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Total Syntheses of Two bis- Allylic-Deuterated DHA Analogues

Mélissa Rosell, Maxime Villa, Thierry Durand, Jean-Marie Galano, Joseph Vercauteren, and Céline Crauste*\[a\]

Abstract: The deuteration of polyunsaturated fatty acids (PUFAs) at their bis-allylic positions is known to limit harmful lipid peroxidation, which leads to the damage of cell membranes. Therefore, to impede toxic lipid peroxidation in retina tissue, we have designed and synthesized two deuterated analogues of docosahexaenoic acid (DHA; C22:6, n-3), which is the main lipid constituent of retina membranes. To avoid its oxidative degradation into toxic carboxyethylpyrrole (CEP) adducts, whilst preserving enzymatic metabolization into a healthy neuroprotective derivative (NPD1), deuterium was incorporated at specific 6- and 6,9-bis-allylic positions. A convergent synthetic strategy, based on a Wittig olefination, was developed to obtain both deuterated DHA species. Common aldehyde intermediates were synthesized from another PUF, eicosapentaenoic acid (EPA; C20:5, n-3). Deuterium atoms were introduced through either the reduction of an ester with a deuterated reagent or a nucleophilic reaction with deuterated paraformaldehyde.

Introduction

Docosahexaenoic acid (DHA; C22:6, n-3), a polyunsaturated fatty acid (PUFA) that is highly enriched in the membranes of retina and brain tissue, is necessary for brain and retina development in infants\[1\] and for the retention of normal function in adults.\[2\] Like many PUFAs, DHA supplementation has been widely studied and has been shown to have a positive effect on several inflammatory diseases.\[3\] Furthermore, DHA supplementation is also considered to be an effective strategy for the prevention of cardiovascular disease\[4\] and neurological disorders.\[5\] However, the effect of DHA on oxidative damage in retina and brain pathologies remains controversial. Conflicting data on DHA supplementation (in clinical studies or in animal models) have shown either beneficial\[6\] or no effect\[7\] on the incidence of retina degeneration, thereby leading to the hypothesis that DHA activity may also depend on DHA metabolites and, thus, on patient metabolism. In fact, because of its chemical structure and the presence of six double bonds and five highly oxidizable bis-allylic positions, this lipid derivative is susceptible to enzymatic and non-enzymatic oxidations. In both cases, conversion occurs through the abstraction of a hydrogen atom from one of the bis-allylic position, as initiated either by reactive oxygen species (ROS; ‘OOR, ‘OR, ‘OH, or NO\[2\]) or by specific enzymes, and leads to an unstable pentadienyl radical that can rearrange in different ways to form cyclic or non-cyclic metabolites. As a result, enzymatic and non-enzymatic conversions of DHA can lead to a large variety of bioactive “mediators” (neuroprotecin, resolin, neuroprostane, neurofurane, neuroketal, etc.), depending on the position of the first abstracted hydrogen atom, the enzyme involved, the partial pressure of oxygen, and the importance of oxidative stress.\[8\]

There is recent compelling evidence that non-enzymatic and enzymatic oxidized metabolites of DHA may contribute to its positive as well as negative biological activities (in vitro and in vivo). These cell mediators, signaling molecules, and biologically active secondary metabolites can either be protective or toxic towards the cells. For example, neuroprotectin D1 (NPD1), which is biosynthesized by 15-lipoxygenase (15-LOX) from enzymatic bis-allylic hydrogen abstraction at the 15 position (followed by epoxide rearrangement and hydrolysis), has shown interesting antioxidant properties in retina cells,\[9\] anti-inflammatory activity,\[10\] and neuroprotective properties\[11\] (Scheme 1). Coming from a non-enzymatic pathway, 4-F\[e\]-neuroprostane is generated by the free-radical oxidation of DHA and has shown powerful antiarrhythmic activity.\[12\] Unfortunately, the ROS-initiated oxidation of PUFAs in cell membranes also leads to lipid peroxides, DNA deterioration, and, thus, is involved in carcinogenesis. The end-products of DHA peroxidation include small electrophilic carbonyl species, such as trans-4-hydroxy-hex-2-enal (4-HHE). Such a reactive aldehyde is prone to nucleophilic attack by cellular components (DNA or proteins), thereby causing irreversible damage to the cells,\[13\] yet it is surprisingly also responsible for antioxidant defenses through the activation of the Nfr2/keap1 pathway in the
In an analogous manner to the formation of trans-4-hydroxy-2-nonenal (4-HNE) through n-6 lipid peroxidation, 4-hydroxy-7-oxo-hept-5-enoic acid (HOHA; Scheme 1) is generated from DHA oxidation, which is, most likely, initiated by H-abstraction at the 6- or 6- and 9-positions. Protein lysyl ε-amino residues readily add onto such HOHA compounds (free acid, lactone, or even phosphatidylethanolamine derivatives) to generate 2-carboxyethylpyrroles (CEP adducts), which induce angiogenesis in the retina, a pathological development that is associated with the advanced stages of age-related macular degeneration (AMD).

Recently, Shchepinov and co-workers prepared analogues of linoleic and linolenic acids that were deuterated at their bis-allylic sites and found that they slowed down bis-allylic abstraction ($k_D/k_H = 23$ in the case of (11,11-D$_2$)-linoleic acid) during the tocopherol-mediated peroxidation process. This result was attributed to the primary kinetic deuterium isotope effect, which was linked to the replacement of two hydrogen atoms on those positions.

Deuterium-labeled compounds can be used in a wide range of applications and their synthesis has been extensively studied. The incorporation of deuterium through H/D exchange has been achieved by using iridium or ruthenium complexes on aliphatic or aromatic substrates, and even at the allylic position after alkene isomerization. However, these methods were not designed to selectively incorporate deuterium atoms at the bis-allylic positions of DHA.

Results and Discussion

Deuterium-labeled compounds can be used in a wide range of applications and their synthesis has been extensively studied. The incorporation of deuterium through H/D exchange has been achieved by using iridium or ruthenium complexes on aliphatic or aromatic substrates, and even at the allylic position after alkene isomerization. However, these methods were not designed to selectively incorporate deuterium atoms at the bis-allylic positions of DHA.

Scheme 1. DHA oxidation yields toxic or beneficial metabolites for retina cells.
To access (6,6-D$_2$)-DHA (1a) and (6,6,9,9-D$_4$)-DHA (1b), we developed a convergent synthetic strategy based on a Wittig reaction for the formation of a double bond at the 7- or 10-positions of the DHA skeleton (Scheme 2). Aldehyde 3a and alkyne 4 were identified as common intermediates in the retrosyntheses of both deuterated PUFAs. Indeed, compound 1a could be obtained from a Wittig olefination reaction between compound 3a and the ylide that is generated from phosphonium salt 2a. Aldehyde 3a could also serve as a precursor to aldehyde 3b, which could be involved in the key Wittig coupling reaction for the synthesis of compound 1b.

Aldehyde 3a could be obtained from the natural PUFA eicosapentaenoic acid (EPA; C20:5, n-3), which would allow us to directly incorporate the remaining four double bonds into the C22 lipid skeleton. Then, deuterium atoms could be incorporated during the syntheses of phosphonium salts 2a and 2b, which could occur from a common deuterated alkyne (synthon 4). This synthon could either be deprotonated and involved in a reaction with oxetane to give compound 2a, or coupled with deuterated propargylic bromide derivative synthon 5 to obtain the second phosphonium salt (2b).

**Synthesis of Deuterated Synthons 4 and 5**

Two methods of deuteration were employed to produce deuterated synthons 4 and 5. The synthesis of synthon 4 (Scheme 3) started with the protection of the methylglycolate with a p-methoxybenzyl (PMB) group under acidic conditions to give compound 6. Reduction$^{[21]}$ of the ester group by using LiAlD$_4$ afforded deuterated intermediate 7, which was converted into the corresponding tosyl ester (8). Finally, nucleophilic substitution with the lithium acetylide ethylenediamine complex (LICCH-EDA)$^{[22]}$ led to the desired deuterated terminal alkyne (4) in 61% yield in four steps.

