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Facile synthesis and stabilization of 2-arachidonylglycerol via its 1,3-phenylboronate ester

Herbert H. Seltzman,* Denise N. Fleming, Gregory D. Hawkins and F. Ivy Carroll

Chemistry and Life Sciences, Research Triangle Institute, Research Triangle Park, N.C. 27709, USA

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Abstract

2-Arachidonylglycerol (2-Ara-Gl) was synthesized via the intermediacy of its 1,3-phenylboronic acid ester. The boronate ester is easily stable enough to enable chromatographic resolution from the corresponding 1-Ara-Gl boronate ester on normal phase elution yet immediately and completely hydrolyzes to 2-Ara-Gl and phenylboronic acid, without isomerization, by simple solution in aqueous-organic solvents. The phenylboronate ester of this 2-acylglycerol has the added advantage of being markedly more stable to both isomerization and oxidation upon storage than the labile 2-Ara-Gl.

Keywords: 2-acylglycerols; 1,3-phenylboronate ester; stability.

2-Arachidonylglycerol (2-Ara-Gl) has been isolated from canine gut and shown to be an endogenous ligand for the central (CB1) and peripheral (CB2) cannabinoid receptors. It shares the physiological actions of Δ^9-THC, the active constituent of marijuana, in binding to transfected CB1 and CB2 receptors, inhibiting cAMP production, eliciting cannabinoid responses in the mouse tetrad of behavioral tests, and inhibiting the electrically evoked contractions of mouse isolated vasa deferentia.[1] Thus, 2-Ara-Gl is of current interest for studies of the neurochemical and immunological systems characterized by the cannabinoid receptors.[2,3]

A chemical synthesis of 2-Ara-Gl would enable ready access to this ligand for biological studies and provide a model for a corresponding radiolabeled synthesis, also of value in biochemical studies. However, synthesis, isolation, storage, and testing of 2-Ara-Gl are all compromised by the instability of the compound to both air oxidation affording olefin rearranged hydroperoxides and to ester rearrangement affording 1-Ara-Gl.[4] Here we report a synthesis that enables an easy release of 2-Ara-Gl without rearrangement to 1-Ara-Gl from a precursor that exhibits excellent resistance to oxidation and ester rearrangement during storage.

Since the mono-acylation of glycerol occurs virtually only at the primary hydroxyl groups, the acylation of 1,3-protected glycerol analogs was investigated.[5,6] 1,3-Benzylideneglycerol has been employed

* Corresponding author. Tel: 1 919 541 6690; fax 1 919 541 8868; e-mail: hhs@rti.org (H. H. Seltzman)

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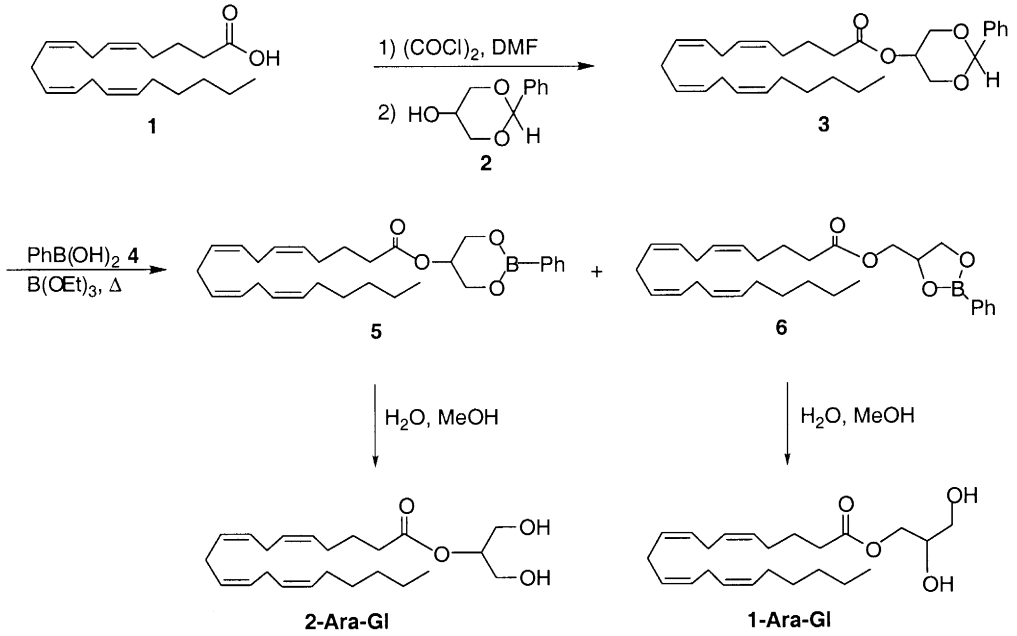
previously in the synthesis of other unsaturated 2-acyl-monoglycerides.[5] In these examples, the removal of the acetal protecting group at the end of the reported syntheses employed boric acid and subsequent mild cleavage of the intermediate boric acid ester with water. This two step mild process circumvented potential reduction of double bonds during an alternative hydrogenolysis or rearrangement to a 1-acylglycerol during standard aqueous acid hydrolysis of the acetal.[5] This suggested that this two-step process using an appropriately substituted intermediate borate ester could provide pure 2-Ara-Gl. Thus, we treated arachidonyl chloride, from commercially available arachidonic acid (**1**), with commercially available *cis*-1,3-*O*-benzylideneglycerol (**2**) to afford 2-arachidonylglycerol 1,3-benzylidene acetal (**3**) in 76% chromatographed yield (Scheme 1).[7] In the case of **3**, we found the subsequent boric acid cleavage to be difficult to drive to completion and to suffer increasing isomerization to 1-Ara-Gl upon longer reaction. Upon hydrolysis, the resulting 1- and 2-Ara-Gl were not readily separable on a preparative scale.



