

## Research Article

# Synthesis of site-specifically deuterated arachidonic acid derivatives containing a remote tritium radiolabel

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## Summary

The synthesis of arachidonic acid derivatives containing site-specifically incorporated deuterium atoms and also a remote tritium label are described. Deuterium incorporation at the C11 and/or C15 position was achieved using Wittig chemistry, while the radiolabel was introduced at a remote position using [ $^3\text{H}$ ]NaBH<sub>4</sub> as the radiolabel source. These compounds can be used to measure secondary kinetic isotope effects for both cyclooxygenase and lipoxygenase enzymes under aerobic turnover with high precision. Copyright © 2006 John Wiley & Sons, Ltd.

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**Key Words:** arachidonic acid; lipoxygenase; isotope effect; radiolabel

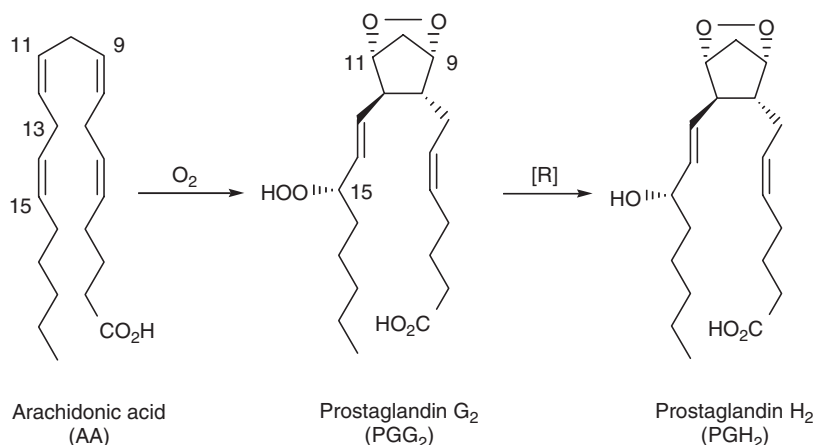
## Introduction

Prostaglandin H synthase (PGHS), or cyclooxygenase (COX), is a bifunctional enzyme that catalyzes the first committed step in the biosynthesis of prostaglandins and thromboxanes.<sup>1–3</sup> In a first reaction the enzyme converts arachidonic acid to PGG<sub>2</sub>, followed by reduction of this intermediate to PGH<sub>2</sub> in a second active site (Scheme 1). Two different isozymes of PGHS have been identified, PGHS-1 and PGHS-2, and they have been linked to numerous biological pathways including inflammation,<sup>4</sup> gastric function<sup>5</sup> and cardiovascular processes.<sup>6</sup>

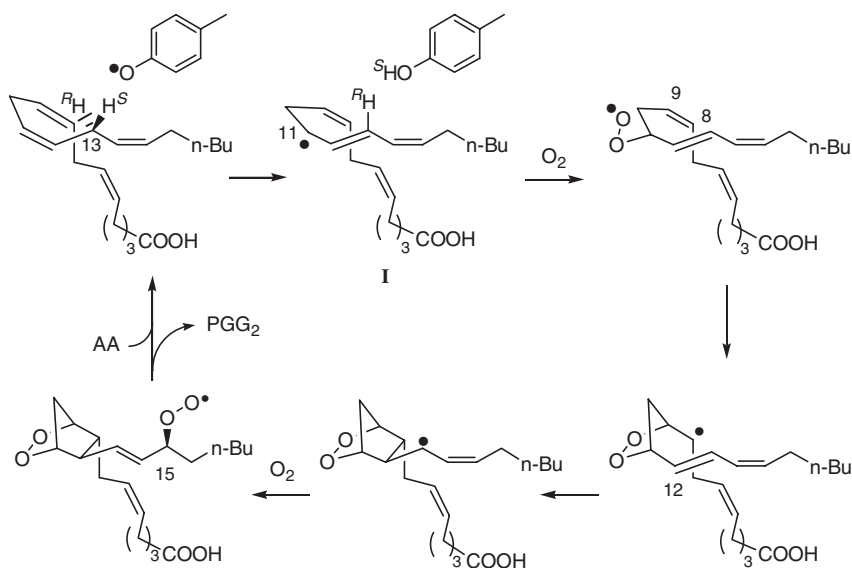
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**Scheme 1. PGHS catalyzed reaction of arachidonic acid forming prostaglandin H<sub>2</sub>**

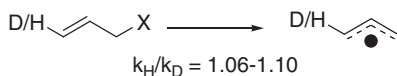


**Scheme 2. Proposed mechanism for prostaglandin H synthase**

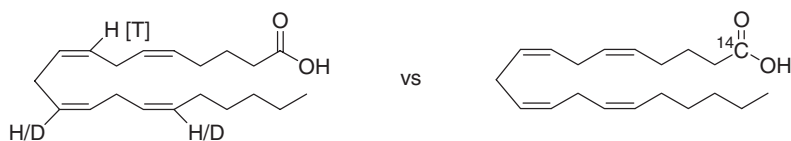
The mechanism of the enzyme as proposed by Hamberg and Samuelsson<sup>7</sup> and modified by Ruf and coworkers with the identification of a tyrosyl radical initiator is shown in Scheme 2.<sup>8–10</sup> Although the proposed mechanism is widely accepted, the experimental characterization of the proposed radical intermediates is still incomplete. The reaction is postulated to be initiated by a hydrogen atom abstraction from the C13 position of arachidonic acid to generate a carbon-based radical intermediate **I**. Characterization of this initial radical intermediate formed under anaerobic conditions has been reported

using EPR spectroscopy and suggests that both isozymes proceed through a pentadienyl radical intermediate.<sup>11,12</sup> However, it is possible that the radical intermediate observed in these studies is not directly on the reaction pathway, but rather the thermodynamically most stable radical that is only formed when oxygen is not present. For example, the pentadienyl radical may be a thermodynamic sink accessed only when an earlier allyl radical cannot react with oxygen. Structural characterization of the initial radical intermediate during aerobic enzyme turnover would require distinction between an allyl and pentadienyl radical.<sup>2</sup> A similar question can be raised for aspirin treated PGHS-2 and PGHS mutants that result in the formation of oxygenation products at C15,<sup>13–15</sup> and for the lipoxygenases, which also abstract a hydrogen atom from a bisallylic position.<sup>16–19</sup>