A different procedure was used to introduce the deuterium atoms into synthon 5 (Scheme 4). After the protection of 5-pentyn-1-ol with a tetrahydropyran (THP) group,$^{[23]}$ terminal alkyne 9 was deprotonated by using a Grignard reagent. Then, nucleophilic attack on deuterated paraformaldehyde afforded alcohol 10, which was further converted into the correspond-

**Synthesis of Phosphonium Salts 2a and 2b**

For the syntheses of compounds 2a and 2b, deuterated alkyne 4 was chosen as a common starting material...
Scheme 5. Synthesis of deuterated phosphonium salts 2a and 2b: a) nBuLi, BF₄₋Et₂O, THF, –78 °C, 79% yield; b) Jones reagent (2.17 equiv), acetone, 0 °C, 94% yield; c) EtOH, H₂SO₄ (cat.), 35 °C, 86% yield; d) Cu, NaI, K₂CO₃, DMF, RT, 75% yield; e) DDQ, CH₂Cl₂/water, RT, 94% yield; f) P₂-Ni, EDA, EtOH, 0 °C, 90% yield (6% over-reduction) for compound 16 and 78% yield (13% over-reduction) for compound 19; g) I₂, PPh₃, imidazole, CH₂Cl₂, 0 °C to RT; h) PPh₃, MeCN, reflux, 87% yield for compound 2a and 93% yield for compound 2b (over two steps).

(Scheme 5). Our synthesis of compound 2a began with ring-opening of the oxetane after the deprotonation of compound 4 by using nBuLi in the presence of Lewis acid BF₄₋Et₂O (79% yield).²⁴ Jones oxidation of alcohol 13 afforded acid 14, which was followed by esterification and deprotection of the PMB group. Reduction of alkyne 15 with the P₂-Ni catalytic system²⁵ (Brown’s catalyst; Ni(OAc)₃, in the presence of NaBH₄, poisoned by ethylenediamine) yielded alkene 16 (90% yield), with a small amount of over-reduction (6%). Then, the iodine derivative was obtained from alcohol 16 by using the Appel reagent and was finally transformed into the corresponding phosphonium salt (2a) through nucleophilic displacement with triphenylphosphine.

The synthesis of compound 2b (Scheme 5) started with the preparation of skipped-diyne unit 17. The coupling reaction between terminal alkyne 4 and deuterated propargylic bromide 5 was achieved by using Caruso conditions in the presence of CuI (75% yield),²⁶ and deprotection of the PMB group gave the tetradeuterated skipped-diyne alcohol (18).

To prevent the formation of over-reduced byproducts during the reduction of skipped-diyne 18 into skipped-diyne 19, three different hydrogenation reactions²⁷ were tested and compared: Lindlar catalyst;²⁸ Rosenmund catalyst;²⁹ and Brown’s catalyst. The proportion of over-reduction was estimated from the integration of the CH₂−OH signal in the ²H NMR spectrum (Figure 1).

Increasing the number of equivalents of ethylenediamine and decreasing the reaction time and temperature allowed us to limit the formation of the over-reduction side-product to 13% (the over-reduced side product was removed during the final purification of compound 1b) with the P₂-Ni catalytic system (78% yield). The Lindlar and Rosenmund catalysts afforded higher proportions of the over-reduction side-product (23% and 32%, respectively; Figure 1) and also suffered from a lack of reproducibility. Finally, phosphonium salt 2b was synthesized from skipped-diyne alcohol 19 in two steps by using similar conditions as for the synthesis of compound 2a.

Figure 1. ¹H NMR signals of the CH₂−OH group during the hydrogenation of compound 18. Percentage over-reduction: a) Lindlar catalyst (Pd/CaCO₃ poisoned with quinoline), 23%; b) Rosenmund catalyst (Pd/BaSO₄ poisoned with quinoline), 32%; c) Brown’s catalyst (Ni(OAc)₃, in the presence of NaBH₄ poisoned with ethylenediamine), 13%.

Synthesis of Aldehydes 3a and 3b

Aldehydes 3a and 3b were prepared from the polyunsaturated fatty acid EPA (Scheme 6). This strategy allowed us to access chemical synths that already contained three or four (Z) double bonds in their backbones. EPA was extracted from cod liver oil by using a process that was developed to concentrate PUFAs starting from fish oil.³⁰ The synthesis of epoxystear 21 began by treating EPA with γ-collidine (2,4,6-trimethylpyridine) in the presence of a small excess of I₂ (2 equiv), as described by Itoh et al.,³¹ to give iodolactone intermediate 20. By using such a procedure, epoxide 21 was obtained in 95% yield in two steps after opening of the iodolactone. This procedure was preferred to that reported by Jakobsen et al.,³¹ in which large excesses of I₂ (8 equiv), KI, and KHCO₃ were required and which led to a low yield of the product and difficult purification of the iodolactone. Aldehyde 3a was finally ob-
We envisaged a synthesis of aldehyde 3a from compound 3b in five steps as described by Wang et al.\textsuperscript{[33]} Thus, the reduction of compound 3a with NaBH₄ afforded homoallylic alcohol 22, which was treated with vanadyl acetylacetonate (VO(acac)₂) and tBuOOH to give intermediate epoxide alcohol 23. Ring-opening of the epoxide was first performed with perchloric acid, which led to an unstable triol intermediate that was treated with sodium periodate to promote oxidative cleavage. The order of addition also played a significant role: more reactive aldehydes afforded the desired aldehyde (3a) in 87% crude yield. This latter compound was used without purification by column chromatography on silica gel because of its susceptibility towards degradation into the corresponding α,β-unsaturated aldehyde. However, purification by aqueous washing and filtration through Celite\textsuperscript{®} allowed us to isolate the desired aldehyde in high purity (which was necessary for a successful Wittig olefination reaction).

We optimized the reaction conditions to obtain the desired aldehyde 3b in high purity from epoxy alcohol 23. Thus, opening of the epoxide and protection of the resulting alcohol by using acetic anhydride, followed by mild saponification with LiOH and oxidative cleavage, led to aldehyde 3b (62% crude yield in three steps without purification by column chromatography on silica gel). Two determining factors were identified for obtaining the aldehyde (3b) in acceptable purity: epoxy alcohol 23 had to be used no more than one week after its preparation (because of possible degradation) and the reaction time of the epoxide-opening/acylation reaction needed to be limited to form only the mono- and diacetylated derivatives, because the triacetylated compound appeared to be less reactive towards saponification.

Conclusion

We have reported the first total syntheses of DHA derivatives that were deuterated at specific 6- or 6,9-bis-allylic positions.
The deuterium atoms were either introduced through the reduction of an ester group by using LiAlD₄ or through the nucleophilic substitution of a deprotonated alkyne on deuterated paraformaldehyde. The key Wittig olefination reactions between deuterated ylides and EPA-derived aldehydes were optimized to highlight the essential parameters that were necessary for obtaining good reproducibility and robustness of this approach. As such, although this strategy comprised multiple steps, the target deuterated DHA analogues were obtained on a scale of hundreds of milligrams, which was sufficient to allow their use in both in vitro and in vivo studies.

These new deuterated DHA compounds could allow us to better understand the beneficial or deleterious effects of DHA-oxidized metabolites and to explain some of the paradoxical studies that have reported no increase in the susceptibility of DHA-enriched cells under specific oxidative conditions. Moreover, these compounds could act as a starting point for the development of new therapeutic DHA analogues that are less prone to deleterious non-enzymatic oxidation, while preserving their enzymatic metabolism into neuroprotectins, for example. Finally, studies on the effect of the incorporation of bis-allylic deuterium in both of the synthesized deuterated DHA analogues, and on their inhibition of lipid peroxidation in retinal cells lines, are underway.