Scheme 1.

The use of phenylboronic acid (**4**), in lieu of boric acid, for the cleavage of the benzylidene acetal gave excellent results. First, the cleavage of the benzaldehyde moiety went to completion to 2-arachidonylglycerol 1,3-phenylboronate ester[8] (**5**) with only modest formation of the 1-arachidonylglycerol 2,3-phenylboronate ester[9] (**6**) (13%). Second, the desired **5** could be easily separated by normal phase chromatography[10] from the minor ester **6**. The 1,3-boronate ester **5**, obtained in 76% chromatographed yield, was characterized and distinguished from the 2,3-boronate ester by NMR analysis. HPLC (reversed phase in MeOH–H_2O) of each of the pure esters **5** and **6** showed only phenylboronic acid and the respective single 1- or 2-Ara-Gl deriving from hydrolysis in the aqueous-organic eluant.[11] Thus, cleavage of either ester is immediate, complete and proceeds without isomerization.

The boronate ester **5** showed no change upon storage (HPLC) over six months. In contrast, the isolated 2-Ara-Gl (see below) showed oxidation in as little as a day (polar components by HPLC) when stored as a resin unless rigorous effort was made to exclude air. When 2-Ara-Gl is stored in various solvents

(MeOH–H_2O, ethanol, CH_2Cl_2), isomerization to 1-Ara-Gl was observed, going near to completion in one or more days even at low temperatures. The stable solid boronate ester **5** serves as an excellent storage form of 2-Ara-Gl.

Cleavage of **5** is immediate and complete by dissolving in 85% MeOH–H_2O with which it is eluted through a C18 reverse phase LoBar chromatography column.[12] 2-Ara-Gl fractions elute without any 1-Ara-Gl or phenylboronic acid (HPLC). The MeOH–H_2O solution can be used as such, in the short term before isomerization becomes significant, or can be partitioned between water and CH_2Cl_2, dried and evaporated to a resin (86% yield) for longer term storage. The NMR spectra[13] is in agreement with that expected for the symmetrical 2-Ara-Gl and that reported[6] and in contrast with 1-Ara-Gl independently prepared from glycerol and arachidonic acid or from cleavage from the boronate ester **6**.

The results presented here demonstrate that 2-arachidonylglycerol-1,3-phenylboronate ester **5** is a versatile intermediate for the synthesis of 2-Ara-Gl that enables: (1) its chromatographic resolution from its isomer **6**, (2) its conversion to 2-Ara-Gl effectively and efficiently under very simple and mild conditions; and (3) provides a means of stable storage for an as needed conversion to the unstable 2-Ara-Gl. The approach suggests its application to the synthesis of other 2-acylglycerols.

