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TITLE:

Synthesis of a Deuterated Standard for the Quantification of 2-Arachidonoylglycerol in *Caenorhabditis elegans*

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KEYWORDS:

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SUMMARY:

This work describes a robust and straightforward method to detect and quantify the endocannabinoid 2-arachidonoylglycerol (2-AG) in *C. elegans*. An analytical deuterated standard us prepared and used for the quantification of 2-AG by isotopic dilution and liquid chromatography-electrospray ionization-tandem mass spectrometry (LC-ESI-MS/MS).

ABSTRACT:

This work presents a method to prepare an analytical standard to analyze 2-arachidonoyl glycerol (2-AG) qualitatively and quantitatively by liquid chromatography-electrospray Ionization-tandem mass spectrometry (LC-ESI-MS/MS). Endocannabinoids are conserved lipid mediators that regulate multiple biological processes in a variety of organisms. In *C. elegans*, 2-AG has been found to possess different roles, including modulation of dauer formation and cholesterol metabolism. This report describes a method to overcome the difficulties associated with the costs and stability of deuterated standards required for 2-AG quantification. The procedure for synthesis of the standard is simple and can be performed in any laboratory,

without the need for organic synthesis expertise or special equipment. In addition, a modification of Folch's method to extract the deuterated standard from *C. elegans* culture is described. Finally, a quantitative and analytic method to detect 2-AG using the stable isotopically labeled analog 1-AG-d_5 is described, which provides reliable results in a fast-chromatographic run. The procedure is useful for studying the multiple roles of 2-AG in *C. elegans* while also being applicable to other studies of metabolites in different organisms.

INTRODUCTION:

Endocannabinoids regulate multiple biological processes in a variety of organisms and are conserved lipid mediators[1]. The first discovered and most well-characterized endocannabinoids are anandamide (arachidonoylethanolamide, AEA) and 2-arachidonoyl glycerol (2-AG). Endocannabinoids play many critical roles, including those involved in brain reward systems as well as drug addiction, memory, mood, and metabolic processes[2]. AEA and 2-AG are only synthesized when needed and have short life spans, and they are degraded through transport protein reuptake and hydrolysis[3].

The use of animal models like *Caenorhabditis elegans* (*C. elegans)* has become important to study the large variety of biological processes including apoptosis, cell signaling, cell cycle, cell polarity, gene regulation, metabolism, ageing, and sex determination[4,5]. Additionally, *C. elegans* is an excellent model for studying the physiological roles of polyunsaturated fatty acids (PUFAs). AEA has been identified in *C. elegans* and is reduced under dietary restriction[6]. This deficiency extends the lifespan of the nematode through a dietary restriction mechanism that can be suppressed by supplementation with the endocannabinoid. Recently, it was discovered that 2-AG and AEA play fundamental roles in the regulation of cholesterol trafficking in *C. elegans*[7]. More importantly, it was determined that supplementation with exogenous 2-AG can rescue dauer arrest, which is caused by the impaired cholesterol trafficking in Niemann-Pick type C1 *C. elegans* mutants.

To gain a better understanding of 2-AG's relationship with cholesterol trafficking and other biological processes in the nematode (i.e., monoaminergic signaling, nociception and locomotion), it is crucial to study this endogenous metabolite and how it is affected under certain environmental and dietary conditions[8-13]. Therefore, it is imperative to design and optimize a method to detect and quantify endogenous 2-AG in *C. elegans* that is simple to use for scientists of different fields, especially those who study the nematode's behavior in relation to this endocannabinoid.

In 2008, Lethonen and coworkers succeeded in identifying 2-AG and AEA in *C. elegans* using LC-MS analytical methods[14]. In 2011, they managed to expand this technique to other endocannabinoids[15]. More recent work has shown other analytical methods that have been successful in detecting and quantifying endocannabinoids in *C. elegans*, including mass spectrometry and GC-MS[16-18], and it has also been reported that similar analytical methods can be expanded to other models[19].

Previously reported analytical methods used for quantifying 2-AG in biological samples usually involve the use of deuterated standards that are commercially acquired and require availability for the purchase[20,21]. Many analytical standards for LC-MS/MS quantification of endocannabinoids are commercially available from different providers. Nevertheless, they are expensive, are sensitive, and become oxidized over time, due to the presence of multiple double bonds. The most common versions of these standards are based on the octa-deuterated arachidonic acid and are suitable for quantification by isotope dilution LC-MS/MS[14,22]. Also, most of these standards are substituted in position 2 of the glycerol, making them unstable under most conditions since they are prone to acyl migration[19,23].

To overcome the difficulties associated with the costs and stability of these deuterated standards, a convenient and simple method is presented to prepare an analytical standard based on glycerol-d_5. The sequence to prepare the penta-deuterated standard requires a three-step procedure that results in the standard 1-AG-d_5, which is stable and does not undergo acyl migration (the main issue when aiming to synthesize 2-monoacylglycerols).

The main objective here is to show a simple and reproducible method to study 2-AG in *C. elegans*, including the synthesis of the analytical deuterated standard, preparation and extraction of the nematode samples, and analysis by LC-MS/MS (**Figure 1**). This synthetic procedure is achievable without the sophisticated organic synthesis knowledge or special equipment, making it suitable for scientists from different fields who are studying *C. elegans* behavior under endocannabinoid influence. The method is also expandable to other study models, making it useful for different targets. The standard, prepared as reported here, has been applied to successfully develop a fast and reliable chromatographic method that allows for effective detection and quantification of 2-AG in a reproducible manner.