**Experimental Section**

**General Methods**

All of the reactions that required anhydrous conditions were performed in oven-dried glassware with magnetic stirring under a nitrogen atmosphere unless otherwise noted. Solvents, anhydrous solvents, and reagents were used as received unless otherwise noted. The reactions were monitored by using TLC on plates that were precoated with silica gel 60 (Merck). The reaction components were visualized by using a 254 nm UV lamp, staining with acidic p-anisaldehyde solution followed by gentle heating, or staining with KMnO₄ solution in EtOH. Organic layers were dried with MgSO₄ unless otherwise stated. Column chromatography was performed on silica gel 40–63 μm. ¹H and ¹³C NMR spectra were recorded at 500 MHz in CDCl₃ (internal reference at δ = 7.26 ppm for ¹H NMR and δ = 77.16 ppm for ¹³C NMR) unless otherwise noted. The NMR spectra were assigned with the help of 2D NMR analysis.

| Table 1. Optimization of Wittig olefination reaction.²[^1]³[^2] |
|---|---|---|---|
| Entry | T [°C] | Aldehyde (equiv) | Product | Yield [%] |
| 1[^b] | –78 | 0.95 | 24a | 6 |
| 2[^b] | –78 to RT | 0.95 | 24a | 12 |
| 3[^c] | –78 to RT | 0.95 | 24a | 31 |
| 4[^d] | –78 to RT | 1.7 | 24a | 40 |
| 5[^e] | –78 to RT | 2 | 24b | 17[^e] |
| 6[^f] | –78 to RT | 1.7 | 24b | 51 |
| 7[^g] | –78 to RT | 1.5 | 24b | 48 |

[^1]: The ylide was generated in situ by the addition of NaHMDS (1 equiv) at –50 °C. [^2]: The ylide was added to the aldehyde at –78 °C. [^3]: The aldehyde was added to the ylide at –78 °C. [^4]: The purity of the aldehyde was below 70% (as estimated by using NMR analysis).

(Methyl 2-[(4-Methoxybenzoyl)oxy]ethanoate (6))

A solution of 4-methoxybenzyl alcohol (8.54 g, 61.80 mmol) in dry Et₂O (50 mL) was added dropwise to a suspension of sodium hydride (60% in mineral oil, 148 mg, 6.18 mmol) in dry Et₂O (30 mL) at RT under an inert atmosphere. After 30 min, the mixture was cooled to 0 °C and trichloroacetonitrile (9.82 g, 68 mmol) was added dropwise. After the addition was complete, the reaction mixture was allowed to warm to RT and stirred for a further 4 h. Then, the solvent was removed under reduced pressure to give the crude intermediate p-methoxybenzyl trichloroacetimidate (PMBTCA). Methyl glycolate (3.90 g, 43.30 mmol) was added to a solution of the intermediate in dry CH₂Cl₂ (210 mL) under an inert atmosphere, the mixture was cooled to 0 °C, and (4-)camphor-10-sulfonic acid (1 g, 4.33 mmol) was added. After the addition was complete, the reaction mixture was allowed to warm to RT and stirred for a further 1 d. The obtained suspension was filtered through a pad of Celite and the filtrate was washed with a saturated aqueous solution of NaHCO₃ (100 mL), dried over Na₂SO₄, filtered, and the solvents were removed under reduced pressure (600 mbar at 40 °C). The residue was purified by column chromatography on silica gel (n-pentane/Et₂O, 9:1 to 6:4) to give the protected alcohol (8; 8.74 g, 96% yield) as a pale-yellow oil.

(1,1-Dideuterio-2-(4-methoxybenzoyloxy) Ethanol (7))

A solution of ester 6 (671 mg, 3.19 mmol) in dry Et₂O (9 mL) was added dropwise to a suspension of lithium aluminum deuteride (LiAlD₄; 134 mg, 3.19 mmol) in dry Et₂O (9 mL) at 0 °C under an inert atmosphere. The mixture was stirred for 2 h at 0 °C and then carefully quenched with an aqueous solution of Rochelle salt (1 M, 5 mL). The layers were separated and the aqueous layer was extracted with Et₂O (3×10 mL). The combined organic layers were dried over Na₂SO₄, filtered, and the solvents were removed under reduced pressure (600 mbar at 40 °C). The residue was purified by column chromatography on silica gel (n-pentane/Et₂O, 1:1) to give deuterated alcohol 7 (535 mg, 91% yield) as a colorless oil.

[^1]: The NMR spectra were assigned with the help of 2D NMR analysis.
[^2]: ¹H COSY, HSQC, and HMBC. The multiplicities are reported as follows: br = broad, m = multiplet, s = singlet, d = doublet, t = triplet, q = quartet, quint = quintuplet, or combinations thereof. MS and HRMS were recorded by using electrospray ionization (ESI) or atmospheric pressure chemical ionization (APCI) techniques with an atmospheric solids analysis probe (ASAP) on Q-TOF mass spectrometers.

[^3]: It was necessary to consider the high volatilities of compounds 4, 6, and 7 during the different workups in their syntheses.

[^4]: Intermediates 3, 4, and 5 were synthesized on a gram scale; the Wittig and hydrogenation reactions for the synthesis of compounds 1 and 2 were synthesized on a scale of hundreds of milligrams.

[^5]: The NMR spectra were assigned with the help of 2D NMR analysis.
[^6]: ¹H NMR (500 MHz, CDCl₃): δ = 7.30–7.28 (m, 2H; CH₂), 6.89–6.87 (m, 2H; CH₂), 4.56 (s, 2H; CH₂CH₃), 4.07 (s, 2H; CH₂), 3.80 (s, 3H; CH₃), 3.75 ppm (s, 3H; CH₂); ¹³C NMR (126 MHz, CDCl₃): δ = 171.0, 159.6, 129.9 (2C), 129.2, 114.0 (2C), 73.1, 66.9, 55.4, 52.0 ppm.
1,1-Dideutero-2-(4-methoxybenzyl)oxy)ethyl 4-Methylbenzenesulfonate (8)

Triethylamine (2.2 mL, 16.30 mmol) and tosyl chloride (3.10 g, 16.30 mmol) were added to a solution of alcohol 7 (1 g, 5.43 mmol) in CH₂Cl₂ (40 mL) at RT under an inert atmosphere. The mixture was stirred for 20 h and then quenched with CH₂Cl₂ (120 mL) and a saturated aqueous solution of NaHCO₃ (20 mL). The layers were separated and the aqueous layer was extracted with CH₂Cl₂ (80 mL). The combined organic layers were dried over Na₂SO₄, filtered, and the solvents were removed under reduced pressure. The residue was purified by column chromatography on silica gel (n-pentane/ Et₂O, 7:3 to 1:1) to give activated alcohol 8 (1.77 g, 97% yield) as a white solid.

R₁ = 0.40 (n-pentane/EtOAc, 7:3); ¹H NMR (500 MHz, CDCl₃); δ = 7.80–7.77 (m, 2H; CH₃(CH=O)); 7.32–7.30 (m, 2H; CH₃(CH=O)); 7.20–7.17 (m, 2H; CH₃(CH=O)); 6.87–6.84 (m, 2H; CH₃(CH=O)); 4.41 (s, 2H; CH₂(OH)); 3.80 (s, 3H; CH₃(CH=O)); 3.62 (s, 2H; CH₂); 2.43 ppm (s, 3H; CH₃(CH=O)); ¹³C NMR (126 MHz, CDCl₃); δ = 159.4, 144.9, 133.1, 129.9 (2C), 129.7, 72.8 ppm (THP); 1H NMR (500 MHz, CDCl₃): δ = 7.82–7.26 (m, 2H; CH₃(CH=O)); 6.89–6.87 (m, 2H; CH₃(CH=O)); 4.49 (s, 2H; CH₂(OH)); 3.80 (s, 3H; CH₃(CH=O)); 3.56 (s, 2H; CH₂); 1.99 ppm (s, 1H; CH); ¹³C NMR (126 MHz, CDCl₃); δ = 159.4, 143.0, 130.2, 129.5 (2C), 113.9 (2C), 81.4, 72.8, 69.4, 67.8, 55.4, 19.4 ppm (quint, J(CD) = 22.7 Hz); HRMS (APASAP+): m/z calcd for C₁₇H₁₅D₂O₂S: 338.1157 [M⁺]; found: 338.1157.

