Synthesis of a Regioselectively Hexadeuterated Linoleic Acid

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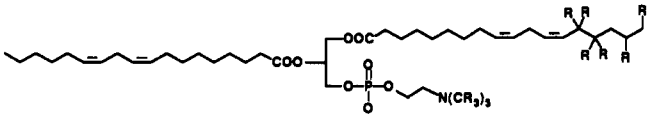
Introduction

"Essential" phospholipids (EPL), i.e., polyenylphosphatidylcholines such as 1,2-dilinoleylphosphatidylcholine (1) are well-known for preventing the development of hypercholesterolaemia and hypertriglyceridaemia by normalizing LDL (low-density lipoproteins) cholesterol,[1a] total cholesterol, as well as triglycerides levels[1b] in serum. EPL promotes cholesterol reverse transport from peripheral tissues to the liver, for instance, by activation of LCAT (lecithin-cholesterol-acyltransferase).[1c] Moreover, indications were obtained for an inhibition of lipid peroxidation by EPL.[1d] All these results point toward a favorable influence of EPL on atherosclerotic changes.

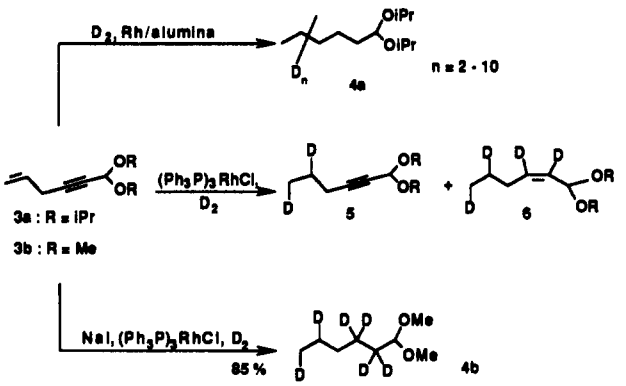


1 : R = H ; 2 : R = D

In order to get more information on the metabolism of EPL 1 after administration in man, we have considered the synthesis of the pentadecadeuterio-1,2-dilinoleylphosphatidylcholine (2) which would be detectable in patient-derived samples by GC–MS. In this paper, we describe the synthesis of 14,14,15,15,17,18-hexadeuteriolinoleic acid (12), a key intermediate to be used in the preparation of 2.

The literature reports several syntheses of deuterio (d_4–d_6) polyunsaturated fatty acids[2–5] (including linoleic acid)[6] with a total deuterium incorporation ranging from only 80 to 89%. Due to the needs of metabolism and mass spectroscopic studies, it was necessary to prepare on a several gram scale a regiospecific d_6 linoleic acid (above 95% deuterium incorporation). We present herein our strategy based on the building up of the cis–cis 1,4-diene unit by two successive Wittig reactions starting from 2,2,3,3,5,6-hexadeuteriohexanal (7).

Scheme I



Results and Discussion

The strategy for the synthesis of key intermediate 7 as its dialkyl acetal **4b** was based on the regioselective reduction of the corresponding 5-hexen-2-ynal dialkyl acetal (**3b**), readily prepared by condensation of Grignard derivative of 1,1-dialkoxy-2-propyne[7] with allyl bromide.

The heterogeneous catalyst rhodium on alumina has been used to perform the catalytic deuterogenation of 5-hexynol without scrambling.[4] However, reduction of **3a** using 5% rhodium on alumina (10–20% w/w) gave compound **4a** (Scheme I) which had undergone positional and H/D scrambling to a statistical distribution of deuterium from d_2 to d_{10} (see Figure 1A–B).

The homogeneous Wilkinson catalyst chlorotris(triphenylphosphine) rhodium is well-known to give syn addition to double bonds without H/D exchange.[8] Reduction of the diisopropyl acetyl **3a** using the Wilkinson catalyst in THF for 5 h gave a mixture of di- and tetradeuterio compounds **5a** and **6a**. Prolonged reaction time produced an intractable mixture of products (Scheme I). Suspecting some steric hindrance effect of the bulky diisopropyl acetal on the triple-bond deuterogenation, we decided to perform the reduction on the corresponding less hindered dimethyl acetal **3b**. Regioselective deuterogenation of enyne **3b**, using the Wilkinson catalyst (Scheme I), led to the desired hexadeuteriohexanal dimethyl acetal (**4b**) in 70–85% yield, depending on reaction conditions.[9] On a large scale (2 g of **3b**), sodium iodide was added to increase the deuteration rate by in situ formation of the iodorhodium complex.[10] Total deuterium