Both computationally and experimentally, a small secondary kinetic isotope effect (KIE) has been reported for the formation of an allyl radical when the substrate is isotopically substituted at the vinyl position (Scheme 3).<sup>20–23</sup> Based on this precedent, the identity of the initial radical intermediate formed during PGHS and lipoxygenase turnover might be determined. If a C11–C13 allyl radical is formed by hydrogen atom abstraction in PGHS, substrate with C11 deuterium labeled would give a normal KIE whereas substrate labeled at C15 would not show a KIE. Conversely, if a C13–C15 allyl radical is formed, substrate with C11 deuterium label would not produce a KIE, whilst C15-labeled substrate would show a normal KIE. If a pentadienyl radical is formed, both compounds would produce a KIE. The predicted magnitude of this secondary isotope effect is only 1.06–1.10,<sup>20–23</sup> and thus a precise method is necessary for these studies. We opted to utilize the approach of remote radiolabeling, where radionuclei are incorporated into a chemically inert position as reporters for relative reaction rates. For these experiments, a tritium label would be incorporated into the deuterated arachidonic acid (AA), whereas <sup>14</sup>C would be used as the remote label in the unlabeled arachidonic acid (Figure 1). In the competition experiment, the enzymatic reaction would be quenched at various extents of conversion, and the starting material and enzymatic product separated by HPLC. The ratio of <sup>14</sup>C to <sup>3</sup>H radiolabel would then be determined for both, and any isotope effect quantified. The first step of hydrogen atom abstraction from C13 is rate limiting and irreversible and, therefore, the presence of a normal KIE should result in an increase in the ratio of <sup>3</sup>H/<sup>14</sup>C in the recovered starting material and a decrease in this ratio in



**Scheme 3. Secondary kinetic isotope effect associated with generation of allyl radical**



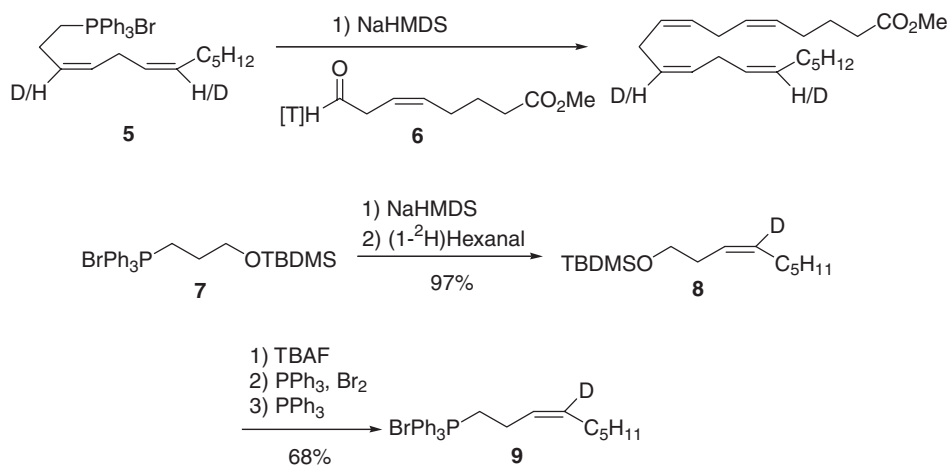
**Figure 1. Competitive experiment for measuring secondary kinetic isotope effects**

the enzyme product. The synthesis of the required radiolabeled substrates for these studies,  $[8\text{-}^3\text{H}]\text{-(}11\text{-}^2\text{H)}\text{AA } \mathbf{1}$  and  $[8\text{-}^3\text{H}]\text{-(}15\text{-}^2\text{H)}\text{AA } \mathbf{2}$ , is described herein.

## Results and discussion

In designing the synthetic scheme for these labeled compounds, we had two primary objectives. First, the route should minimize the transformations necessary with radiolabeled intermediates. The incorporation of the tritium label was envisioned using a Wittig reaction of a deuterated phosphonium salt with a labeled aldehyde (Scheme 4). This reaction is known to proceed with good yield and stereocontrol of the alkene product.<sup>11,24</sup> Not only is the incorporation of the radiolabel late in the synthesis, but also the methyl arachidonate product requires only a basic hydrolysis to yield the desired product.

The labeled aldehyde partner **6** was prepared by reduction of unlabeled aldehyde with  $[^3\text{H}]\text{NaBH}_4$  under Luche conditions,<sup>25</sup> followed by oxidation with Dess-Martin periodinane.<sup>26</sup> This procedure minimized the procedural steps requiring only extraction to isolate the radiolabeled aldehyde.

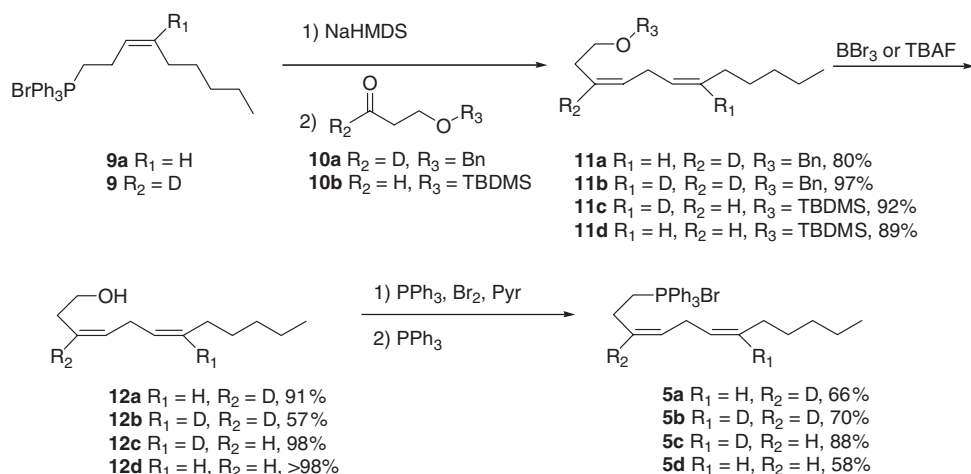


**Scheme 4. Synthetic approach for radiolabeled arachidonic acids and synthesis of monodeuterated phosphonium salt **9****

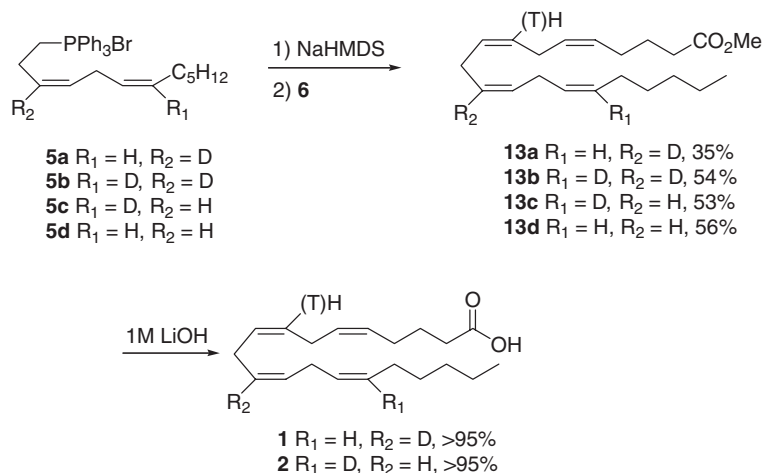
The elimination of silica gel chromatography or distillation greatly reduced the number of laboratory procedures necessary and, therefore, the risk of contamination. After this two-step procedure, the radiolabeled aldehyde **6** was isolated in good purity, with less than 5% isomerization to the  $\alpha,\beta$ -unsaturated aldehyde. This impurity was removed after reaction with phosphonium salt **5**.