1-((But-3-yn-1,1-dideutero-1-xyloxy)methyl)-4-methoxybenzene (4)

A solution of activated alcohol 8 (664 mg, 1.96 mmol) in dry DMSO (4 mL) was added dropwise to a suspension of LiC₂H₂EDA (90%, 300 mg, 2.95 mmol) in dry DMSO (2 mL) at 10 °C under an inert atmosphere. The mixture was stirred for 15 min at 10 °C and then allowed to warm to RT. Then, the mixture was stirred for a further 4 h and carefully quenched with CH₂Cl₂ (20 mL), water (10 mL), and brine (10 mL). The layers were separated and the aqueous layer was extracted with CH₂Cl₂ (4 x 10 mL). The combined organic layers were dried over Na₂SO₄, filtered, and the solvents were removed under reduced pressure (500 mbar at 40 °C). The residue was purified by column chromatography on silica gel (n-pentane/Et₂O, 95:5) to give deuterated alkyne 4 (290 mg, 77% yield) as a pale-yellow oil.

R₁ = 0.30 (n-pentane/Et₂O, 9:1); ¹H NMR (500 MHz, CDCl₃); δ = 7.28–7.26 (m, 2H; CH₃(CH=O)); 6.89–6.87 (m, 2H; CH₃(CH=O)); 4.49 (s, 2H; CH₂(OH)); 3.80 (s, 3H; CH₃(CH=O)); 3.56 (s, 2H; CH₂); 1.99 ppm (s, 1H; CH); ¹³C NMR (126 MHz, CDCl₃); δ = 159.4, 143.0, 130.2, 129.5 (2C), 113.9 (2C), 81.4, 72.8, 69.4, 67.8, 55.4, 19.4 ppm (quint, J(CD) = 20.3 Hz); HRMS (APASAP+): m/z calcd for C₁₇H₁₅D₂O₂S: 338.1119 [M⁺]; found: 338.1118.

2-(Pent-4-yn-1-yloxy)tetrahydro-2H-pyran (9)

A solution of 2,4-dihydropyran (DHP; 5.78 g, 68.80 mmol) in CH₂Cl₂ (10 mL) was added dropwise to a solution of pent-4-yn-1-ol (2.29 g, 77.40 mmol) in THF (20 mL) at 0 °C. Asolution of DHP (5.78 g, 68.80 mmol) in CH₂Cl₂ (20 mL) was added dropwise to a solution of pent-4-yn-1-ol (2.29 g, 77.40 mmol) in THF (20 mL) at 0 °C. The mixture was stirred for a further 15 min and then cooled to 0 °C. The mixture was cooled to 0 °C and carefully quenched with Et₂O (20 mL) and a saturated aqueous solution of NaHCO₃ (10 mL). The solid was washed with Et₂O (9 x 20 mL). The combined organic layers were washed with brine (2 x 10 mL), dried over Na₂SO₄, filtered, and the solvents were removed under reduced pressure. The residue was purified by column chromatography on silica gel (n-pentane/Et₂O, 95:5) to give deuterated alkyne 9 (1.02 g, 86% yield) as a colorless oil.

R₁ = 0.16 (n-pentane/Et₂O, 7:3); ¹H NMR (500 MHz, CDCl₃); δ = 4.57 (dd, J(2H) = 4.2, 3.0 Hz, 1H; CH₂(CH=O)); 3.86–3.77 (m, 2H; CH₂(CH=O)); 1.99 ppm (s, 1H; CH); ¹³C NMR (126 MHz, CDCl₃); δ = 159.4, 143.0, 130.2, 129.5 (2C), 113.9 (2C), 81.4, 72.8, 69.4, 67.8, 55.4, 19.4 ppm (quint, J(CD) = 20.3 Hz); HRMS (APASAP+): m/z calcd for C₁₇H₁₅D₂O₂S: 338.1119 [M⁺]; found: 338.1118.

2-(6-Bromo-6,6-dideuteroisoxazol-4-yn-1-yl)tetrahydro-2H-pyran (11)

A solution of triphenylphosphine (13.80 g, 52.60 mmol) in CH₂Cl₂ (50 mL) was added dropwise under an inert atmosphere to a solution of tetrabromomethane (8.72 g, 26.30 mmol) in CH₂Cl₂ (50 mL) at −40 °C. Then, solutions of deuterated alcohol 10 (5.26 g, 26.30 mmol) and imidazole (3.58 g, 52.60 mmol) in CH₂Cl₂ (50 mL each) were added dropwise to the mixture at −40 °C. The reaction was stirred at −40 °C for 2 h and then quenched with a saturated aqueous solution of NaHCO₃ (25 mL) and an aqueous solution of Na₂SO₄ (10%, 25 mL). The layers were separated and the aqueous layer was extracted with CH₂Cl₂ (2 x 50 mL). The combined organic layers were washed with water (2 x 100 mL), dried over Na₂SO₄, filtered, and the solvents were removed under reduced pressure. The residue was triturated with Et₂O (50 mL) and the suspension was filtered through a pad of Celite® to remove most of the white solid (triphenylphosphine oxide), which was washed with Et₂O (3 x 50 mL). The filtrate was concentrated under reduced pressure and the residue was purified by column chromatography on silica gel (n-pentane/Et₂O, 9:1 to 7:3) to give deuterated bromide derivative 11 (6.92 g, 60% yield) as a colorless oil.

R₁ = 0.39 (n-pentane/EtOAc, 9:1); ¹H NMR (500 MHz, CDCl₃); δ = 4.59 (m, 1H; CH₂(CH=O)); 3.88–3.77 (m, 2H; CH₂(CH=O)); 3.53–3.43 (m, 2H; CH₂(CH=O)); 2.37 (t, J(2H) = 7.1 Hz, 2H; CH₂(CH=O)); 1.84–1.77 (m, 3H; CH₂(CH=O)); 1.73–1.70 (m, 1H; CH₂(CH=O)); 1.60–1.50 ppm (m, 4H; CH₂(CH=O)).
13C NMR (126 MHz, CDCl$_3$): $\delta$ = 98.9, 87.6, 75.5, 65.9, 62.3, 30.7, 28.6, 25.6, 19.6, 16.0, 15.5 ppm (quat, 1;CD)=24.3 Hz); HRMS (ASAP+): m/z calcd for C$_9$H$_5$D$_2$BrO$_2$: 263.0616; found: 263.0616.

6,6-Dideutero-6,6-dieuteriohex-4-ynoic Acid (12)

Jones reagent (2.17 m, 35 mL) was added dropwise to a solution of deuterated bromide derivative 11 (3.95 g, 15 mmol) in acetone (40 mL) at 0°C and the mixture was stirred at 0°C for 30 min. Then, the reaction was quenched with propan-2-ol (45 mL) and Et$_2$O (200 mL). The suspension is filtered through a pad of Celite® and the green solid was washed with Et$_2$O (4 x 100 mL). The filtrate was washed with acidified brine (2 x 100 mL, pH 1), dried over Na$_2$SO$_4$, filtered, and the solvents were removed under reduced pressure. The obtained residue was purified by column chromatography on silica gel (8:2 to 6:4, n-pentane/ Et$_2$O) to give acid 12 (2.51 g, 87% yield) as a white solid.

6,6-Dideutero-6,6-dieuteriohept-4-ynoic Acid (14)

Jones reagent (2.17 m, 0.28 mL) was added dropwise to a solution of alcohol 13 (100 mg, 0.40 mmol) in acetonitrile (6 mL) at 0°C. The mixture was stirred at 0°C for 1 h. Then, the reaction was quenched with propan-2-ol (0.50 mL) and the suspension was filtered through a pad of Celite® and the green solid was washed with EtOAc (15 mL). The filtrate was washed with acidified water (5 mL; pH 1) and acidified brine (10 mL; pH 1), dried over Na$_2$SO$_4$, filtered, and the solvents were removed under reduced pressure. The obtained residue was purified by column chromatography on silica gel (n-pentane/EtOAc, 9:1 to 6:4) to give acid 14 (100 mg, 94% yield) as a white solid.