PROTOCOL:

1. 1-AG-d_5 preparation

NOTE: For obtaining 1-AG-d_5 as a deuterated internal standard for quantification assays, follow the protocol as detailed below.

1.1. Differential protection

1.1.1. To only protect primary alcohols, first add 38 mg of glycerol-d_8 to a 10 mL reaction tube using a Pasteur pipette and add a magnetic stirrer.

1.1.2. Add 5 mL of anhydrous dichloromethane (DCM) using a 5 mL Hamilton syringe, and fill the tube with dry N_2 to yield an inert atmosphere.

1.1.3. Prepare a bath using a shallow Dewar flask filled with distilled ethyl acetate.

1.1.4. Fit the hermetically closed reaction tube inside the bath and cool it by slowly adding liquid N_2 to the ethyl acetate until the solvent is frozen.

CAUTION: Liquid violently boils at room temperature (RT) and can cause severe burns when contacting eyes and skin.

1.1.5. Add 54 mg of anhydrous collidine using a Hamilton syringe.

CAUTION: Collidine is volatile and has a very strong and unpleasant scent.

1.1.6. Add 70 mg of tert-butyldimethylsilyl chloride and stir the entire solution for 3 h at -78 °C on a magnetic stirrer.

1.1.7. After 3 h, leave the reaction to warm at RT and keep stirring for an additional 12 h.

1.1.8. Add 2 mL of brine to quench the reaction.

1.1.9. Extract the solution 3x with 2 mL of distilled dichloromethane using a separating funnel, saving the organic extract each time.

1.1.10. Combine the three organic extracts and dry them over sodium sulfate.

1.1.11. Evaporate the dichloromethane under reduced pressure in a vacuum rotary evaporator carefully to avoid solvent projections.

1.1.12. Purify the crude mixture by column chromatography using silica gel as the stationary phase and a 10% increasing hexane/ethyl acetate gradient, starting from 100% hexane and finishing with 100% ethyl acetate.

1.1.13. Combine the product-containing fractions and remove the solvent under reduced pressure in a vacuum rotary evaporator to obtain the pure 1-O,3-O-bis-(TBDMS) glycerol-d_5 as a colorless liquid.

1.2. Esterification

1.3.1. Add 10 mg of the 1-O,3-O-bis(TBDMS)-glycerol-d_5 (previously synthesized) to a 10 mL reaction tube using a Pasteur pipette and add a magnetic stirrer.

1.3.2. Add 2 mL of anhydrous dichloromethane using a 5 mL Hamilton syringe, and fill the tube with dry N_2 to yield an inert atmosphere.

1.3.3. Cool the solution to 0 °C using an ice bath.

1.3.4. Add 36 mg of arachidonic acid using a multi-volume adjustable micropipette and stir.

1.3.5. Add 15 mg of 4-dimethylaminopyridine and stir.

1.3.6. Add 15 mg of N,N'-diisopropylcarbodiimide using a multi-volume adjustable micropipette and stir.

1.3.7. Let the mixture react at 0 °C for 3 h.

1.3.8. After 3 h, leave the reaction to warm at RT and keep stirring for an additional 12 h.

1.3.9. Add 2 mL of water to quench the reaction.

1.3.10. Extract the organic solution 3x with 2 mL of distilled dichloromethane (DCM) using a separating funnel.

1.3.11. Place the three organic extracts in the same tube and dry them over sodium sulfate.

1.3.12. Evaporate the dichloromethane under reduced pressure in a vacuum rotary evaporator carefully to avoid solvent projections.

1.3.13. Purify the crude mixture by column chromatography using silica gel as the stationary phase and a 10% increasing hexane/ ethyl acetate gradient, starting from 100% hexane and finishing with 50% hexane/50% ethyl acetate.

1.3.14. Combine the product-containing fractions and remove the solvent under reduced pressure in a vacuum rotary evaporator to obtain the pure 1-O, 3-O-bis(TBDMS)-2-AG-d_5 as a yellowish liquid.

1.4. Deprotection

1.4.1. Add 15 mg of the 1-O,3-O-bis(TBDMS)-2-AG-d_5 (previously synthesized) to a 10 mL reaction tube using a Pasteur pipette and add a magnetic stirrer.

1.4.2. Add 2 mL of anhydrous THF using a 5 mL Hamilton syringe, and fill the tube with dry N_2 to yield an inert atmosphere.

1.4.3. Cool the solution to 0 °C using an ice bath.

1.4.4. Add 150 µL dropwise of 1 M tetrabutylammonium fluoride solution in THF using a Hamilton syringe.

1.4.5. Let the reaction warm to RT and stir for 1 h.

1.4.6. After 1 h, add 2 mL of water to quench the reaction.

1.4.7. Extract the solution 3x with 2 mL of distilled dichloromethane using a separating funnel.

1.4.8. Combine the three organic extracts and dry them over sodium sulfate.

1.4.9. Evaporate the dichloromethane under reduced pressure in a vacuum rotary evaporator to obtain the pure 1-AG-d_5 as a yellowish liquid.