The second objective of the synthetic route was the generation of phosphonium salts **5** with a high degree of deuterium incorporation. These compounds were synthesized utilizing Wittig reactions for the key transformations.  $[8\text{-}^3\text{H}]\text{-(15-}^2\text{H)}\text{AA}$  was prepared starting with reaction of previously reported phosphonium salt **7**<sup>27</sup> with  $(1\text{-}^2\text{H})$ hexanal. Deprotection of the substituted alkene **8** with a solution of tetrabutylammonium fluoride provided the alcohol in good yield (Scheme 4). Conversion to the phosphonium salt **9** was accomplished by bromination followed by treatment with triphenylphosphine. At this point, both the substituted phosphonium and the literature reported unlabeled phosphonium salt<sup>28</sup> **9a** were used in a series of Wittig reactions using either protiated<sup>24</sup> or deuterated aldehydes **10a, b** to generate the unlabeled, monodeuterated, and dideuterated skipped dienes **11** (Scheme 5). The substituted aldehyde **10a** was formed by reduction of the corresponding methyl ester with lithium aluminum deuteride followed by pyridinium chlorochromate oxidation.

Deprotection of **11a, b** with boron tribromide proceeded in good yield with no isomerization of the skipped double bonds. Similarly, deprotection of **11c, d** with a solution of tetrabutylammonium fluoride gave alcohols **12c, d** with >98%  $^2\text{H}$  incorporation for **12c**. Subsequent bromination and reaction with triphenylphosphine produced the phosphonium salts **5**. A final Wittig reaction



**Scheme 5. Synthesis of substituted phosphonium salts**



### Scheme 6. Synthesis of radiolabeled arachidonic acids

utilizing the phosphonium salts **5** and radiolabeled aldehyde **6** yielded methyl arachidonates **13a-d** (Scheme 6). Hydrolysis with a 1 M solution of LiOH (aq) and THF (1:2) afforded the final arachidonic acid derivatives **1** and **2**. These compounds were purified using consecutive C18 and silver-ion reversed phase HPLC to remove the small amount of stereoisomers and the specific radioactivities were determined by liquid scintillation counting. Concentrations of arachidonic acid in stock solutions were determined by complete conversion to HPETE with soybean lipoxygenase and spectrophotometric determination of the HPETE concentration. The methyl ester of [8-<sup>3</sup>H]-(11,15-<sup>2</sup>H<sub>2</sub>)AA **13b** will, after hydrolysis, be used to confirm the results obtained with the monodeuterated substrates **1** and **2** and the methyl ester of [8-<sup>3</sup>H]AA **13d** will be used to ensure that the remote tritium label itself does not induce any secondary KIE.

## Experimental

### Phosphonium salt of 1-bromo-(4-<sup>2</sup>H)non-3-ene (**9**)

The synthesis of phosphonium salt **9** and **9a** was based on a previously reported synthetic scheme.<sup>12</sup>

### (3-<sup>2</sup>H)Dodeca-3,6-dienyloxymethyl-benzene (**11a**)

A dry flask was charged with phosphonium salt **9a** (335.2 mg, 0.70 mmol). After purging with Ar, THF (10 ml) was added and the solution was cooled to -78°C. A 1.0 M solution of NaHMDS in THF (600 µl, 0.60 mmol) was added and the solution was stirred for 1 h at -78°C. The substituted aldehyde **10a** (98.6 mg, 0.60 mmol) in 2 ml THF was added very slowly. The reaction was

stirred at  $-78^{\circ}\text{C}$  for 3 h, and then slowly warmed to room temperature. After 10 h, the reaction was quenched with water and extracted with ether ( $3 \times 30\text{ ml}$ ). The combined organic layers were dried with  $\text{MgSO}_4$ , filtered and concentrated. The resulting oil was purified by flash chromatography (100% pentane) yielding 130.6 mg (80%) of **11a** as a clear oil.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  0.91 (t,  $J = 7.1\text{ Hz}$ , 3H), 1.21–1.42 (m, 6H), 2.07 (q,  $J = 7.1\text{ Hz}$ , 2H), 2.44 (t,  $J = 7.0\text{ Hz}$ , 2H), 2.83 (t,  $J = 6.9\text{ Hz}$ , 2H), 3.52 (t,  $J = 7.0\text{ Hz}$ , 2H), 4.55 (s, 2H), 5.32–5.51 (m, 3H), 7.25–7.38 (m, 5H). HRMS (CI,  $M + 1$ ) for  $\text{C}_{18}\text{H}_{36}^2\text{HOSi}$  calculated 298.2676, found 298.2668.