Ethyl 6-Bromo-6,6-dieuteriohex-4-ynoate (5)

Concentrated H$_2$SO$_4$ (250 mL) was added to a solution of acid 12 (1.93 g, 9.48 mmol) in EtOH (80 mL) and the mixture was stirred at reflux for 1 d. The reaction was quenched with a saturated aqueous solution of NaHCO$_3$ (20 mL) and the aqueous layer was extracted with n-pentane/Et$_2$O (1:1, 4 x 100 mL). The combined organic layers were washed with brine (100 mL), dried over Na$_2$SO$_4$, and the solvents were removed under reduced pressure. The residue was purified by column chromatography on silica gel (n-pentane/Et$_2$O, 95:5) to give deuterated ester 5 (2.07 g, 90% yield) as a pale-yellow oil.

6,6-Dideutero-6,6-dieuteriohept-4-yne-1-ol (13)

A solution of nBuLi (1.6 m in n-hexane, 1.64 mL) was slowly added to a solution of alkyn 4 (500 mg, 2.06 mmol) in dry THF (3 mL) at –78°C under an inert atmosphere and the mixture was stirred at –78°C for 30 min. Then, a solution of oxetane (85 mL, 1.30 mmol) in dry THF (2.50 mL) was slowly added to the mixture, followed by the slow addition of BF$_3$·Et$_2$O (320 mL, 2.52 mmol). The reaction was stirred at –78°C for 30 min and quenched with EtOAc (10 mL), a saturated aqueous solution of NH$_4$Cl (5 mL) and brine (5 mL). The layers were separated and the aqueous layer was extracted with EtOAc (2 x 10 mL). The combined organic layers were dried over Na$_2$SO$_4$, filtered, and the solvents were removed under reduced pressure. The obtained residue was purified by column chromatography on silica gel (n-pentane/Et$_2$O, 9:1 to 5:5) to give deuterated alcohol 13 (258 mg, 79% yield) as a pale-yellow oil.

6,6-Dideutero-7-(4-methoxybenzylxoy)hept-4-yn-1-ol (15)

Concentrated H$_2$SO$_4$ (350 mL) was added to a solution of acid 14 (100 mg, 0.38 mmol) in EtOH (7 mL) and the mixture was stirred at 40°C for 4 d under an inert atmosphere. The reaction was quenched with a saturated aqueous solution of NaHCO$_3$ (10 mL) and water (10 mL) and the mixture was extracted with n-pentane/ Et$_2$O 1:1 (4 x 10 mL). The combined organic layers were washed with brine (100 mL), dried over Na$_2$SO$_4$, and the solvents were removed under reduced pressure. The residue was purified by column chromatography on silica gel (n-pentane/Et$_2$O, 7:3 to 1:1) to give ester 15 (2.07 g, 86% yield) as a pale-yellow oil.

Ethyl 6,6-Dideutero-7-hydroxyhept-4-yne-4-ynoate (16)

A solution of NaBH$_4$ in EtOH (0.50 m, 0.56 mL) was added dropwise to a solution of NitoAc$_4$·H$_2$O (139 mg, 0.56 mmol) in EtOH (25 mL) at 0°C under a hydrogen atmosphere. The mixture was stirred at 0°C for 15 min and ethylenediamine (0.22 mL, 3.35 mmol) was added. After a further 15 min at 0°C, a solution of deuterated alkyn 15 (384 mg, 2.23 mmol) in EtOH (15 mL) was added. Before and after each addition, three cycles of vacuum/H$_2$ addition were performed. The reaction was stirred for 40 min at 0°C under a hydrogen atmosphere and then quenched with a saturated aqueous solution of NH$_4$Cl (30 mL) and brine (30 mL). The reaction mixture was extracted with Et$_2$O (3 x 50 mL) and the combined organic layers were dried over Na$_2$SO$_4$, filtered, and the solvents were removed under reduced pressure. The residue was purified by column chromatography on silica gel (n-pentane/Et$_2$O, 6:4) to give alkenel 16 (371 mg, 90% yield) as a pale-yellow oil (with 5% of the over-reduced byproduct).

6,6-Dideutero-7-(4-methoxybenzylxoy)hept-4-ynoic Acid (14)

Concentrated H$_2$SO$_4$ (350 mL) was added dropwise to a solution of alcohol 13 (100 mg, 0.40 mmol) in acetonitrile (6 mL) at 0°C. The mixture was stirred at 0°C for 1 h. Then, the reaction was quenched with propan-2-ol (0.50 mL) and the suspension was filtered through a pad of Celite® and the green solid was washed with EtOAc (15 mL). The filtrate was washed with acidified water (5 mL; pH 1) and acidified brine (10 mL; pH 1), dried over Na$_2$SO$_4$, filtered, and the solvents were removed under reduced pressure. The obtained residue was purified by column chromatography on silica gel (n-pentane/EtOAc, 9:1 to 6:4) to give acid 14 (100 mg, 94% yield) as a white solid.

Ethyl 6,6-Dideutero-7-hydroxyhept-4-yne-4-ynoate (15)

Concentrated H$_2$SO$_4$ (350 mL) was added to a solution of acid 14 (100 mg, 0.38 mmol) in EtOH (7 mL) and the mixture was stirred at 40°C for 4 d under an inert atmosphere. The reaction was quenched with a saturated aqueous solution of NaHCO$_3$ (10 mL) and water (10 mL) and the mixture was extracted with n-pentane/ Et$_2$O 1:1 (4 x 10 mL). The combined organic layers were washed with brine (100 mL), dried over Na$_2$SO$_4$, and the solvents were removed under reduced pressure. The residue was purified by column chromatography on silica gel (n-pentane/Et$_2$O, 7:3 to 1:1) to give ester 15 (2.07 g, 86% yield) as a pale-yellow oil.

6,6-Dideutero-6,6-dieuteriohept-4-ynoic Acid (14)

Concentrated H$_2$SO$_4$ (350 mL) was added to a solution of acid 14 (100 mg, 0.38 mmol) in EtOH (7 mL) and the mixture was stirred at 40°C for 4 d under an inert atmosphere. The reaction was quenched with a saturated aqueous solution of NaHCO$_3$ (10 mL) and water (10 mL) and the mixture was extracted with n-pentane/ Et$_2$O 1:1 (4 x 10 mL). The combined organic layers were washed with brine (100 mL), dried over Na$_2$SO$_4$, and the solvents were removed under reduced pressure. The residue was purified by column chromatography on silica gel (n-pentane/Et$_2$O, 7:3 to 1:1) to give ester 15 (2.07 g, 86% yield) as a pale-yellow oil.
(m, 4H; CH₂), 1.22 ppm (t, J(H,H) = 7 Hz, 3H; CH₃outer); ¹³C NMR (126 MHz, CDCl₃): δ = 173.4, 130.8, 127.1, 62.0, 60.5, 34.1, 30.2 ppm; H RMS: z = 2.0 Hz, 19.2 Hz, 14.3 ppm.

(42) Ethyl 6,6-Dideutero-7-iodohept-4-enoate (16)

Imidazole (348 mg, 5.11 mmol) and triphenylphosphine (671 mg, 2.56 mmol) were added to a solution of 1 (538 mg, 89 % yield) as a pale-yellow oil. The residue was purified by column chromatography on silica gel (95:5 n-pentane/EtO, 9:1) to give iodide derivative 16 (538 mg, 89 % yield) as a pale-yellow oil.