1.5. Monitor all reactions by thin layer chromatography performed on silica gel 60 F_{254} pre-coated aluminum sheets. Visualize the bands under a 254 nm UV lamp after staining with an ethanolic solution of 4-anisaldehyde.

2. Preparation of standard stock and measuring solutions

2.1. Dissolve 1 mg of the internal standard 1-AG-d_5 in 1 mL of ACN and sonicate for 1 min to obtain the 1,000 ppm standard stock solution.

2.2. To prepare the 1,000 ppb solution used for quantification in worms, first prepare a 10 ppm solution: take 10 µL of the stock solution using a Hamilton syringe and dilute it to a final volume of 1 mL by adding 990 µL of ACN.

2.3. Take 100 µL from the solution produced in step 2.2 using a Hamilton syringe, and dilute it to a final volume of 1 mL by adding 900 µL of ACN to obtain the 1,000 ppb solution used for the quantification.

2.4. Sonicate for 1 min between each step to ensure complete solubilization. Store the solutions at -78 °C to maintain the concentrations and integrity of the standards. After the standard solution is used, flow some nitrogen before closing the vial to prevent oxidation.

3. Growth and maintenance of *C. elegans*

NOTE: Seed the nematode growth medium (NGM) agar plates with *E. coli* OP50 and propagate the worms on these plates.

3.1. Mix 3 g of NaCl with 17 g of agar.

3.2. Add 2.5 g of peptone, then add 975 mL of H_2O.

3.3. Autoclave for 50 min, then cool the flask to 55 °C.

3.4. Mix the following: 1 mL of 1 M $CaCl_2$, 1 mL of 1 M $MgSO_4$, 25 mL of 1 M KH_2PO_4 buffer (all of which have been previously autoclaved), and 1 mL of 5 mg/mL cholesterol in ethanol.

3.5. While maintaining a sterile environment, dispense the NGM solution into 60 mm Petri

plates, filling the plates to two-thirds of their volume. Store the plates at 4 °C.

3.6. Streak the *E. coli* bacterial culture from a -80 °C glycerol stock onto the LB agar plate. Let it grow on the plate overnight at 37 °C.

3.7. Pick up a single colony to inoculate 100 mL of liquid LB overnight at 37 °C with agitation.

NOTE: It is not necessary to check the O.D. because this strain can reach stationary phase over this time.

3.8. Remove the stored NGM plates, remove the lids in the laminar flow hood, and leave open to allow evaporation of excess moisture from the plates.

3.9. Once the plates are dried, use a Pasteur pipette to add 100 µL of OP50 *E. coli* to the center of the plate without spreading.

3.10. Leave the OP50 *E. coli* lawn to grow overnight at RT or at 37 °C for 8 h.

3.11. Add the desired number worm embryos obtained by hypochlorite treatment or "bleaching" (Section 4).

NOTE: Cool the plates to RT before the addition of worms.

4. Bleaching technique for synchronizing *C elegans* cultures

4.1. Seed and chunk worms onto 6 cm NGM plates.

4.2. Leave the worms growing for 2–3 days to obtain sufficient numbers of eggs and gravid adults on the plate.

4.3. Once there are enough eggs/adults, pour 5 mL of M9 onto the plate.

4.4. Transfer the worms to a 15 mL centrifuge tube using a glass pipette.

4.5. Centrifuge the tube for 2 min at 2,000 *x g* and pellet the worms.

4.6. Suction out most of the M9, avoiding disturbance of the worm pellet.

4.7. Add 3 mL of bleaching solution (2:1:1 ratio of NaOH:NaOCl:H_2O).

4.8. Invert gently to mix the solution for 5 min or until the number of intact adult worms decreases.

CAUTION: Do not bleach for more than 5 min.

4.9. Centrifuge for 1 min at 2,000 *x g* and suction most of the bleaching solution without disturbing the worm pellet.

4.10. Add 15 mL of M9 and mix well.

4.11. Centrifuge again at 2000 *x g* for 1 min.

4.12. Suction out most of the M9 without disturbing the worm pellet.

4.13. Repeat steps 4.10–4.12 one or two more times.

4.14. Add 5 mL of fresh M9 and agitate.

4.15. Let the eggs hatch overnight with gentle rocking.

5. Worm sample preparation

5.1. Let the N2 embryos obtained by the bleaching procedure hatch overnight in M9 buffer (5 mL in a 15 mL centrifuge tube) at 20 °C.

5.2. Harvest the synchronized L1s by centrifuging the tube for 2 min at 2,000 *x g*.

5.3. Wash the worms with M9 buffer 1x, then quantify the number of live L1 worms.

5.4. Seed approximately 10,000 worms into NGM plates (10 cm diameter) with 1 mL of OP50 *E. coli* (previously dried).

5.5. Incubate the plates for 48 h at 20 °C until worms reach the L4 stage.

5.6. Harvest the worms using cold M9 buffer in a 15 mL centrifuge tube, wash them 1x, then and transfer them to a 1.5 mL tube.

5.7. Pellet the worms by centrifugation at 2,000 *x g* for 1 min, eliminate most of the supernatant, immerse the tubes in liquid nitrogen, and store at -80 °C.

6. Lipid extraction

6.1. Thaw approximately 100 µL of frozen worm pellets belonging to N2 on ice, add 1.3 mL of methanol, and sonicate the sample for 4 min.