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(1) (a) Zierenberg, O.; Assmann, G.; Schmitz, G.; Rosseneu, M. *Atherosclerosis* **1981**, *39*, 527. (b) Wong, E. K.; Nicolosi, R. J.; Low, P. A.; Herd, J. A.; Hayes, K. C. *Lipids* **1980**, *15*, 428. (c) Rosseneu, M.; Declercq, B.; Vandamme, D.; Vercaemst, R.; Soetewey, F.; Peeters, H.; Blaton, V. *Atherosclerosis* **1979**, *32*, 141. (d) Jaeschke, H.; Werner, C.; Wendel, A. *Chem.-Biol. Interact.* **1987**, *64*, 127.

(2) Synthesis of methyl 15,15,16,16-tetradeuterio-6,9,12-octadecatrienoate: Rakoff, H. *Chem. Phys. Lipids* **1984**, *35*, 117.

(3) Synthesis of methyl 17,17,18,18-tetradeuterio-5,8,11-eicosatrienoate: Adlof, R. O.; Emken, E. A. *J. Label. Compd. Radiopharm.* **1987**, *24*, 699.

(4) Synthesis of 19,19,20,20,20-pentadeuterioarachidonic acid: Marron, B. E.; Spanevello, R. A.; Elisseou, M. E.; Serhan, C. N.; Nicolaou, K. C. *J. Org. Chem.* **1989**, *54*, 5522.

(5) Synthesis of methyl 9,10,15,15,16,16-hexadeuterio-12-octadecenoate: Rakoff, H.; Emken, E. A. *J. Label. Compd. Radiopharm.* **1978**, *15*, 233; *Ibid.* **1982**, *19*, 1135.

(6) Synthesis of 9,10,12,13-tetradeuterio- and 9,10,11,11,12,13-hexadeuteriolinoleic acids: Evans, J. C.; Jackson, S. K.; Rowlands, C. C.; Barratt, M. D. *Biochim. Biophys. Acta* **1985**, *835*, 421.

(7) Ward, J. P.; Van Dorp, D. A. *Rec. Trav. Chim.* **1985**, *85*, 117.

(8) Osborn, J. A.; Jardine, F. H.; Young, J. F.; Wilkinson, G. J. *J. Chem. Soc. A* **1966**, 1711.

(9) Particularly on a 2-g scale the reaction was, sometimes, incomplete, and beside hexanal dimethyl acetal **4b** we identified the di- and tetradeuteriated intermediates 2-hexynal dimethyl acetal **5b** and 2-hexenal dimethyl acetal **6b** in the relative ratio of 85/15. In order to remove these unsaturated acetals the following sequence was carried out: addition of bromine and methyl orthoformate (to prevent hydrolysis of acetal) led to di- and tetrabromides and unchanged hexanal-d_6, neutralization of acid with excess triethylamine, and distillation at room temperature under vacuum and flash chromatography.

(10) Birch, A. J.; Williamson, D. H. In *Organic Reactions*; Dauben, W. G., Ed.; Wiley and Sons: New York, 1976; Vol. 24, p 23.