Ethyl 6,6,9,9-Tetradecenoic acid-4,7-diynoate (18)

A solution of terminal alkyne 4 (1.63 g, 8.50 mmol) in DMF (40 mL) was added to a solution of Cul (3.40 g, 17.80 mmol), Na₂SO₃ (2.57 g, 17.80 mmol), and K₂CO₃ (3.52 g, 25.50 mmol) in DMF (25 mL) at 20°C. A solution of bromide derivative 5 (1.88 g, 8.50 mmol) in DMF (30 mL) was immediately added to the mixture at 20°C and the mixture was stirred at 20°C for 15 h and then quenched with a saturated aqueous solution of NH₄Cl (100 mL) and brine (100 mL). The aqueous layer was extracted with EtOAc (3 x 200 mL). The combined organic layers were washed with brine (2 x 50 mL) and water (50 mL), dried over Na₂SO₄, filtered, and the solvents were removed under reduced pressure. The residue was purified by column chromatography on silica gel (n-pentane/EtO, 9:1) to give the coupling product (17; 2.12 g, 75 % yield) as a pale-yellow oil.

Ethyl 6,6,9,9-Tetradecenoic acid-4,7-diynoate (19)

A solution of NaBH₄ in EtOH (0.5 M, 0.56 mL) was added dropwise to a solution of Ni(OAc)₂·4H₂O (38 mg, 0.15 mmol) in EtOH (25 mL) at 0°C under a hydrogen atmosphere. The mixture was stirred at 0°C for 15 min and a solution of ethylenediamine in EtOH (0.5 M, 1.88 mL) was added. After a further 15 min at 0°C, a solution of diyne 18 (100 mg, 0.47 mmol) in EtOH (15 mL) was added. Before and after each addition of the reagent, three cycles of vacuum/H₂ addition were performed. The reaction was stirred for 40 min at 0°C under a hydrogen atmosphere and then quenched with a saturated aqueous solution of NH₄Cl (15 mL) and brine (20 mL). The aqueous layer was extracted with EtOAc (3 x 30 mL). The combined organic layers were dried over Na₂SO₄, filtered, and the solvents were removed under reduced pressure. The residue was purified by column chromatography on silica gel (6:4 n-pentane/EtO) to give a mixture of products (19; 191 mg, 75 % yield) as a pale-yellow oil.
HRMS (ESI+): m/z calcd for C_{12}H_{13}O_{3}P: 217.1742 [M+H]^+; found: 217.1741.

(42,72)-Ethyl 6,6,9,9-Tetradecatetra-10-iododeca-4,7-dienoate (19)

Imidazole (429 mg, 6.30 mmol) and triphenylphosphine (826 mg, 3.15 mmol) were added to a solution of 1 (800 mg, 3.15 mmol) in CHCl\(_3\) (7 mL) under an inert atmosphere. The mixture was cooled to 0 °C and a solution of alcohol 19 (447 mg, 2.10 mmol) in CHCl\(_3\) (7 mL) was added. After the addition was complete, the reaction mixture was allowed to warm to RT and stirred for a further 4 h. Then, the reaction was quenched with a saturated aqueous solution of Na\(_2\)SO\(_4\) (20 mL), water (20 mL), and CHCl\(_3\) (15 mL). The layers were separated and the aqueous layer was extracted with CHCl\(_3\) (15 mL). The combined organic layers were dried over Na\(_2\)SO\(_4\), filtered, and the solvents were removed under reduced pressure. The residue was purified by column chromatography on silica gel (n-pentane/MeOH, 95:5) to give the iodide derivative (634 mg, 93% yield) as a pale-yellow oil.

\[ R_{f} = 0.55 \] (n-pentane/EtOAc, 95:5); \[ ^1H \text{NMR (500 MHz, CDCl}_3\]): \[ \delta = 5.49 (d, J(H,H) = 11 Hz, 1H; CD_2-CH-CD-CH_2), 3.58-3.33 (m, 3H; CD_2-CH), 4.13 (q, J(H,H) = 7 Hz, 2H; CH_3, 3.12 (s, 2H; CH_3), 2.41-2.33 (m, 4H; CH_2), 1.25 ppm (t, J(H,H) = 7 Hz, 3H; CH_3)]

Methyl 4-(3-((2Z,5Z,8Z,11Z)-Tetradeca-2,5,8,11-tetraen-1-yl)oxiran-2-yl)butanoate (21)

Triphenylphosphine (583 mg, 2.22 mmol) was added to a solution of the iodide derivative (604 mg, 1.85 mmol) in MeCN (12 mL) at RT under an inert atmosphere. The mixture was stirred at reflux for 17 h and the solvent was removed under reduced pressure. The residue was purified by column chromatography on silica gel (CH\(_2\)Cl\(_2\)/MeOH, 98:2 to 9:1) to give phosphonium salt 2b (903 mg, 74% yield) as a yellow oil.

\[ R_{f} = 0.21 \] (n-pentane/MeOH, 95:5); \[ ^1H \text{NMR (500 MHz, CDCl}_3\]): \[ \delta = 5.42-5.30 (m, 30, 10H; CH), 2.85-2.80 (m, 8H; CH_3), 6.05, 34.3, 30.9 (q, J(C,D) = 19.7 Hz), 25.3 (q, J(C,D) = 19.1 Hz), 23.0, 14.4, 5.1 ppm; HRMS (APCI+): m/z calcd for C_{25}H_{28}O_2P: 327.0759 [M+H]^+; found: 327.0762.

\[(3Z,6Z)-(2,2,5,5-Tetradecatetra-10-ethoxy-10-oxodeca-3,6-dien-1-yl)triphenylphosphonium iodide (2b)\]

Methyl 4-(3-((2Z,5Z,8Z,11Z)-Tetradeca-2,5,8,11-tetraen-1-yl)oxiran-2-yl)butanoate (21)

\[ \gamma\text{-Collidine (2,4,6-trimethylpyridine, 0.26 mL, 2 mmol) and I}_2 (250 mg, 1mmol) were added to a solution of eicosapentaenoic acids (3.6 mmol) in MeCN (15 mL) at 0 °C under an inert atmosphere. The mixture was stirred for 1 h at 0 °C and then quenched with a saturated aqueous solution of Na\(_2\)SO\(_4\) (10 mL) and EtOAc (30 mL). The layers were separated and the organic layer was washed with brine (3 × 20 mL) and water (10 mL). The combined organic layers were dried over Na\(_2\)SO\(_4\), filtered, and the solvents were removed under reduced pressure. The crude iodolactone was immediately used in the next step without further purification.

K\(_2\)CO\(_3\), (150 mg, 0.99 mmol) was added to a solution of the crude iodolactone (400 mg) in MeOH (10 mL) at RT under an inert atmosphere. The mixture was stirred for 4 h at RT and quenched with brine (15 mL) and water (20 mL). The aqueous layer was extracted with n-pentane (4 × 30 mL). The combined organic layers were dried over Na\(_2\)SO\(_4\), filtered, and the solvents were removed under reduced pressure. The residue was purified by column chromatography on silica gel (n-pentane/MeOH, 95:5) to give epoxide 21 (158 mg, 95% yield) as a pale-yellow oil.

\[ R_{f} = 0.25 \] (n-pentane/MeOH, 95:5); \[ ^1H \text{NMR (500 MHz, CDCl}_3\]): \[ \delta = 5.53-5.27 (m, 8H; CH), 3.66 (s, 3H; CH_3), 2.95-2.92 (m, 2H; CH_3), 2.34-2.78 (m, 6H; CH_3), 2.41-2.35 (m, 3H; CH_2), 1.86-1.77 (m, 2H; CH_3), 1.68-1.77 (m, 2H; CH_3), 0.96 ppm (t, J(H,H) = 7.5 Hz, 3H; CH_3)]

\[ ^13C \text{NMR (125 MHz, CDCl}_3\): \[ \delta = 173.8, 132.2, 130.7, 128.7, 128.6, 127.8, 127.8, 127.1, 124.4, 56.7, 56.3, 51.7, 33.7, 27.3, 26.3, 25.9, 25.7, 25.6, 22.1, 20.7, 14.4 ppm.