6.2. Add 2.6 mL of chloroform, and 1.3 mL of 0.5 M KCl/0.08 M H_3PO_4 to a final ratio of 1:2:1, 1,000 ppb of the internal standard 1-AG-d_5, and butylated hydroxytoluene as an antioxidant agent at a final concentration of 50 µg/mL.

6.3. Vortex the samples for 1 min and sonicate in an ultrasonic water bath for 15 min on ice.

6.4. Vortex the samples 2x for 1 min and centrifuge for 10 min at 2,000 *x g* to induce the phase separation.

6.5. Collect the lower phase and collect it in a clean tube, dry it under nitrogen, and resuspend the solid residue in 100 µL of ACN.

7. Endocannabinoid analysis by HPLC-MS/MS

7.1. Use liquid chromatography coupled with an ESI triple quadrupole mass spectrometer to detect and quantify 2-AG from nematode samples.

7.2. Use the following ratio for reversed-phase HPLC: from 0.0–0.5 min H_2O:ACN (40:60), from 0.5–6.5 min H_2O:ACN (40:60) to (25:75), from 6.5–7.5 min H_2O:ACN (25:75), from 7.5–8.0 min H_2O:ACN (25:75) to (40:60); from 8.0–12.0 min H_2O:ACN (40:60).

7.3. Maintain the column temperature at 40 °C and set the autosampler tray temperature to 10 °C.

7.4. Set the following ionization conditions: positive-ion mode; drying gas (N_2) temperature = = 300 °C; drying gas flow rate = 10 L/min; nebulizer pressure = 10 UA; and cap. voltage = 4 kV.

7.5. For the analyte detection, use MRM with the following transitions: 379.2 m/z to 289.2 m/z for 2-AG; and 384.2 m/z to 289.2 m/z for 1-AG-d_5.

8. Endocannabinoid quantification in worms

8.1. Use deuterated internal standard 1-AG-d_5 and calculate the peak area ratios of the analyte to the internal standard.

8.2. Use the following transitions: 384.2 m/z to 287.2 m/z for 2-AG; and 379.2 m/z to 287.2 m/z for 1-AG-d_5.

8.3. Calculate the concentration of the endogenous 2-AG by comparing to the peak area ratios of the deuterated standard using the concentration value of the standard.

REPRESENTATIVE RESULTS:

An isotopically labeled analog was successfully synthesized from commercially available d_8-glycerol and arachidonic acid using a 3-step synthetic method (**Figure 2, Figure 3**). These steps are straightforward and do not require sophisticated equipment, specially controlled conditions, or expensive reagents. Thus, this method is robust and may be successfully

extended to synthesize monoacylglycerides containing different fatty acids.

1-AG-d_5 was structurally characterized using nuclear magnetic spectroscopy. ^{1}H NMR showed the characteristic multiplet at 5.44 ppm to 4.93 ppm, which integrates for the eight vinyl protons of the arachidonoyl chain and triplet at 2.40 ppm, corresponding to the two protons of the alpha position to the carbonyl group. In ^{2}D NMR, it is also possible to see a 2.9 ppm to 2.7 ppm multiplet assignable to the five deuterium of the glycerol portion.

The chemically synthesized 1-AG-d_5 was used as an internal standard in *C. elegans* samples. The standard was added to the samples before extraction then extracted with the endogenous lipids, using a straightforward method adapted from Folch[24]. This modified method provides a high recovery value of the standard, as shown by HPLC quantification.

The method was optimized using the transitions 1) 384.2 m/z to 287.2 m/z for 2-AG and 2) 379.2 m/z to 287.2 m/z for 1-AG-d_5, in which the glycerol molecules are lost **(Figure 4)**. The limits of detection (LOD) and quantification (LOQ) were calculated for the standard using a calibration curve, resulting in values of 5 ppb and 16.6 ppb, respectively. The retention time for the standard was 6.8 min.

2-AG endogenous from the *C. elegans* samples was successfully detected and quantified by isotopic dilution with the chemically synthesized 1-AG-d_5 using HPLC-MS/MS **(Figure 5)**.

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Since the original concentrations of the deuterated standards in samples 1 and 3 were each 1,000 ppb, from the peak area ratio it was possible to calculate the endogenous concentration of 2-AG at 340 ppb for sample 1 and 360 ppm for sample 3, yielding an average of 350 ppm **(Table 1)**.

FIGURE AND TABLE LEGENDS:

Figure 1: Summary of synthesis, worm sampling, and quantification. To achieve successful quantification of the endogenous 2-AG, it was necessary to synthesize its deuterated analog using a three-step sequence. Afterwards, it was added to worm samples, extracted, and analyzed by HPLC-MS/MS. Used as an internal standard, the synthetic of 1-AG-d_5 was the tool used to quantify the endogenous metabolite.

Figure 2: Synthetic scheme for obtaining 1-AG-d_5. A mass of 10 mg of the deuterated analog was obtained using the three-step method involving 1) protection of the glycerol-d_8, 2)

acylation with arachidonic acid, and 3) deprotection.

Figure 3: Chemical structure of the isotopically labeled 2-AG analog.

Figure 4: Selected fragmentations for quantification of 1-AG-d_5 and 2-AG.