*(3- $^2\text{H}$ -7- $^2\text{H}$ )Dodeca-3,6-dienyloxymethyl-benzene (11b)*

The procedure described for the synthesis of **11a** was followed using phosphonium salt **9** (246 mg, 0.52 mmol) and aldehyde **10a** (90.2 mg, 0.55 mmol). After purification by column chromatography (5% ether in pentane), **11b** (145.5 mg, 97%) was obtained as a clear oil.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  0.89 (t,  $J = 7.0\text{ Hz}$ , 3H), 1.22–1.39 (m, 6H), 2.04 (bt,  $J = 7.1\text{ Hz}$ , 2H), 2.41 (d,  $J = 7.0\text{ Hz}$ , 2H), 2.80 (t,  $J = 7.3\text{ Hz}$ , 2H), 3.49 (t,  $J = 7.1\text{ Hz}$ , 2H), 4.53 (s, 2H), 5.33 (tt,  $J = 1.5\text{ Hz}$ , 7.3 Hz, 1H), 5.45 (tt,  $J = 1.5\text{ Hz}$ , 7.3 Hz, 1H), 7.25–7.38 (m, 5H).  $^{13}\text{C}$  NMR (100.6 MHz,  $\text{CDCl}_3$ )  $\delta$  14.3 ( $\text{CH}_3$ ), 22.8 ( $\text{CH}_2$ ), 25.9 ( $\text{CH}_2$ ), 27.3 ( $\text{CH}_2$ ), 28.1 ( $\text{CH}_2$ ), 29.5 ( $\text{CH}_2$ ), 31.8 ( $\text{CH}_2$ ), 70.1 ( $\text{CH}_2$ ), 73.1 ( $\text{CH}_2$ ), 127.7 (CH), 127.8 (CH), 127.9 (CH), 128.6 (CH), 130.3 (CH), 138.7 (Cq). IR ( $\text{cm}^{-1}$ ): 3029, 2928, 2855, 2355, 2323, 2221, 1633, 1454, 1362. HRMS (EI,  $M +$ ) for  $\text{C}_{19}\text{H}_{26}^2\text{H}_2\text{O}$  calculated 274.2266, found 274.2270.

*tert-Butyl-dodeca-3,6-(7- $^2\text{H}$ )dienyloxy-dimethylsilane (11c)*

A dry flask was charged with phosphonium salt **9** (521.3 mg, 1.11 mmol) and purged with  $\text{N}_2$ . THF (25 ml) was added and the reaction was cooled to  $-78^{\circ}\text{C}$ . A 1.0 M solution of NaHMDS (1.0 ml, 1.00 mmol) was added dropwise and the reaction was stirred for 1 h. A solution of the aldehyde **10b** (291.2 mg, 1.55 mmol) in THF (2 ml) was added slowly over 30 min. The reaction was stirred at  $-78^{\circ}\text{C}$  for 3 h, and then slowly warmed to room temperature. After an additional 10 h, the reaction was quenched with water (40 ml) and extracted with ether ( $3 \times 50\text{ ml}$ ). The combined organic layers were washed with water ( $1 \times 10\text{ ml}$ ), dried with  $\text{MgSO}_4$ , filtered and concentrated. The resulting oil was purified by flash chromatography (3% ether in pentane) yielding **11c** (274.0 mg, 92%) as a clear oil.  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ )  $\delta$  0.06 (s, 6H), 0.90 (s, 9H), 1.24–1.39 (m, 6H), 2.04 (t,  $J = 7.1\text{ Hz}$ , 2H), 2.30 (q,  $J = 6.8\text{ Hz}$ , 2H), 2.79 (t,  $J = 6.9\text{ Hz}$ , 2H), 3.62 (t,  $J = 7.0\text{ Hz}$ , 2H), 5.33 (t,  $J = 7.2\text{ Hz}$ , 1H), 5.36–5.46 (m, 2H).  $^{13}\text{C}$  NMR (125.6 MHz,  $\text{CDCl}_3$ )  $\delta$  -5.1 ( $\text{CH}_3$ ), 14.3 ( $\text{CH}_3$ ), 18.6 (Cq), 22.8 ( $\text{CH}_2$ ), 25.9 ( $\text{CH}_2$ ), 26.2 ( $\text{CH}_3$ ), 27.3 ( $\text{CH}_2$ ),

29.5 (CH<sub>2</sub>), 31.3 (CH<sub>2</sub>), 31.7 (CH<sub>2</sub>), 63.1 (CH<sub>2</sub>), 126.0 (CH), 127.8 (CH), 130.3 (CH). IR (cm<sup>-1</sup>): 3014, 2957, 2929, 2858, 1472. HRMS (CI, M + 1) for C<sub>18</sub>H<sub>36</sub><sup>2</sup>HOSi calculated 298.2676, found 298.2668.

*tert*-Butyl-dodeca-3,6-dienyloxy-dimethylsilane (**11d**)

The procedure described for the synthesis of **11c** was followed using phosphonium salt **9a** (819.4 mg, 1.75 mmol) and aldehyde **10b** (291.2 mg, 1.55 mmol). After purification by column chromatography (3% ether in pentane), **11d** (345.1 mg, 89%) was obtained as a clear oil. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 0.06 (s, 6H), 0.88–0.91 (m, 12H), 1.24–1.39 (m, 6H), 2.05 (q, *J* = 7.0 Hz, 2H), 2.30 (q, *J* = 6.8 Hz, 2H), 2.80 (t, *J* = 6.4 Hz, 2H), 3.61 (t, *J* = 7.0 Hz, 2H), 5.29–5.47 (m, 4H). <sup>13</sup>C NMR (100.6 MHz, CDCl<sub>3</sub>) δ –5.0 (CH<sub>3</sub>), 14.3 (CH<sub>3</sub>), 18.6 (Cq), 22.8 (CH<sub>2</sub>), 26.0 (CH<sub>2</sub>), 26.2 (CH<sub>3</sub>), 27.4 (CH<sub>2</sub>), 29.6 (CH<sub>2</sub>), 31.4 (CH<sub>2</sub>), 31.8 (CH<sub>2</sub>), 63.1 (CH<sub>2</sub>), 126.0 (CH), 127.9 (CH), 130.2 (CH), 130.6 (CH).

(3-<sup>2</sup>H)Dodeca-3,6-dien-1-ol (**12a**)

A dry flask was charged with the protected alcohol **11a** (106.8 mg, 0.390 mmol) and purged with N<sub>2</sub>. CH<sub>2</sub>Cl<sub>2</sub> (8 ml) was added and the solution was cooled to –78°C. A 1.0 M solution of boron tribromide in CH<sub>2</sub>Cl<sub>2</sub> (390 μl, 0.390 mmol) was added dropwise. After 15 min, an additional 100 μl of the 1.0 M solution of BBr<sub>3</sub> was added, and the reaction stirred at –78°C. After 10 min, the reaction was quenched with methanol (1 ml) and the reaction warmed to room temperature. A saturated solution of NaHCO<sub>3</sub> in water (5 ml, aq) was added and the aqueous layer extracted with ether (4 × 30 ml). The combined organic layers were dried with MgSO<sub>4</sub>, filtered and concentrated. The resulting oil was purified by flash chromatography (25% ether in pentane) yielding **12a** (65.0 mg, 91%) as a clear oil. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ 0.88 (t, *J* = 7.0 Hz, 3H), 1.22–1.38 (m, 6H), 1.59 (s, 1H), 2.04 (q, *J* = 7.2 Hz, 2H), 2.35 (t, *J* = 6.5 Hz, 2H), 2.81 (t, *J* = 7.2 Hz, 2H), 3.65 (t, *J* = 6.5 Hz, 2H), 5.28–5.34 (m, 1H), 5.37–5.43 (m, 1H), 5.53 (t, *J* = 7.4 Hz, 1H). <sup>13</sup>C NMR (125.6 MHz, CDCl<sub>3</sub>) δ 14.3 (CH<sub>3</sub>), 22.8 (CH<sub>2</sub>), 25.9 (CH<sub>2</sub>), 27.4 (CH<sub>2</sub>), 29.5 (CH<sub>2</sub>), 30.9 (CH<sub>2</sub>), 31.7 (CH<sub>2</sub>), 62.4 (CH<sub>2</sub>), 125.2 (t, *J* = 23.5 Hz, CD), 127.6 (CH), 130.9 (CH), 131.6 (CH). IR (cm<sup>-1</sup>): 3339 (b), 3012, 2927, 2857, 1654, 1636, 1458. HRMS (CI, M + 1) for C<sub>12</sub>H<sub>21</sub>DO calculated 183.1733, found 183.1735.