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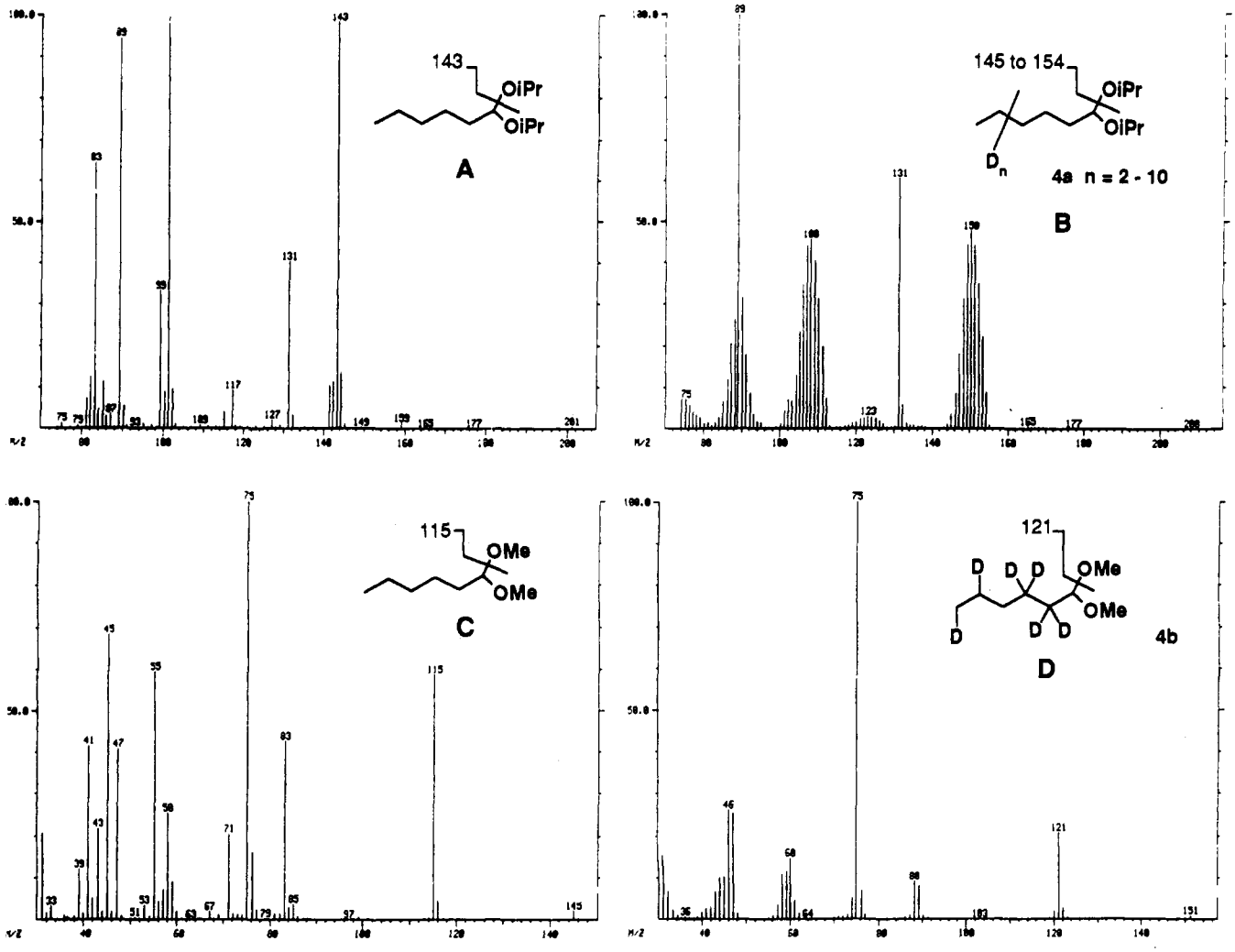
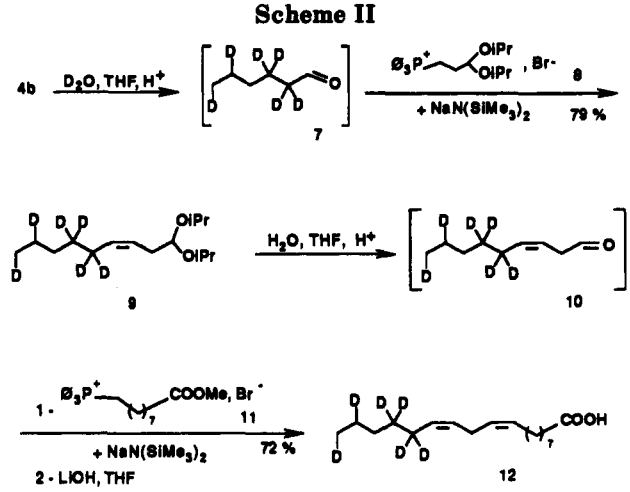


Figure 1. Mass spectra of A, hexanal diisopropyl acetal, B, polydeuterated hexanal diisopropyl acetal **4a**, C, hexanal dimethyl acetal, and D, hexadeuterated hexanal dimethylyl acetal **4b**.

incorporation was above 98% by mass spectroscopy[11] (see Figure 1C–D) and no scrambling and/or H/D exchange was detected by NMR spectroscopy. In ^{13}C NMR spectroscopy, carbon atoms C2 and C3, each bearing two deuterium atoms, appear as quintets while C5 and C6, each bearing one deuterium atom, appear as triplets.