Figure 5: HPLC chromatograms for 1-AG-d_5 and 1-AG as pure standards and internal standards in a worm sample. It was possible to analyze retention times and see that 1) the worm appears not to have endogenous 1-AG and 2) it would only have 2-AG, but the standard 1-AG-d_5 will still work as a good analytical standard for quantification by isotopic dilution. The transitions used were: 384.2 m/z to 287.2 m/z for 2-AG, and 379.2 m/z to 287.2 m/z for 1-AG-d_5.

Table 1: Peak area ratios for the deuterated standard and endogenous 2-AG. The ratios were calculated as a quotient between the peak areas of 2-AG and 1-AG-d_5, respectively, for two isolated samples, both with deuterated standard added prior to extraction.

DISCUSSION:

Endocannabinoids are a class of lipids that have been implicated in the regulation of dauer formation in *C. elegans*[7]. More specifically, the synthesis of polyunsaturated fatty acids (PUFAs) is important for cholesterol trafficking and the reproductive development of worms. It is revealed here that 2-AG, an arachidonic acid containing endocannabinoid, is responsible for restituting the dauer larva to its normal cycle in worms that have impaired cholesterol metabolism[7].

Given the recently discovered importance of 2-AG in the enhancement of cholesterol trafficking and other biological processes and how little is known about how lipids influence this process, a reliable detection method for this endocannabinoid is necessary. The successful development of this simple and robust synthetic method to obtain the deuterated analog 1-AG-d_5 is a key step in this protocol.

Most of the reported methods to quantify monoacylglycerols involve the use of commercially available analytical standards, which are usually expensive and unstable under regular storage conditions. This makes them inconvenient for researchers who require larger quantities of standards and fresh stocks. They are also unreachable for lower budget laboratories. However, this method overcomes this obstacle by proposing synthesis of the standard using more accessible starting materials.

It is also remarkable that contrary to other reported methods (which use deuterated analytical standards of 2-substituted monoacylglycerols that suffer acyl-migration under many conditions, so that two chromatographic peaks are seen and affect the relative quantification by isotopic dilution[25]), this method efficiently uses a 1-substituted deuterated analytical standard, which is a single isomer and does not undergo acyl-migration.

The synthetic method is straightforward and requires no sophisticated conditions, making it ideal for any laboratory having minimal equipment, budget, and access to reactants. It is also a simple technique that can be used by any scientist working in the field, without the need for special training in organic synthesis. The worm sample preparation is the conventional method, without further complications. Finally, the lipid extraction method to obtain the final samples is a modification from Folch's protocol[24] that allows for better recovery values, since it does not require chromatographic column purification.

The critical step is to ensure that the sample preparation and lipid extraction are performed adequately to achieve good and detectable recovery of the standard. It is also important to 1) produce fresh stock solutions monthly to maintain conditions of the standard and 2) check by NMR-spectroscopy or LC-MS that the standard is still pure and has not undergone oxidation or degradation. The only limitation of this technique relies in its expansion to other studies that may have endogenous 2-AG concentrations lower than the presented LOQ. In this case, the method should be modified to ensure that the concentration falls between the limits.

In the case of failure during the protocol in which 1) there is no visible chromatographic signal of the standard or 2) the recovery value of the standard after extraction is lower than expected, it is recommended to repeat sample preparation and lipid extraction. Since the synthetic route involves synthesis of a protected deuterated glycerol building block that is finally acylated with arachidonic acid in the last step, this method can be expanded to the synthesis of deuterated standards of other monoacylglycerols, diacylglicerols, phospholipids, and structurally related metabolites.

In summary, this new procedure describes a straightforward and reproducible method for detecting and quantifying 2-AG, which will help address some of the unanswered questions regarding the role of this endocannabinoid in *C. elegans.*

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DISCLOSURES:

The authors declare no conflicts of interest.