(3-<sup>2</sup>H-7-<sup>2</sup>H)Dodeca-3,6-dien-1-ol (**12b**)

The procedure described for the synthesis of **12a** was followed using compound **11b**. Purification by column chromatography (30% ether in pentane), yielded **12b** (40.0 mg, 57%) as a clear oil. <sup>1</sup>H NMR (500 MHz,



$\text{CDCl}_3$ )  $\delta$  0.88 (t,  $J = 7.0$  Hz, 3H), 1.24–1.37 (m, 6H), 1.51 (bs, 1H), 2.04 (t,  $J = 7.2$  Hz, 2H), 2.35 (t,  $J = 6.4$  Hz, 2H), 2.81 (t,  $J = 7.1$  Hz, 2H), 3.65 (t,  $J = 6.5$  Hz, 2H), 5.32 (tt,  $J = 1.4$  Hz, 7.3 Hz, 1H), 5.54 (tt,  $J = 1.4$  Hz, 7.4 Hz, 1H).  $^{13}\text{C}$  NMR (125.6 MHz,  $\text{CDCl}_3$ )  $\delta$  14.3 ( $\text{CH}_3$ ), 22.8 ( $\text{CH}_2$ ), 25.9 ( $\text{CH}_2$ ), 27.3 ( $\text{CH}_2$ ), 29.5 ( $\text{CH}_2$ ), 30.9 ( $\text{CH}_2$ ), 31.7 ( $\text{CH}_2$ ), 62.4 ( $\text{CH}_2$ ), 125.2 (t,  $J = 23.0$  Hz, CD), 127.5 (s, CH), 130.5 (t,  $J = 23.5$  Hz, CD), 131.7 (s, CH). IR ( $\text{cm}^{-1}$ ): 3339 (b), 2957, 2929, 2858, 1458. HRMS (EI,  $\text{M}^+$ ) for  $\text{C}_{12}\text{H}_{20}\text{D}_2\text{O}$  calculated 184.1796, found 184.1794.

*(7- $^2\text{H}$ )Dodeca-3,6-dien-1-ol (12c)*

A dry flask was charged with protected alcohol **11c** (258.1 mg, 0.86 mmol) and purged with  $\text{N}_2$ .  $\text{CH}_2\text{Cl}_2$  (5 ml) was added and the reaction was cooled to  $0^\circ\text{C}$ . A 1.0 M solution of TBAF in THF (1.20 ml, 1.20 mmol) was then added dropwise. The reaction was stirred at  $0^\circ\text{C}$  for 15 min, then warmed to room temperature. After an additional 24 h, the solvent was removed under vacuum. The resulting oil was purified by flash chromatography (25% ether in pentane) yielding **12c** (155.0 mg, 98%) as a clear oil.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  0.89 (t,  $J = 7.0$  Hz, 3H), 1.24–1.41 (m, 6H), 2.04 (t,  $J = 7.1$  Hz, 2H), 2.36 (q,  $J = 6.9$  Hz, 2H), 2.82 (t,  $J = 7.3$  Hz, 2H), 3.66 (q,  $J = 6.2$  Hz, 2H), 5.32 (tt,  $J = 1.4$  Hz, 7.3 Hz, 1H), 5.35–5.43 (m, 1H), 5.52–5.58 (m, 1H).  $^{13}\text{C}$  NMR (100.6 MHz,  $\text{CDCl}_3$ )  $\delta$  14.3 ( $\text{CH}_3$ ), 22.8 ( $\text{CH}_2$ ), 25.9 ( $\text{CH}_2$ ), 27.3 ( $\text{CH}_2$ ), 29.5 ( $\text{CH}_2$ ), 31.0 ( $\text{CH}_2$ ), 31.7 ( $\text{CH}_2$ ), 62.5 ( $\text{CH}_2$ ), 125.5 (CH), 127.4 (CH), 131.8 (CH).

*Dodeca-3,6-dien-1-ol (12d)*

The procedure described for the synthesis of **12c** was followed using compound **11d**. After purification by column chromatography (30% ether in pentane), **12d** was obtained as a clear oil (quant., 127.7 mg).  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ )  $\delta$  0.88 (t,  $J = 7.0$  Hz, 3H), 1.23–1.46 (m, 6H), 2.05 (q,  $J = 7.1$  Hz, 2H), 2.36 (q,  $J = 6.9$  Hz, 2H), 2.82 (t,  $J = 7.1$  Hz, 2H), 3.65 (q,  $J = 6.3$  Hz, 2H), 5.28–5.43 (m, 3H), 5.51–5.57 (m, 1H).  $^{13}\text{C}$  NMR (125.6 MHz,  $\text{CDCl}_3$ )  $\delta$  14.3 ( $\text{CH}_3$ ), 22.8 ( $\text{CH}_2$ ), 25.9 ( $\text{CH}_2$ ), 27.4 ( $\text{CH}_2$ ), 29.5 ( $\text{CH}_2$ ), 31.0 ( $\text{CH}_2$ ), 31.7 ( $\text{CH}_2$ ), 62.4 ( $\text{CH}_2$ ), 125.5 (CH), 127.6 (CH), 130.9 (CH), 131.8 (CH).