Starting from **4b**, the first Wittig reaction involved a three-carbon homologation of aldehyde **7** by using (3,3-diisopropoxypropyl)triphenylphosphonium bromide (**8**).[12] This homologating reagent bearing a very sensitive acetal leads, after Wittig reaction and deprotection, to a cis-β,γ-ethylenic aldehyde without migration of the double bond[13] (Scheme II). To avoid any H/D exchange at the α position of the carbonyl group, hydrolysis of acetal **4b** was performed in D_2O/THF in the presence of anhydrous camphorsulfonic acid. Then, the resulting hexadeuteriohexanal **7** was thoroughly dried by a slow azeotropic distillation of benzene and added to the ylide solution from **8**. Purification by two flash chromatographies gave hexadeuterio *cis*-3-nonenal diisopropyl acetal **9** in 79% yield. Total deuterium incorporation was unchanged (>98% by MS[11] and NMR analyses), and the integrity of

Scheme II



the double-bond positions was checked by GC and ^{13}C NMR analyses (two lines for ethylenic carbon atoms).

Mild hydrolysis of acetal **9**[13] gave the corresponding pure aldehyde **10** which underwent Wittig condensation with phosphorane from **11** (prepared in three steps from azelaic monomethyl ester in 46% overall yield)[14] and alkaline hydrolysis (LiOH) led to hexadeuterio linoleic acid **12** in 65–72% yield.[11]

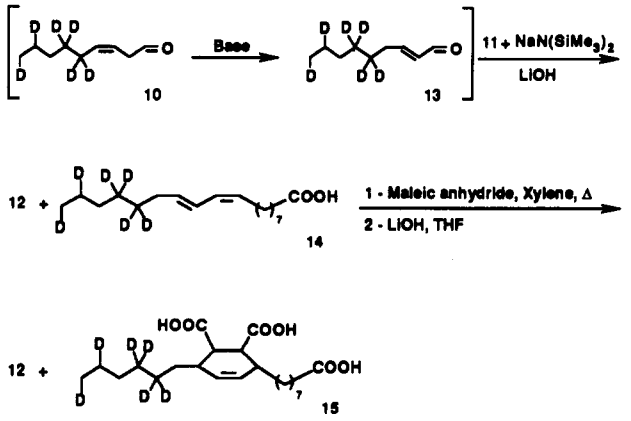
(11) In all MS spectra done for each sample of compounds **4b**, **9**, and **12**, the presence of either d_5 or other deuterium distribution was not detectable (≤1–2% d_5).

(12) Viala, J.; Santelli, M. *Synthesis* **1988**, 395.

(13) Viala, J.; Santelli, M. *J. Org. Chem.* **1988**, *110*, 2953. Viala, J.; Munier, P.; Santelli, M. *Tetrahedron* **1991**, *47*, 3347.

(14) Génard, S.; Patin, H. *Bull. Soc. Chim. Fr.* **1991**, *128*, 397.

Scheme III



On a large reaction scale (over 10 g of phosphonium **11**), it was, sometimes, difficult to remove all of the moisture from the Wittig salt, and cis-β,γ-ethylenic aldehyde **10**, partly, underwent isomerization into trans-α,β-ethylenic aldehyde **13** before the Wittig reaction, giving 3–20% of conjugated dienic acid **14** which was difficult to separate from **12** (Scheme III). The presence of **14** was determined easily by TLC (UV) and ^{13}C or ^{1}H NMR analyses. The purification was accomplished chemically by in situ Diels–Alder reaction of **14** with maleic anhydride in refluxing xylene. After disappearance of the UV-active spot on TLC plates, basic hydrolysis and acidification to pH 4 led to a mixture of **12** and the water-soluble triacid **15**. Pure hexadeuterio linoleic acid **12** was then obtained by simple extraction.

Conclusion

This synthesis of 14,14,15,15,17,18-hexadeuteriolinoleic acid **12** started from a new synthon **7** which is conveniently prepared on a several gram scale. Deuterium atoms were regiospecifically introduced at once. Total label incorporation was above 98%. Intermediates **7** and **9** will be versatile hexadeuterio synthons, useful for the preparation of many d_6-labeled polyunsaturated fatty acids and their metabolites, by the same strategy.

Experimental Section

General Methods. ^{1}H NMR and ^{13}C NMR spectra were recorded at 200.13 and 50.32 MHz, respectively, in $CDCl_3$ solutions. For ^{13}C NMR spectra, assignments were confirmed by *J*-modulated spin echo. All reactions were carried out under a positive argon atmosphere. All glassware was dried at 180 °C and cooled in a desiccator under argon. THF was distilled from CaH_2 before use, and CH_2Cl_2 was dried over P_2O_5. Wittig reactions run at −100 °C were cooled in 95% ethanol with liquid nitrogen and slowly warmed to 0 °C over 4 h. Solutions of reaction products were dried over anhydrous $MgSO_4$ for 5 min and concentrated. All reactions were monitored by TLC carried out on E. Merck 60F-254 silica gel plates. The purity (>97%) of key intermediates **4b**, **9**, and **12** was checked by GC. Microanalyses were performed by the Department of Chemistry for **4b**, **9**, and **12**; carbon and hydrogen results were within ±0.4% of theory.