Extraction of EPA from Cod Liver Oil

Commercial cod liver oil (7.00 g; Cooper, France) was dissolved in a mixture of ETOH/H$_2$O (95:5, 35 mL) in the presence of NaOH (2.1 g) under an argon atmosphere. The mixture was protected from light by using aluminum foil and heated at 82 °C for 2 h. The ethanolic fraction was evaporated under reduced pressure and the residue was dissolved in n-hexane (40 mL) after heating. Then, water (35 mL) was added to the organic layer and the unsaponifiable material was removed by repeated extraction of the aqueous phase with n-hexane (4 × 40 mL). The aqueous phase was acidified to pH 2 by using an aqueous solution of HCl (60.0 mL). The fatty acids were extracted with n-hexane (4 × 35 mL) and the organic phase was concentrated under reduced pressure to give the crude fatty acids (6.47 g) as an oil. Urea (19.4 g) and EtOH (80 mL) were added to the crude residue. The mixture was protected from light by using aluminum foil and heated at 60–70 °C until it turned into a homogeneous clear solution. The mixture was allowed to cool to RT and then cooled at 4 °C for 24 h. The resulting crystals were separated from the liquid by filtration. The filtrate was diluted with water (50 mL) and acidified to pH 4–5 with an aqueous solution of HCl (60.0 mL). n-Hexane (100 mL) was added and the solution was thoroughly stirred for 1 h. The n-hexane layer that contained the liberated fatty acids was separated from the aqueous layer and washed with water (3 × 60 mL). The organic phase was dried with Na$_2$SO$_4$ and concentrated under reduced pressure to give a crude mixture of PUFA's (820 mg), which was purified by preparative HPLC (column: Atlantis Prep OBDM; particle size: 10 μm; dimensions: 19 mm × 250 mm; water/MeOH, 13:87; isotropic flow; photodiode array detector (PDA) detection: 217 nm) to give pure EPA (1.33 g).

\[ R_{f} = 0.21 \] (n-pentane/MeOAc, 8:2); \[ ^1H \text{NMR (500 MHz, CDCl}_3\]): \[ \delta = 5.42-5.30 (m, 30, 10H; CH), 2.85-2.80 (m, 8H; CH_3), 3.84, 2.65, 1.20 ppm (t, J(H,H) = 7 Hz, 3H; CH_3), 2.20-2.14 (m, 4H; CH_3)]

\[ ^13C \text{NMR (126 MHz, CDCl}_3\): \[ \delta = 173.2, 130.4, 128.8, 128.4 (2C), 60.5, 33.9, 17.1742 \]
(3Z,6Z,9Z,12Z)-Pentadeca-3,6,9,12-tetraenal (3a)

Acetic anhydride (9 mL) was added to a solution of epoxide 21 (4.48 g, 13.47 mmol) in acetic acid (90 mL) at RT under an inert atmosphere. The mixture was stirred at 45 °C for 2.5 h and the solvents were removed under reduced pressure. The crude dicetate was immediately used in the next step without further purification. A solution of LiOH·H₂O (2.70 g, 64.35 mmol) in water (100 mL) was added to a solution of the dicetate in MeOH (100 mL) at RT under an inert atmosphere. The mixture was stirred at RT for 21 h, cooled to 0 °C, and carefully quenched with an aqueous solution of HCl (10%) to pH 1. The aqueous layer was extracted with Et₂O (2 × 100 mL). The combined organic layers were dried over Na₂SO₄, filtered, and the solvents were removed under reduced pressure. The crude diol was immediately used in the next step without further purification.

A solution of sodium periodate (6.68 g, 31.23 mmol) in water (30 mL) was added to a solution of the crude diol in MeOH (180 mL) at RT under an inert atmosphere. The mixture was stirred at RT for 3 h and then quenched with brine (150 mL). The aqueous layer was extracted with n-pentane (3 × 100 mL) and the combined organic layers were successively washed with a saturated aqueous solution of NaHCO₃ (100 mL), brine, and water. The organic layer was dried over Na₂SO₄, filtered, and the solvents were removed under reduced pressure. Then, the residue was dissolved in Et₂O (10 mL) and finally filtered through a plug of Celite® and the solvents were removed under reduced pressure to give crude aldehyde 3a (2.57 g, 87% yield). Purification by column chromatography on normal-phase silica gel was not possible because of isomerization of the desired aldehyde into the cis-β-unsaturated aldehyde.

Rᵣ = 0.47 (n-pentane/EtOAc, 9:1); ¹H NMR (500 MHz, CDCl₃): δ = 9.68 (t, ¹J(H,H) = 2 Hz, 1H; CHO), 5.72–5.67 (m, 1H; CH), 5.62–5.56 (m, 1H; CH), 5.44–5.38 (m, 6H; CH), 3.24–3.06 (m, 2H; CH₂–CHO), 2.83–2.78 (m, 4H; 2H; CH₂–CH₂–CHO), 2.1–2.01 (m, 2H; CH₂–CH₂–CHO), 0.97 ppm (t, ¹J(H,H) = 7.5 Hz, 3H; CH₃); ¹³C NMR (126 MHz, CDCl₃): δ = 199.5, 133.3, 132.3, 129.0, 128.9, 127.7, 127.1, 118.8, 42.6, 26.1, 25.8, 25.7, 20.7, 14.4 ppm.

(3Z,6Z,9Z,12Z)-Pentadeca-3,6,9,12-tetraen-1-ol (22)

NaBH₄ (1.47 g, 38.86 mmol) was added to a solution of aldehyde 20 (100 mL) and the solution was stirred at RT for 3 h and then quenched with brine (150 mL). The aqueous layer was extracted with CH₂Cl₂ (3 × 80 mL). The combined organic layers were dried over Na₂SO₄, filtered, and the solvents were removed under reduced pressure. The residue was purified by column chromatography on silica gel (n-pentane/EtOAc, 9:1 to 7:3) to give alcohol 22 (1.64 g, 71% yield) as a colorless oil.

Rᵣ = 0.18 (n-pentane/EtOAc, 9:1); ¹H NMR (500 MHz, CDCl₃): δ = 5.58–5.53 (m, 1H; CH), 5.44–5.29 (m, 7H; CH), 3.66 (t, ¹J(H,H) = 6.5 Hz, 2H; CH₂–O), 2.87–2.80 (m, 6H; CH₂–CH₂–CHO), 2.39–2.34 (m, 2H; CH₂–CH₂–CHO), 1.93–1.85 (m, 1H; CH₂–CH₂–CHO), 1.77–1.70 (m, 1H; CH₂–CH₂–CHO), 0.97 ppm (t, ¹J(H,H) = 7.5 Hz, 3H; CH₂–CH₂–CHO); ¹³C NMR (126 MHz, CDCl₃): δ = 132.2, 131.3, 128.8, 128.5, 128.1, 128.0, 127.1, 125.8, 62.4, 31.0, 25.9, 25.8, 25.7, 20.7, 14.4 ppm.

(2Z,5Z,8Z)-Undeca-2,5,8-trien-1-yl)oxiran-2-yl) Ethanol (23)

Vanadyl acetylacetone (100 mg, 0.37 mmol) was added to a solution of homoallylic alcohol 22 (1.64 g, 7.44 mmol) in toluene (60 mL) at 0 °C under an inert atmosphere. The mixture was stirred at 0 °C for 5 min and a portion of BuOOH (5.5 mL in n-decane, 2.7 mL) was added dropwise. The mixture was stirred at RT for 1.5 h and a second portion of BuOOH was added. The mixture was stirred for 4.5 h and then quenched with an aqueous solution of Na₂SO₄ (20%, 100 mL) and water (100 mL). The aqueous layer was extracted with CH₂Cl₂ (3 × 60 mL). The combined organic layers were dried over Na₂SO₄, filtered, and the solvents were removed under reduced pressure. The residue was purified by column chromatography on silica gel (n-pentane/EtOAc, 9:1 to 7:3) to give epoxide 23 (1.29 g, 73% yield) as a pale-yellow oil.