*Phosphonium salt of 1-bromo-(3- $^2\text{H}$ )dodeca-3,6-diene (5a)*

The synthesis of phosphonium salt **5a** was based on a previously reported synthetic scheme.<sup>12</sup>

*Phosphonium salt of 1-bromo-(3- $^2\text{H}$ -7- $^2\text{H}$ )non-3-ene (5b)*

The procedure described for the synthesis of (3- $^2\text{H}$ )-1-bromo-dodeca-3,6-diene<sup>12</sup> was followed using compound (**12b**). After purification by column

chromatography (100% pentane), 1-bromo-(3-<sup>2</sup>H-7-<sup>2</sup>H)dodeca-3,6-diene was obtained in 83% yield (41.7 mg) as a clear oil. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ 0.89 (t, *J* = 6.9 Hz, 3H), 1.24–1.39 (m, 6H), 2.04 (t, *J* = 7.1 Hz, 2H), 2.65 (t, *J* = 7.1 Hz, 2H), 2.79 (t, *J* = 7.3 Hz, 2H), 3.37 (t, *J* = 7.2 Hz, 2H), 5.32 (tt, *J* = 1.4 Hz, 7.3 Hz, 1H), 5.52 (tt, *J* = 1.4 Hz, 7.4 Hz, 1H). <sup>13</sup>C NMR (125.6 MHz, CDCl<sub>3</sub>) δ 14.3 (CH<sub>3</sub>), 22.8 (CH<sub>2</sub>), 25.9 (CH<sub>2</sub>), 27.3 (CH<sub>2</sub>), 29.5 (CH<sub>2</sub>), 30.9 (CH<sub>2</sub>), 31.7 (CH<sub>2</sub>), 32.6 (CH<sub>2</sub>), 125.9 (t, *J* = 24.0 Hz, CD), 127.2 (s, CH), 130.7 (t, *J* = 23.0 Hz, CD), 131.4 (s, CH).

1-Bromo-(3-<sup>2</sup>H-7-<sup>2</sup>H)dodeca-3,6-diene was converted to phosphonium salt **5b** as reported previously.<sup>12</sup> After purification by column chromatography, **5b** was obtained in 84% yield (65.9 mg) as a gummy oil. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ 0.80 (t, *J* = 7.2 Hz, 3H), 1.10–1.24 (m, 6H), 1.80 (t, *J* = 7.1 Hz, 2H), 2.37–2.43 (m, 2H), 2.47 (t, *J* = 7.4 Hz, 2H), 3.76–3.83 (m, 2H), 5.08 (t, *J* = 7.1 Hz, 1H), 5.30 (t, *J* = 7.4 Hz, 1H), 7.63–7.67 (m, 6H), 7.72–7.82 (m, 9H). <sup>13</sup>C NMR (125.6 MHz, CDCl<sub>3</sub>) δ 14.2 (CH<sub>3</sub>), 20.5 (d, *J* = 3.8 Hz, CH<sub>2</sub>), 22.7 (CH<sub>2</sub>), 23.1 (d, *J* = 47.9 Hz, CH<sub>2</sub>), 25.6 (CH<sub>2</sub>), δ 27.2 (CH<sub>2</sub>), 29.3 (CH<sub>2</sub>), 31.6 (CH<sub>2</sub>), 118.3 (d, *J* = 85.6 Hz, Cq), 126.7 (CH), 130.7 (CH), 130.8 (d, *J* = 12.8 Hz, CH), 133.9 (d, *J* = 10.2 Hz, CH), 135.3 (d, *J* = 2.8 Hz, CH). <sup>31</sup>P NMR (202.3 MHz, CDCl<sub>3</sub>) δ 25.2 (s).

*Phosphonium salt of 1-bromo-(7-<sup>2</sup>H)dodeca-3,6-diene (5c)*

The procedure described for the synthesis of 1-bromo-(3-<sup>2</sup>H)dodeca-3,6-diene<sup>12</sup> was followed using compound **12c**. After purification by column chromatography (100% pentane), 1-bromo-(7-<sup>2</sup>H)dodeca-3,6-diene was obtained in 88% yield (71.5 mg) as a clear oil. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ 0.89 (t, *J* = 7.0 Hz, 3H), 1.24–1.39 (m, 6H), 2.04 (t, *J* = 7.2 Hz, 2H), 2.65 (q, *J* = 7.2 Hz, 2H), 2.80 (t, *J* = 7.3 Hz, 2H), 3.38 (t, *J* = 7.1 Hz, 2H), 5.32 (t, *J* = 7.3 Hz, 1H), 5.35–5.42 (m, 1H), 5.48–5.55 (m, 1H). <sup>13</sup>C NMR (125.6 MHz, CDCl<sub>3</sub>) δ 14.3 (CH<sub>3</sub>), 22.8 (CH<sub>2</sub>), 26.0 (CH<sub>2</sub>), 27.3 (CH<sub>2</sub>), 29.5 (CH<sub>2</sub>), 31.0 (CH<sub>2</sub>), 31.7 (CH<sub>2</sub>), 32.6 (CH<sub>2</sub>), 126.3 (CH), 127.2 (CH), 130.7 (t, *J* = 23.5 Hz, CD), 131.5 (CH). IR (cm<sup>-1</sup>): 3015, 2958, 2928, 2856, 1458, 1435. HRMS (CI, M + 1) for C<sub>12</sub>H<sub>20</sub>DBr calculated 245.0889, found 245.0884.

(7-<sup>2</sup>H)-1-Bromo-dodeca-3,6-diene was converted to phosphonium salt **5c** as reported previously.<sup>12</sup> After purification by column chromatography, **5c** (132.2 mg, 92%) was obtained as a gummy oil. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 0.79 (t, *J* = 7.1 Hz, 3H), 1.10–1.25 (m, 6H), 1.80 (q, *J* = 7.1 Hz, 2H), 2.36–2.43 (m, 2H), 2.47 (t, *J* = 7.3 Hz, 2H), 3.65–3.73 (m, 2H), 5.04–5.12 (m, 1H), 5.22–5.28 (m, 1H), 5.31 (t, *J* = 7.4 Hz, 1H), 7.63–7.81 (m, 15H). <sup>13</sup>C NMR (100.6 MHz, CDCl<sub>3</sub>) δ 14.3 (CH<sub>3</sub>), 20.4 (d, *J* = 3.7 Hz, CH<sub>2</sub>), 22.7 (CH<sub>2</sub>), 23.0 (d, *J* = 48.8 Hz, CH<sub>2</sub>), 25.7 (CH<sub>2</sub>), 27.3 (CH<sub>2</sub>), 29.3 (CH<sub>2</sub>), 31.6 (CH<sub>2</sub>), 118.2 (d, *J* = 86.2 Hz, CH), 126.8 (CH), 130.79 (d, *J* = 12.9 Hz, CH), 130.81 (CH),

131.1 (CH), 133.8 (d,  $J = 10.8$  Hz, CH), 135.4 (d,  $J = 3.1$  Hz, CH).  $^{31}\text{P}$  NMR (161.9 MHz,  $\text{CDCl}_3$ )  $\delta$  25.0 (s). HRMS (ESI, M-Br) for  $\text{C}_{30}\text{H}_{35}\text{DP}$  calculated 428.2617, found 428.2639.