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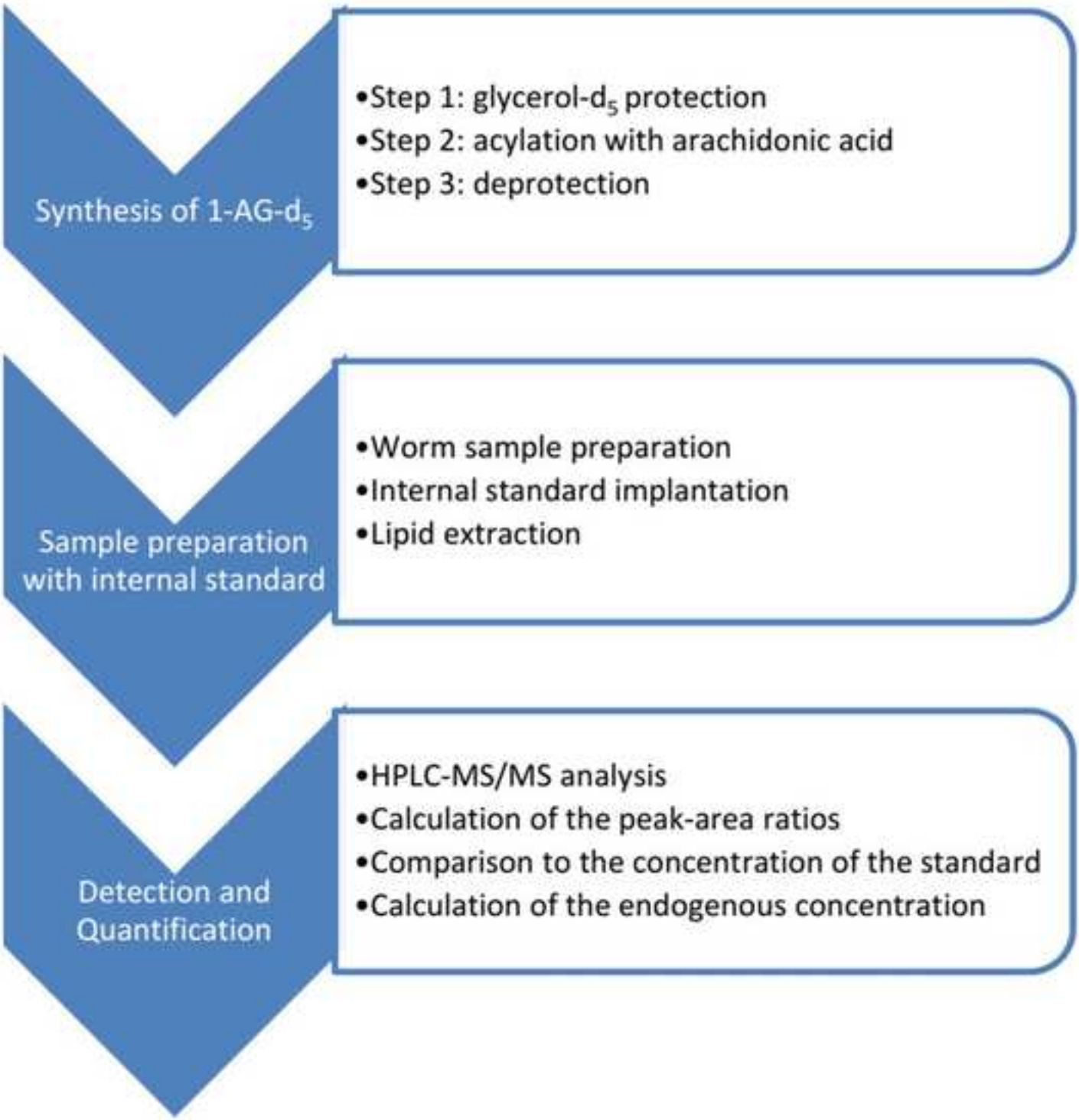
Figure 1



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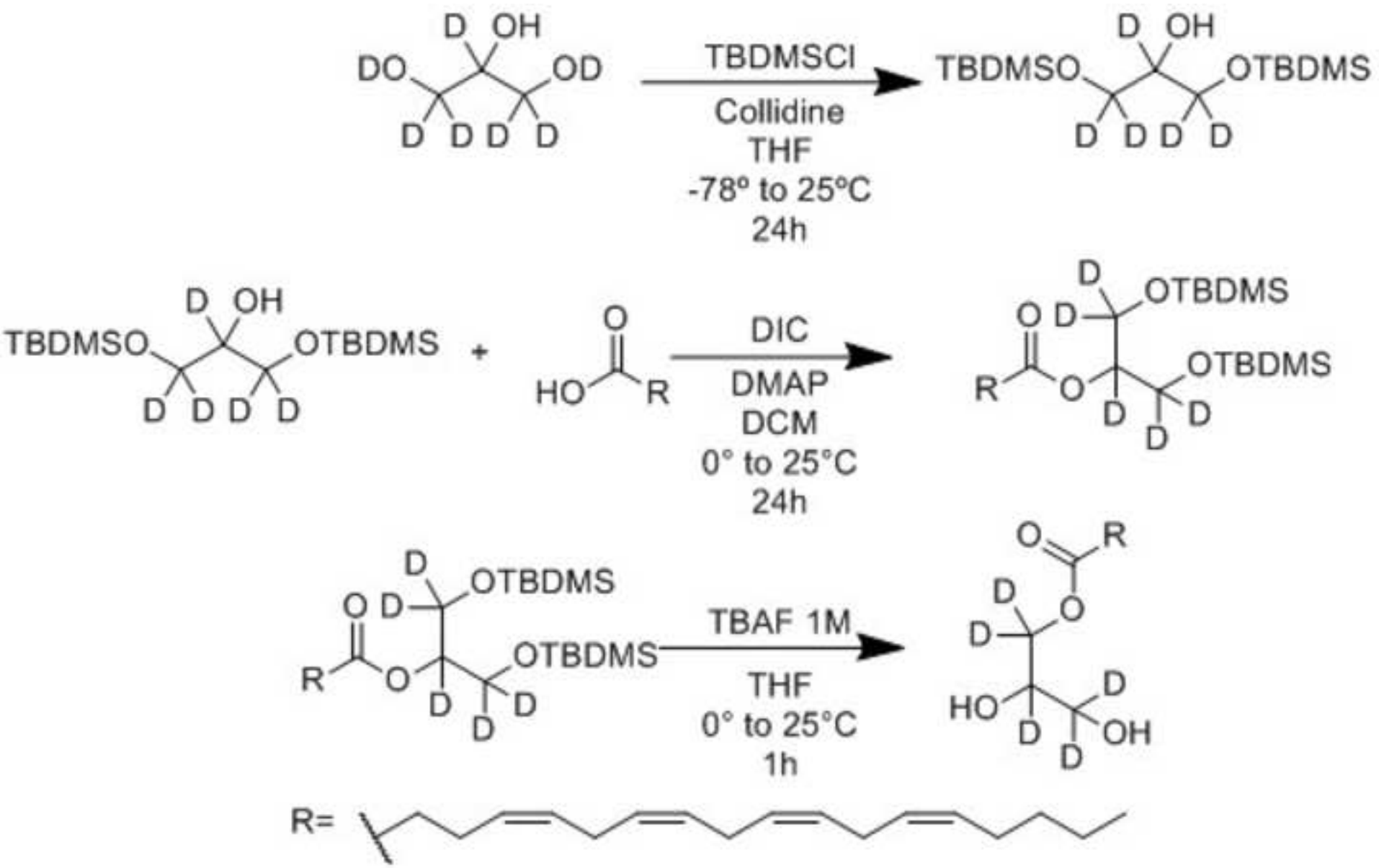

