoroacetyl)-2-arachidonoylglycerol **2**. Compound **2**, from which 2-arachidonoylglycerol is generated under mild conditions, can be envisaged as a convenient storage form of 2-AG (protection of 1- and 3-hydroxyl groups as trifluoroacetyl esters should prevent scrambling of the arachidonoyl moiety) or as a possible prodrug for this lipid mediator.

Efficacy of this approach for the synthesis of 2-arachidonoyl glycerol **3** was assessed by subjecting (±)-glycidyl arachidonate **1** (obtained in one step from commercially available (±)-glycidol and arachidonic acid in 95% yield[15,16]) to the transformations shown in Scheme 1. To this end, compound **1** in dichloromethane was treated with TFAA (4 equiv.) at room temperature for 1 h. ^{1}H and ^{13}C NMR spectroscopy revealed that under these conditions the conversion of **1** to 1,3-bis(trifluoroacetate) **2**[17] was quantitative and completely regioselective (>99%). Thus, intermediate **2** can be either directly used for a subsequent transformation, or isolated (~94% yield, see Experimental) and stored for several months (−20°C, under argon) without detectable alterations of its spectral characteristics (^{1}H and ^{13}C NMR spectroscopy).

Since trifluoroacetate esters are known to undergo smooth transesterification with alcohols,[19] as a final step of this synthetic protocol we treated 1,3-bis(trifluoroacetate) **2** in a CH_2Cl_2–pentane with pyridine (10 equiv.) and methanol (15 equiv.). The reaction was quantitative (completion within 3 h) and after removal of volatile products via evaporation, isomerically homogenous 2-arachidonoylglycerol **3**[18] (purity >99%, ^{1}H and ^{13}C NMR spectroscopy) was obtained in 92% overall yield (calculated on **1**) with no necessity for additional purification.

*A typical procedure for the preparation of **2** and **3***: To a solution of glycidyl arachidonate **1** (0.180 g, 0.50 mmol), in alcohol-free dichloromethane (2.0 mL), trifluoroacetic anhydride (TFAA, 0.278 mL, 2.00 mmol), in alcohol-free dichloromethane (2.0 mL), was added at −20°C, and the reaction mixture was kept at room temperature for 1 h. The solvent and unreacted TFAA were removed under reduced pressure (bath temp. 40°C), the residue was dissolved in toluene (5.0 mL) and passed through a silica gel pad (~5 g) prepared in the same solvent. The support was washed with toluene (100 mL) and the solvent was removed under reduced pressure to provide 1,3-bis(trifluoroacetyl)-2-arachidonoylglycerol **2** as a yellowish oil. Yield, 0.267 g (94%, purity >99%, ^{1}H NMR spectroscopy).

To produce 2-arachidonoylglycerol **3**, to a solution of **2** in pentane–CH_2Cl_2 (2:1, v/v, 5.0 mL), pyridine (0.40 mL, 5.0 mmol) and methanol (0.30 mL, 7.5 mmol) in the same solvent (5.0 mL) were added at −20°C, and the reaction mixture was left at room temperature for 3 h. After evaporating solvents, **3** was obtained as a yellowish oil. Yield, 0.175 g (92%, calculated on **1**; purity >99%, ^{1}H NMR spectroscopy).

In conclusion, we have developed an efficient synthetic strategy based on a novel, regioselective transformation of glycidyl arachidonate **1** into 2-arachidonoyl-1,3-bis(trifluoroacetyl)glycerol **2** intermediate, from which 2-arachidonoylglycerol **3** can be retrieved under mild conditions. The main features of this new synthetic protocol are: (i) highly effective and practically quantitative, one-pot synthesis of 2-arachidonoylglycerol **3** under mild reaction conditions; (ii) the produced compounds **2** and **3** are of high purity, which alleviates problems of their additional purification, and thus the extent of acyl migration (and other side-reactions) is minimised; (iii) glycidyl arachidonate **1** and intermediate **2** can be considered as convenient storage forms of 2-AG; (iv) the method makes use of commercially available reactants and it is easy to scale-up.