Rᵣ = 0.26 (n-pentane/EtOAc, 6:4); ¹H NMR (500 MHz, CDCl₃): δ = 5.54–5.28 (m, 6H; CH), 3.90–3.82 (m, 2H; CH₂–O), 3.11 (dt, ¹J(H,H) = 4.5, 8 Hz, 1H; CH₂–O), 3.01–2.97 (m, 1H; CH–CH₂–O), 2.85–2.79 (m, 4H; CH₂–CH₂–O), 2.46–2.40 (m, 1H; CH–CH₂–O), 2.28–2.22 (m, 1H; CH–CH₂–O); ¹³C NMR (126 MHz, CDCl₃): δ = 132.3, 130.9, 129.0, 127.5, 127.0, 124.1, 60.8, 56.1, 55.1, 30.6, 26.5, 25.9, 25.7, 20.7, 14.4 ppm.
A solution of sodium bis(trimethylsilyl)amide (NaHMDS; 2.0 mol in THF, 0.50 mL) was added dropwise to a solution of deuterated phosphonium salt (2a; 548 mg, 1.0 mmol) in THF (9 mL) at −50 °C under an inert atmosphere. Then, the reaction was stirred at −20 °C for 30 min and cooled to −78 °C. A solution of aldehyde 3a (371 mg, 1.70 mmol) in THF (9 mL) was cooled to −78 °C and added through a cannula to the orange mixture. The reaction was allowed to warm to RT over 1.5 h and then stirred for a further 21 h. The reaction was quenched with a saturated aqueous solution of NH₄Cl (10 mL). The aqueous layer was extracted with n-pentane/EtOAc (8:2, 4×20 mL). The combined organic layers were dried over MgSO₄, filtered, and the solvents were removed under reduced pressure. The residue was purified by column chromatography on silica gel (n-pentane/EtO₂, 98:2) to give the deuterated DHA ethyl ester (24a; 143 mg, 40 % yield) as a yellow oil.

Rᵣ = 0.51 (n-pentane/ EtOAc, 9:1); ¹H NMR (500 MHz, CDCl₃); δ = 5.40–5.29 (m, 12 H; CH₂); 4.12 (q, 3J(H,H) = 7 Hz, 2H; CH₂CH₂); 2.85–2.79 (m, 8H; CH₂CH₂); 2.40–2.33 (m, 4H; CH₂CH₂CO); 2.08–2.04 (m, 2H; CH₂CH₂); 1.25 (t, 3J(H,H) = 7 Hz, 3H; CH₃); 0.96 ppm (t, 3J(H,H) = 7.5 Hz, 3H; CH₃); ¹³C NMR (126 MHz, CDCl₃); δ = 173.2, 132.1, 129.2, 128.6, 128.3 (2C), 128.3, 128.2, 128.1 (2C), 128.0, 127.1, 60.4, 34.4, 25.7 (2C), 25.7, 25.6, 25.1 (quint, 3J(C,D) = 19.4 Hz), 22.9, 20.7, 14.4, 14.3 ppm; HRMS (APSA+): m/z calc for C₆₃H₁₀8D₁₀O₅: 359.2919 [M+H]$^+$; found: 359.2918.

### (4Z,7Z,10Z,13Z,16Z,19Z)-Ethyl 6,6-Dideuterodocos-4,7,10,13,16,19-hexaenoate (24a)

A solution of LiOH·H₂O (109 mg, 2.60 mmol) in water (1 mL) was added to a solution of ester (2a; 143 mg, 0.40 mmol) in EtOH (1 mL) under an inert atmosphere. The mixture was heated at 65 °C for 1 h and an additional portion of LiOH·H₂O (50 mg, 0.20 mmol) was added. After heating at 65 °C for 7 h, the reaction was cooled to RT and quenched with water (10 mL) and an aqueous solution of HCl (1.0 M) to pH 2. The aqueous layer was extracted with n-pentane/EtOAc (6:4, 3×10 mL). The combined organic layers were dried over MgSO₄, filtered, and the solvents were removed under reduced pressure. The residue was purified by preparative HPLC to remove traces amounts of over-reduced byproducts (column: Waters Symmetry Prep$^{TM}$ (C₁₂); particle size: 7 μm; dimensions: 7.8 mm×300 mm; water/MeCN, 17:83; isocratic flow; UV detection: 217 nm) to give pure deuterated DHA.

Rᵣ = 0.50 (n-pentane/ EtOAc, 6:4); ¹H NMR (500 MHz, CDCl₃); δ = 5.43–5.29 (m, 12 H; CH₂); 2.86–2.80 (m, 8H; CH₂CH₂), 2.43–2.38 (m, 4H; H₂–CH–CH₂–CO), 2.10–2.04 (m, 2H; CH₂–CH₂–CO), 0.99 ppm (t, 3J(H,H) = 7.5 Hz, 3H; CH₃); ¹³C NMR (126 MHz, CDCl₃); δ = 179.5, 132.2, 129.6, 128.7, 128.5, 128.4, 128.4, 128.2, 128.2 (2C), 128.0 (2C), 127.7, 127.1, 34.1, 25.8 (2C), 25.7, 25.7, 25.1 (quint, 3J(C,D) = 19.2 Hz), 22.6, 20.7, 14.4 ppm; HRMS (ESI–): m/z calc for C₆₃H₁₀8D₁₀O₅: 329.2450 [M–H]$^-$; found: 329.2446; HPLC (column: Bridge$^{®}$ BEH (C₁₂); particle size: 2.5 μm; dimensions: 2.1×100 mm; water/MeOH/20:80; isocratic flow; PDA detection: 200–800 nm); tᵣ = 6.36 min.

### (4Z,7Z,10Z,13Z,16Z,19Z)-Ethyl 6,6,9,9-Tetradideuterodocos-4,7,10,13,16,19-hexaenoate (24b)

A solution of NaHMDS (2.0 mol in THF, 85 mL) was added dropwise to a solution of phosphonium salt (2b; 100 mg, 0.17 mmol) in THF (1.50 mL) at −50 °C under an inert atmosphere. The reaction was stirred at −20 °C for 30 min and then cooled to −78 °C. A solution of aldehyde 3b (51 mg, 1.70 mmol) in THF (1.50 mL) was cooled to −78 °C and added through a cannula to the orange mixture. The reaction was allowed to warm to RT over 1.5 h and then stirred for 21 h. The reaction was quenched with a saturated aqueous solution of NH₄Cl (10 mL). The aqueous layer was extracted with n-pentane/EtOAc (9:1, 4×5 mL). The combined organic layers were dried over MgSO₄, filtered, and the solvents were removed under reduced pressure. The residue was purified by column chromatography on silica gel (n-pentane/EtO₂, 98:2) to give DHA ethyl ester (24b; 31 mg, 51 % yield) as a yellow oil.

Rᵣ = 0.53 (n-pentane/ EtOAc, 9:1); ¹H NMR (500 MHz, CDCl₃); δ = 5.40–5.32 (m, 12 H; CH₂); 4.12 (q, 3J(H,H) = 7 Hz, 2H; CH₂CH₂); 2.86–2.80 (m, 6H; CH₂CH₂); 2.39–2.34 (m, 4H, CH₂–CH₂–CO), 2.08–2.05 (m, 2H; CH₂–CH₂–CO), 1.25 (t, 3J(H,H) = 7 Hz, 3H; CH₃); 0.97 ppm (t, 3J(H,H) = 7.5 Hz, 3H; CH₃); ¹³C NMR (126 MHz, CDCl₃); δ = 173.2, 132.1, 129.2, 128.7, 128.4, 128.3, 128.2, 128.1 (2C), 128.0, 127.1, 60.5, 34.4, 25.7, 25.5, 25.1 (quint, 3J(C,D) = 19.4 Hz), 25.1 (quint, 1J(C,D) = 19.5 Hz), 22.9, 20.7, 14.4, 14.4 ppm; HRMS (APSA+): m/z calc for C₆₃H₁₀8D₁₀O₅: 361.3045 [M+H]$^+$; found: 361.3046.
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