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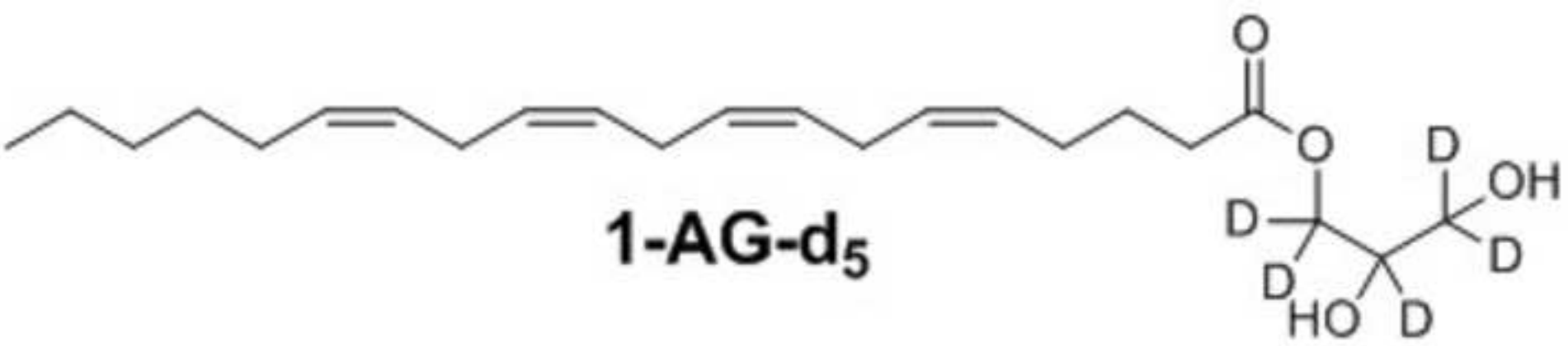

1-AG-d_5

Figure 4

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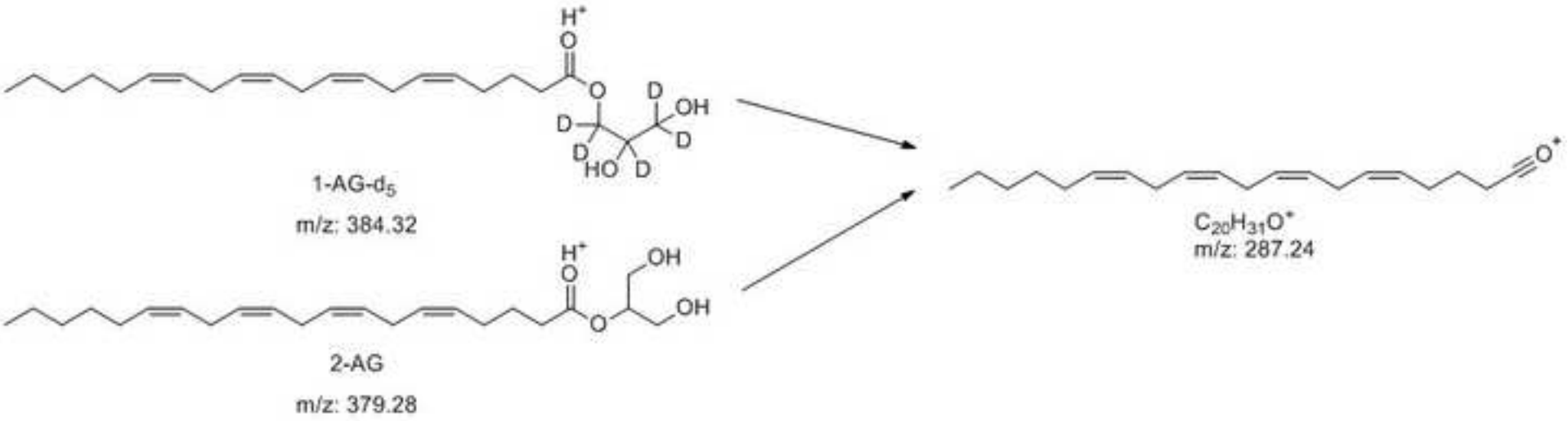