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16. Yellowish oil; R_f (pentane:EtOAc = 90:10, v/v) = 0.33; anal. calcd for $C_{23}H_{36}O_3$ (360.54): C, 76.62; H, 10.06. Found: C, 76.70; H, 10.04. ^{1}H NMR δ_H (in ppm, $CDCl_3$, 400 MHz) 0.88 (t, J = 6.8 Hz, 20-CH_3, 3H); 1.22–1.40 (m, 17-19-CH_2, 6H); 1.71 (p, J = 7.5 Hz, 3-CH_2, 2H); 2.05, 2.11 (m, 16-CH_2, 4-CH_2, 4H); 2.36 (t, J = 7.5 Hz, 2-CH_2, 2H); 2.64 (dd, J = 2.6, 2.6 Hz, C(O)CH_2CHC$H_a$$H_b$O, 1H); 2.77–2.86 (m, 7, 10, 13-CH_2, C(O)CH_2CHC$H_a$$H_b$O, 7H); 3.19 (m, C(O)$CH_2CHCH_2$O, 1H); 3.91 (dd, J = 6.2, 6.2 Hz, OCH_2CHC$H_a$$H_b$OC(O), 1H); 4.40 (dd, J = 3.3, 2.9 Hz, OCH_2CHC$H_a$$H_b$OC(O), 1H); 5.28–5.44 (m, CH=CH, 8H); ^{13}C NMR δ_C (in ppm, $CDCl_3$, 100 MHz) 14.28 (20-CH_3); 22.78 (19-C); 24.91 (3-C); 25.83 (7, 10, 13-C); 26.73 (16-C); 27.43 (4-C); 29.54 (18-C); 31.73 (17-C); 33.62 (2-C); 127.76, 128.07, 128.35, 128.45, 128.80, 129.04, 129.19, 130.71 (5, 6, 8, 9, 11, 12, 14, 15-C); 173.47 (1-C): arachidonoyl fragment; 44.87 (1-C); 49.56 (2-C); 65.05 (3-C): oxirane-2-methyl fragment.
17. Yellowish oil; R_f (pentane:toluene:EtOAc = 40:50:10, v/v/v) = 0.57; anal. calcd for $C_{27}H_{36}O_6F_6$ (570.57): C, 56.84; H, 6.36. Found: C, 56.93; H, 6.30. ^{1}H NMR δ_H (in ppm, $CDCl_3$, 400 MHz) 0.89 (t, J = 6.9 Hz, 20-CH_3, 3H); 1.22–1.40 (m, 17-19-CH_2, 6H); 1.70 (p, J = 7.3 Hz, 3-CH_2, 2H); 2.05, 2.12 (m, 16-CH_2, 4-CH_2, 4H); 2.36 (t, J = 7.5 Hz, 2-CH_2, 2H); 2.72–2.90 (m, 7, 10, 13-CH_2, 6H); 4.46 (dd, J = 5.5, 5.5 Hz, C(O)O$CH_a$$H_bCHCH_a$$H_b$OC(O), 2H); 4.63 (dd, J = 4.2, 4.4 Hz, C(O)O$CH_a$$H_bCHCH_a$$H_b$OC(O), 2H); 5.27–5.46 (m, CH=CH, CH_2CHCH_2, 9H); ^{13}C NMR δ_C (in ppm, $CDCl_3$, 100 MHz) 14.26 (20-CH_3); 22.78 (19-C); 24.70 (3-C); 25.81 (7, 10, 13-C); 26.57 (16-C); 27.43 (4-C); 29.53 (18-C); 31.73 (17-C); 33.41 (2-C); 127.73, 128.03, 128.22, 128.54, 128.68, 128.83, 129.44, 130.72 (5, 6, 8, 9, 11, 12, 14, 15-C); 172.51 (1-C): arachidonoyl fragment; 114.50 (q, J = 285.3 Hz, 2-C); 157.17 (q, J = 43.5 Hz, 1-C): trifluoroacetyl fragment; 64.92 (1-C, 3-C); 67.22 (2-C): glycerol fragment.
18. Yellowish oil; R_f (pentane:toluene:EtOAc = 30:20:50, v/v/v) = 0.33; anal. calcd for $C_{23}H_{38}O_4$ (378.56): C, 72.98; H, 10.12. Found: C, 73.00; H, 10.18. ^{1}H NMR δ_H (in ppm, $CDCl_3$, 400 MHz) 0.88 (t, J = 6.8 Hz, 20-CH_3, 3H); 1.22–1.41 (m, 17-19-CH_2, 6H); 1.73 (p, J = 7.3 Hz, 3-CH_2, 2H); 2.05, 2.13 (m, 16-CH_2, 4-CH_2, 4H); 2.39 (t, J = 7.6 Hz, 2-CH_2, 2H); 2.73–2.89 (m, 7, 10, 13-CH_2, 6H); 3.82 (d, J = 4.8 Hz, OCH_2CHCH_2O, 4H); 4.92 (tt, J = 4.6, 4.6 Hz, OCH_2CH, 1H); 5.28–5.46 (m, CH=CH, 8H); ^{13}C NMR δ_C (in ppm, $CDCl_3$, 100 MHz) 14.29 (20-CH_3); 22.78 (19-C); 24.97 (3-C); 25.83 (7, 10, 13-C); 26.71 (16-C); 27.43 (4-C); 29.54 (18-C); 31.74 (17-C); 33.90 (2-C); 127.75, 128.06, 128.32, 128.54, 128.85, 128.99, 129.26, 130.76 (5, 6, 8, 9, 11, 12, 14, 15-C); 174.03 (1-C): arachidonoyl fragment; 62.67 (1-C, 3-C); 75.28 (2-C): glycerol fragment.
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