Materials. D_2 was purchased from Air Liquide, (99%), $LiAlH_4$ from Fluka, and other chemicals from Aldrich Chemical Co. (3,3-Diisopropoxypropyl)triphenylphosphonium bromide (**8**) was prepared according to the method reported previously.[12]

5-Hexen-2-ynal Dimethyl Acetal (3b). To a 1.5 M solution of EtMgBr in THF (400 mL, 0.6 mol, 1.5 equiv) was added at 10 °C 1,1-dimethoxy-2-propyne (41.5 g, 0.41 mol, 1 equiv) in THF (60 mL). Then, the mixture was stirred at 35 °C for 5 h and cooled to 0 °C. After addition of CuCl (2 g, 20 mmol, 0.05 equiv) and stirring for 15 min, pure allyl bromide (71 mL, 0.82 mol, 2 equiv) was slowly added at 0 °C. The reaction mixture was warmed to 30 °C and maintained overnight at this temperature. Quenching at 0 °C with saturated NH_4Cl solution (100 mL), acidification to pH 7 with 1 N HCl, extraction with ether (3 × 60 mL), drying over $MgSO_4$, concentration, and distillation gave pure acetal **3b** (44.3 g, 0.31 mol, 77%) as a colorless liquid: bp 65 °C (25 mm); R_f 0.40 (silica, ether/pentane = 1/1); ^{1}H NMR δ 5.78 (ddt, J = 17.0, 9.9, 5.3 Hz, 1 H, H_2C=C*H*), 5.29 (br d, J = 17.0 Hz, 1 H, H_{cis}HC=CH), 5.14 (br s, 1 H, *H*C(OCH$_3$)$_2$), 5.10 (br d, J = 9.9 Hz, 1 H, H_{trans}HC=CH), 3.34 (s, 6 H, 2 × OC*H*$_3$), 3.01 (br d, J = 5.3 Hz, 2 H, C*H*$_2$); IR (film) 3105, 2800, 2775, 2310, 2285, 1650, 1480, 1430, 1370, 1350, 1160, 1100, 1060 cm^{-1}.

[2,2,3,3,5,6-$^{2}H_6$]-Hexanal Dimethyl Acetal (4b). A suspension of NaI (1.8 g, 100 w/w) in THF (65 mL) at −30 °C under vigorous stirring was saturated with D_2 by evacuating and filling with D_2 three times. Wilkinson catalyst (271 mg, 15% w/w) was added, and the flask was again evacuated and filled with D_2 three times. Warming at 30 °C allowed dissolution of catalyst and NaI. After being cooled to 0 °C, enyne **3b** (1.8 g, 12.8 mmol) was added via a syringe, and the reaction mixture warmed at 45 °C for 6 h (at 40 °C there was precipitation of NaCl). The dark brown mixture was concentrated, and pentane (20 mL) was added allowing precipitation of rhodium derivatives. Filtration through a column of silica gel (eluent ether/pentane = 1/10) gave pure hexadeuteriohexanal dimethyl acetal (**4b**) (1.65 g, 10.9 mmol, 85%) as colorless liquid: R_f 0.48 (silica, ether/pentane = 1/4); ^{1}H NMR δ 4.33 (br s, 1 H, *H*C(OCH$_3$)$_2$), 3.29 (s, 6 H, 2 × OC*H*$_3$), 1.24 (br s, 3 H, *H*CDC*H*$_2$), 0.84 (br s, 2 H, *H*$_2$CD); ^{13}C NMR δ 104.52 (s, 1 C, *C*(OCH$_3$)$_2$), 52.41 (s, 2 C, 2 × OCH$_3$)), 31.53 (quintet, J = 19.2 Hz, 1 C, CH$_2$*C*D$_2$), 31.30 (s, 1 C, *C*H$_2$), 23.28 (quintet, J = 19.2 Hz, 1 C, *C*D$_2$C(OCH$_3$)$_2$), 22.03 (t, J = 19.1 Hz, 1 C, *C*HD), 13.48 (t, J = 19.1 Hz, 1 C, *C*H$_2$D); IR (film) 2980, 2910, 2205, 2130, 1465, 1370, 1360, 1330, 1130, 1030 cm^{-1}; MS (EI) *m/e* (relative intensity) 121 (M$^+$ − OCH$_3$, 21), 89 (121 − CH$_3$OH, 8), 75 (CH(OCH$_3$), 100).