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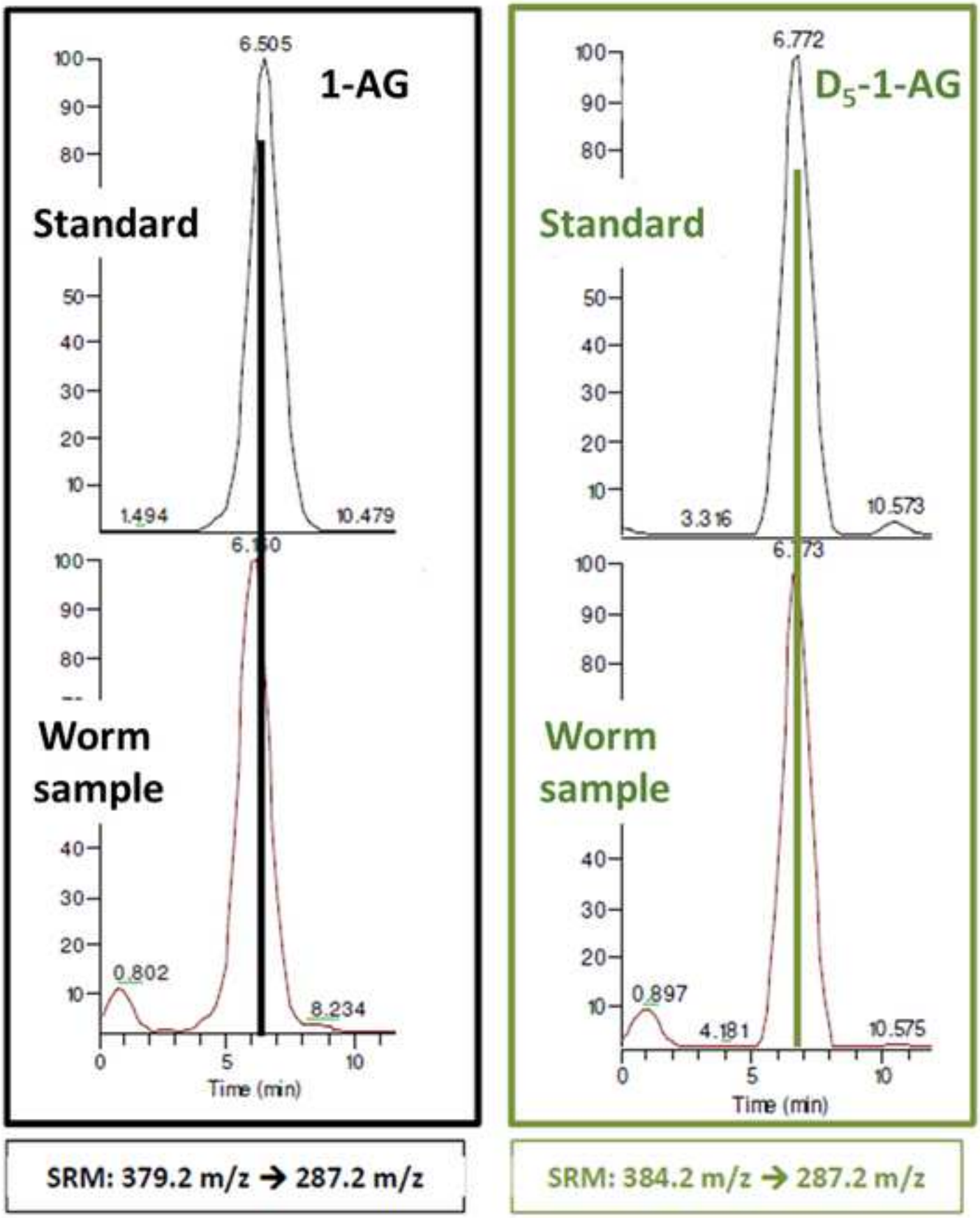

Table 1

	1-AG-d_5	2-AG	Ratio (2-AG/1-AG-d_5)
Sample 1	71964.74	210616.08	0.34
Sample 3	74311.36	205648.43	0.36

Name of Material/ Equipment	Company	Catalog Number	Comments/Description
4-dimethylaminopyridine	Sigma-Aldrich	107700	reagent grade, 99%
antioxidant BHT	Sigma-Aldrich	W21805	
Arachidonic acid	Sigma-Aldrich	10931	
Glycerol-d_8	Sigma-Aldrich	447498	
Mass detector Triple Quadrupole	Thermo Scientific		TSQ Quantum Access Max
N,N'-diisopropylcarbodiimide	Sigma-Aldrich	D125407	
NMR spectrometer	Bruker		Avance II 300 MHz
reversed-phase HPLC column	Thermo Fisher	25003-052130	C18 Hypersil-GOLD (50 x 2.1 mm)
tert-Butyldimethylsilyl chloride	Sigma-Aldrich	190500	reagent grade, 97%
tetrabutylammonium fluoride	Sigma-Aldrich	216143	1.0M in THF
UHPLC System	Thermo Scientific		Ultimate 3000 RSLC Dionex
worm strain N2 Bristol	Caenorhabditis Genetics Center (CGC)		

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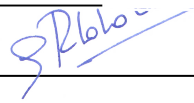
Signature:	
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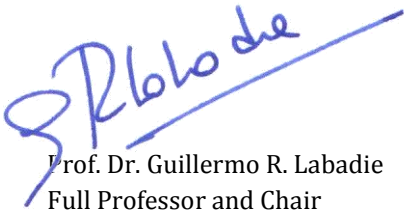

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