*Phosphonium salt of 1-bromo-dodeca-3,6-diene (5d)*

The synthesis of phosphonium salt **5d** was based on a previously reported synthetic scheme.<sup>29</sup>

*Methyl [1- $^3\text{H}$ ]8-oxo-oct-5-enoate (6)*

A dry flask was charged with methyl 8-oxo-oct-5-enoate<sup>11,12</sup> (54.6 mg, 0.32 mmol) and purged with  $\text{N}_2$ . THF (10 ml) was added and the solution was cooled to  $0^\circ\text{C}$ . Cerium chloride (97.4 mg, 0.38 mmol) was added in one portion and the reaction was stirred for 45 min, then the  $[\text{T}]\text{NaBH}_4$  (6.1 mg, 0.16 mmol,  $\sim 200$  mCi/mmol) was added. The reaction was stirred at  $0^\circ\text{C}$  for 4 h, and then slowly warmed to room temperature overnight. After 10 h, the reaction was quenched with water (10 ml) and extracted with ether ( $6 \times 30$  ml). The combined organic layers were dried with  $\text{MgSO}_4$ , filtered and concentrated. The resulting oil was used without further purification.

The labeled alcohol was purged with  $\text{N}_2$ , and  $\text{CH}_2\text{Cl}_2$  (10 ml) was added. The solution was cooled to  $0^\circ\text{C}$  and then Dess-Martin periodinane (275.6 mg, 0.64 mmol) was added in one portion. The reaction was stirred at  $0^\circ\text{C}$  for 15 min, and then slowly warmed to room temperature. After an additional 1 h, the reaction was quenched with water (20 ml) and extracted with pentane ( $6 \times 30$  ml). The combined organic layers were washed with water ( $4 \times 20$  ml), dried with  $\text{MgSO}_4$ , filtered and concentrated yielding 37.9 mg (70%) of **6** as a clear oil. The labeled aldehyde was used without further purification.  $^1\text{H}$  NMR (100.6 MHz,  $\text{CDCl}_3$ )  $\delta$  1.71 (quintet,  $J = 7.3$  Hz, 2H), 2.09 (q,  $J = 7.3$  Hz, 2H), 2.32 (t,  $J = 7.4$  Hz, 2H), 3.18–3.21 (m, 2H), 3.67 (s, 3H), 5.56–5.72 (m, 2H), 9.67 (t,  $J = 1.8$  Hz, 1H).  $^1\text{H}$  NMR spectroscopy indicates only trace  $\alpha,\beta$ -unsaturated aldehyde present ( $< 5\%$ ).

*Methyl [8- $^3\text{H}$ ]-( $11\text{-}^2\text{H}$ )arachidonate (13a)*

A dry flask was charged with phosphonium salt **5a** (39.0 mg, 0.077 mmol) and dried by azeotroping with benzene ( $3 \times 10$  ml). THF (5 ml) was added and the solution was cooled to  $-78^\circ\text{C}$ . A 1.0 M solution of NaHMDS in THF (65  $\mu\text{l}$ , 0.065 mmol) was added dropwise and the reaction was stirred for 1 h. A solution of aldehyde **6** (10.1 mg, 0.059 mmol) in THF (500  $\mu\text{l}$ ) was added dropwise over  $\sim 15$  min. The reaction was stirred at  $-78^\circ\text{C}$  for 3 h, and then slowly warmed to room temperature. After an additional 9 h, the reaction was quenched with water (10 ml) and extracted with ether ( $5 \times 30$  ml). The combined organic layers were dried with  $\text{MgSO}_4$ , filtered and concentrated.

The resulting oil was purified by flash chromatography (3% ether in pentane) yielding **13a** (7.3 mg, 35%) as a clear oil.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  0.88 (t,  $J = 7.0$  Hz, 3H), 1.24–1.39 (m, 6H), 1.70 (p,  $J = 7.4$  Hz, 2H), 2.05 (q,  $J = 7.0$  Hz, 2H), 2.11 (q,  $J = 6.8$  Hz, 2H), 2.32 (t,  $J = 7.5$  Hz, 2H), 2.77–2.85 (m, 6H), 3.66 (s, 3H), 5.29–5.44 (m, 7H).  $^{13}\text{C}$  NMR (100.6 MHz,  $\text{CDCl}_3$ )  $\delta$  14.3 ( $\text{CH}_3$ ), 22.8 ( $\text{CH}_2$ ), 25.0 ( $\text{CH}_2$ ), 25.82 ( $\text{CH}_2$ ), 25.83 ( $\text{CH}_2$ ), 26.8 ( $\text{CH}_2$ ), 27.4 ( $\text{CH}_2$ ), 29.6 ( $\text{CH}_2$ ), 31.7 ( $\text{CH}_2$ ), 33.7 ( $\text{CH}_2$ ), 51.7 ( $\text{CH}_3$ ), 127.8 (CH), 128.38 (CH), 128.41 (CH), 128.7 (CH), 129.09 (CH), 129.15 (CH), 130.7 (CH), 174.3 (Cq).

*Methyl [8- $^3\text{H}$ ]- (11,15- $^2\text{H}_2$ )arachidonate (**13b**)*

The procedure described for the synthesis of **13a** was followed using phosphonium salt **5b** (140.3 mg, 0.275 mmol) and aldehyde **6** (25.3 mg, 0.148 mmol). After purification by column chromatography (3% ether in pentane), **13b** (25.8 mg, 54%) was obtained as a clear oil.  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ )  $\delta$  0.88 (t,  $J = 7.1$  Hz, 3H), 1.24–1.38 (m, 6H), 1.71 (p,  $J = 7.4$  Hz, 2H), 2.05 (t,  $J = 7.4$  Hz, 2H), 2.11 (q,  $J = 7.0$  Hz, 2H), 2.32 (t,  $J = 7.5$  Hz, 2H), 2.78–2.85 (m, 6H), 3.67 (s, 3H), 5.31–5.43 (m, 6H). Specific radioactivity 44.8 mCi/mmol.