Acknowledgements

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6. Han, L.; Razdan, R. K. *Tetrahedron Lett.* **1999**, *40*, 1631–1634. A 1,3-disilyl ether of glycerol has recently been reported for the synthesis of 2-Ara-Gl, for which the deblocking of the final intermediate proceeds under different conditions (low temperature, *n*-Bu_4NF, HOAc buffer, overnight) and yields than the phenylboronate ester of this work does (ambient temperature, 85% MeOH–H_2O, immediate).
7. 2-Arachidonyl-*cis*-1,3-*O*-benzylideneglycerol: ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.52–7.48 (m, 2H, ArH_2), 7.39–7.34 (m, 3H, ArH_3), 5.55 (s, 1H, CHPh), 5.38 (m, 8H, vinyl H), 4.71 (s, 1H, 2′-H), 4.28, 4.16 (d,d, 4H, J=13.5 Hz, eq./ax. 1′/3′-H), 2.82–2.77 (m, 6H, diallylic), 2.45 (t, 2H, J=7.6 Hz, 2-CH_2), 2.18–2.00 (m, 4H, allylic-H), 1.75 (p, 2H, J=7.3 Hz, 3-CH_2), 1.37–1.17 (m, 6H, 17-19-CH_2), 0.88 (t, 3H, J=6.7 Hz, 20-CH_3). ^{13}C NMR ($CDCl_3$) δ 173.6, 137.8, 130.4, 129.0, 128.9, 128.5, 128.3, 128.2, 127.9, 127.5, 126.0, 101.2, 69.4, 65.8, 33.7, 31.5, 29.3, 27.1, 26.5, 25.6, 24.7, 22.5. MS (DIP, 14 eV) *m/e* 466 (M^+).
8. 2-Arachidonylglycerol 1,3-phenylboronate ester: ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.8 (dd, 2H, J=8.1, 1.5 Hz, phenyl), 7.44–7.36 (m, 3H, phenyl), 5.45–5.32 (m, 8H, vinyl), 5.18 (p, 1H, J=2.2 Hz, 2′-CH), 4.28 (dd, 2H, J=12, 2.2 Hz, 1′- and 3′-CH[ax. or eq.]), 4.19 (dd, 2H, J=12, 2.2 Hz, 1′- and 3′-CH[eq. or ax.]), 2.84–2.76 (m, 6H, diallylic), 2.36 (t, 2H, J=7.8 Hz, 2-CH_2), 2.13–2.01 (m, 4H, allylic), 1.69 (p, 2H, J=7.5 Hz, 3-CH_2), 1.37–1.28 (m, 6H, 17–19 CH_2), 0.88 (t, 3H, J=6.8 Hz, 20-CH_3).
9. 1-Arachidonylglycerol 2,3-phenylboronate ester: ^{1}H NMR (300 MHz, $CDCl_3$): 7.28 (d, 2H, J=9 Hz, ArH_2), 7.46 (d, 1H, J=3 Hz, ArH), 7.38 (t, 2H, J=7.5 Hz, ArH_2′), 5.37 (m, 8H, vinyl H_8), 4.80 (m, 1H, 2′-H), 4.45 (t, 1H, J=9.1 Hz, 3′-H_a), 4.30 (dd, 1H, J=4.0, 11.9 Hz, 1′-H_a), 4.21 (dd, 1H, J=5.1, 11.9 Hz, 1′-H_b), 4.14 (dd, 1H, J=9.1 Hz, 3′-H_b), 2.86–2.75 (m, 6H, 7,

10, 13-CH_2), 2.35 (t, 2H, J=7.5 Hz, 2-CH_2), 2.14–1.98 (m, 4H, 4, 16-CH_2), 1.69 (p, 2H, J=7.5 Hz, 3-CH_2), 1.40–1.22 (m, 6H, 17-19-CH_2), 0.89 (t, 3H, J=12 Hz, 20-CH_3).

10. In chemical transformations, boronate esters have been used as protecting groups, which have generally been isolated by crystallization and distillation. The use of adsorption chromatography to separate boronate ester derivatives of compounds is less often seen. Chromatographically they have been used mostly for GC and for TLC where it is employed in the eluant to alter the mobility of various polyols. For a review of the applications of boronate esters, see: Ferrier, R. J. *Adv. in Carbohy. Chem. Biochem*. **1978**, *35*, 31–80.
11. Waters Resolve C18 Radial Compression column 10 um (part No. WAT084720), 85% MeOH–H_2O, 214 nm detection, 2 mL/min, Rt: 2-Ara-Gl 12.5 min, 1-Ara-Gl 13.9 min.
12. Merck LoBar C18 reversed phase size A column.
13. 2-Arachidonylglycerol (2-Ara-Gl): ^{1}H NMR ($CDCl_3$, 250 MHz): 5.37 (m, 8H, vinyl), 4.91 (p, 1H, J=4.72 Hz, 2′-CH), 3.82 (d, 4H, J=4.72 Hz, 1′,3′-CH_2), 2.81 (m, 6H, diallylic), 2.38 (t, 2H, J=7.5 Hz, 2-CH_2), 2.02 (m, 4H, allylic), 1.71 (p, 2H, J=7.4 Hz, 3-CH_2), 1.31 (m, 6H, 17-19-CH_2), 0.87 (t, 3H, J=6.7 Hz, 20-CH_3); CMR ($CDCl_3$): 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, 25.64, 25.62, 24.8, 22.6, 14.1.