*Methyl [8- $^3\text{H}$ ]- (15- $^2\text{H}$ )arachidonate (**13c**)*

The procedure described for the synthesis of **13a** was followed using phosphonium salt **5c** (41.6 mg, 0.081 mmol) and aldehyde **6** (10.1 mg, 0.059 mmol). After purification by column chromatography (3% ether in pentane), **13c** (10.0 mg, 53%) was obtained as a clear oil.  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ )  $\delta$  0.89 (t,  $J = 7.0$  Hz, 3H), 1.24–1.38 (m, 6H), 1.70 (p,  $J = 7.4$  Hz, 2H), 2.05 (t,  $J = 7.3$  Hz, 2H), 2.11 (q,  $J = 7.0$  Hz, 2H), 2.32 (t,  $J = 7.5$  Hz, 2H), 2.76–2.85 (m, 6H), 3.67 (s, 3H), 5.31–5.43 (m, 7H).  $^{13}\text{C}$  NMR (125.6 MHz,  $\text{CDCl}_3$ )  $\delta$  14.3 ( $\text{CH}_3$ ), 22.8 ( $\text{CH}_2$ ), 25.0 ( $\text{CH}_2$ ), 25.80 ( $\text{CH}_2$ ), 25.81 ( $\text{CH}_2$ ), 26.7 ( $\text{CH}_2$ ), 27.3 ( $\text{CH}_2$ ), 29.5 ( $\text{CH}_2$ ), 31.7 ( $\text{CH}_2$ ), 33.6 ( $\text{CH}_2$ ), 51.7 ( $\text{CH}_3$ ), 127.6 (CH), 128.1 (CH), 128.40 (CH), 128.43 (CH), 128.8 (CH), 129.1 (CH), 129.2 (CH), 174.3 (Cq).

*Methyl [8- $^3\text{H}$ ]arachidonate (**13d**)*

The procedure described for the synthesis of **13a** was followed using phosphonium salt **5d** (68.9 mg, 0.135 mmol) and aldehyde **6** (12.6 mg, 0.074 mmol). After purification by column chromatography (3% ether in pentane), **13d** (13.3 mg, 56%) was obtained as a clear oil.  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ )  $\delta$  0.88 (t,  $J = 7.0$  Hz, 3H), 1.22–1.39 (m, 6H), 1.71 (p,  $J = 7.4$  Hz, 2H), 2.05 (q,  $J = 7.2$  Hz, 2H), 2.11 (q,  $J = 7.0$  Hz, 2H), 2.32 (t,  $J = 7.5$  Hz, 2H), 2.78–2.86 (m, 6H), 3.67 (s, 3H), 5.30–5.43 (m, 8H). Specific activity 45 mCi/mmol.

*[8-<sup>3</sup>H]-(11-<sup>2</sup>H) Arachidonic acid (1)*

A dry flask was charged with methyl arachidonate **13a** (3.6 mg, 0.011 mmol) and purged with N<sub>2</sub>. THF (2 ml) was added and the solution was cooled to 0°C. A 1.0 M solution of LiOH (aq) (500 µl) was added and the reaction was stirred for 1 h. The reaction was slowly warmed to room temperature and stirred for an additional 25 h. The reaction was quenched with a 1 M HCl (aq) solution (2 ml) and extracted with ether (6 × 20 ml). The combined organic layers were dried with MgSO<sub>4</sub>, filtered and concentrated. The resulting oil was purified by flash chromatography (40% ether in pentane) yielding **1** (~4.0 mg, quant.) as a clear oil. The final compound was then further purified by HPLC using first a Varian Microsorb C18 column followed by a Chromsper 5 Lipids silver-ion column (both 250 × 4.6 mm). <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ 0.88 (t, *J* = 7.0 Hz, 3H), 1.24–1.38 (m, 6H), 1.72 (p, *J* = 7.4 Hz, 2H), 2.05 (q, *J* = 7.1 Hz, 2H), 2.14 (q, *J* = 7.1 Hz, 2H), 2.37 (t, *J* = 7.5 Hz, 2H), 2.79–2.85 (m, 6H), 5.30–5.44 (m, 6H). <sup>13</sup>C NMR (125.6 MHz, CDCl<sub>3</sub>) δ 14.3 (CH<sub>3</sub>), 22.8 (CH<sub>2</sub>), 24.7 (CH<sub>2</sub>), 25.7 (CH<sub>2</sub>), 25.8 (CH<sub>2</sub>), 26.6 (CH<sub>2</sub>), 27.4 (CH<sub>2</sub>), 29.5 (CH<sub>2</sub>), 30.5 (CH<sub>2</sub>), 31.7 (CH<sub>2</sub>), 33.2 (CH<sub>2</sub>), 127.8 (CH), 128.3 (CH), 128.5 (CH), 128.7 (CH), 129.0 (CH), 129.3 (CH), 130.7 (CH). Specific activity 96.9 mCi/mmol.

*[8-<sup>3</sup>H]-(15-<sup>2</sup>H) Arachidonic acid (2)*

The procedure described for the synthesis of **1** was followed using compound **13c** (4.3 mg). After purification by column chromatography (40% ether in pentane), **2** (quant., ~5.2 mg) was obtained as a clear oil. The final compound was then purified by HPLC as described for **1**. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ 0.89 (t, *J* = 7.0 Hz, CH<sub>3</sub>), 1.25–1.39 (m, 6H), 1.72 (p, *J* = 7.4 Hz, 2H), 2.05 (t, *J* = 7.2 Hz, 2H), 2.13 (q, *J* = 7.0 Hz, 2H), 2.37 (t, *J* = 7.5 Hz, 2H), 2.79–2.86 (m, 6H), 5.31–5.46 (m, 7H). <sup>13</sup>C NMR (125.6 MHz, CDCl<sub>3</sub>) δ 14.3 (CH<sub>3</sub>), 22.8 (CH<sub>2</sub>), 24.7 (CH<sub>2</sub>), 25.8 (CH<sub>2</sub>), 25.8 (CH<sub>2</sub>), 26.6 (CH<sub>2</sub>), 27.3 (CH<sub>2</sub>), 29.5 (CH<sub>2</sub>), 30.5 (CH<sub>2</sub>), 31.7 (CH<sub>2</sub>), 33.2 (CH<sub>2</sub>), 127.6 (CH), 128.1 (CH), 128.3 (CH), 128.5 (CH), 128.8 (CH), 129.0 (CH), 129.3 (CH). Specific activity 97 mCi/mmol.

## Conclusion

The syntheses of four deuterium-labeled arachidonic acid derivatives containing a C8 remote tritium label are described. These substrates can be used to measure the secondary kinetic isotope effect of both soybean lipoxygenase and PGHS to provide evidence for the structure of the initial radical intermediate formed during enzyme turnover.

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