[2,2,3,3,5,6-$^{2}H_6$]-Hexanal (7). A solution of acetal **4b** (2.8 g, 18.4 mmol, 1 equiv), D_2O (9 mL), and camphorsulfonic acid (641 mg, 2.76 mmol, 0.15 equiv) in THF (150 mL) was refluxed for 0.5 h. After the solution was cooled to 0 °C, diluted with pentane (150 mL) and washed with brine (2 × 15 mL), the combined aqueous layers were extracted with ether (40 mL). All organic layers were dried, and solvents were slowly distilled. Then, benzene (25 mL) was added to the remaining solution and distilled. Crude hexanal **7** was transferred into a pear-shaped flask containing 4-Å molecular sieves and diluted with THF (5 mL) just before being used in the next step: ^{1}H NMR δ 9.76 (s, 1 H, C*H*O), 1.35 (br s, 3 H, *H*CDC*H*$_2$), 0.97 (br s, 2 H, *H*$_2$CD); ^{13}C NMR δ 202.73 (s, 1 C, *C*HO), 30.87 (s, 1 C, *C*H$_2$), 21.83 (t, J = 19.6 Hz, 1 C, *C*HD), 13.37 (t, J = 19.6 Hz, 1 C, *C*H$_2$D); IR (film) 2975, 2940, 2860, 2180, 1715 cm^{-1}.

[5,5,6,6,8,9-$^{2}H_6$]-*cis*-3-Nonenal Diisopropyl Acetal (9). To a suspension of phosphonium salt **8**[13] (21.7 g, 43.4 mmol, 2.3 equiv) in THF (290 mL) at −40 °C was added a 1 M THF solution of sodium bis(trimethylsilyl)amide (41 mL, 41 mmol, 2.2 equiv). The orange solution of ylide was stirred at rt for 4 h and cooled to −100 °C. A solution of deuterated hexanal **7** was added to the ylide. The pear-shaped flask was rinsed twice with THF (5 mL). After slow warming to 0 °C over 4 h, the reaction mixture was hydrolyzed with saturated NH_4Cl solution (20 mL) and water (20 mL), diluted with ether (100 mL), and washed with brine (10 mL). The aqueous layers were extracted with ether (2 × 100 mL), and the combined extracts were washed with brine (10 mL). All organic solutions were dried and concentrated giving a thick and dark oil. Two flash chromatographies over 70–230-mesh silica gel (eluent ether/pentane = 1/10) and 230–400-mesh silica gel (eluent ether/pentane = 1/50) led to pure nonenal diisopropyl acetal **9** (3.6 g, 14.5 mmol, 79%) as a colorless oil: R_f 0.81 (silica, ether/pentane = 1/4); ^{1}H NMR δ 5.47–5.29 (m, 2 H, C*H*=C*H*), 4.51 (t, J = 5.6 Hz, 1 H, *H*C(OiPr)$_2$), 3.84 (septet, J = 6.2 Hz, 2 H, 2 × C*H*(CH$_3$)$_2$), 2.31 (br t, J = 5.6 Hz, 2 H, C*H*$_2$CH(OiPr)$_2$), 1.23 (br s, 3 H, CHDC*H*$_2$), 1.16 (d, J = 6.2 Hz, 6 H, 2 × C*H*$_3$), 1.11 (d, J = 6.2 Hz, 6 H, 2 × C*H*$_3$), 0.83 (br s, 2 H, C*H*$_2$D); ^{13}C NMR δ 132.04 (s, 1 C, *C*HCH$_2$), 124.35 (s, 1 C, CD$_2$*C*H), 100.15 (s, 1 C, *C*(OiPr)$_2$), 67.79 (s, 2 C, 2 × *C*H(CH$_3$)$_2$), 33.81 (s, 1 C, CH$_2$*C*H(OiPr)$_2$), 31.20 (s, 1 C, *C*H$_2$CD$_2$), 23.42 (s, 2 C, 2 × *C*H$_3$),

22.60 (s, 2 C, 2 × CH_3), 22.10 (t, J = 19.1 Hz, 1 C, CHD), 13.70 (t, J = 19.1 Hz, 1 C, CH_2D); IR (film) 2980, 2920, 2190, 1470, 1380, 1370, 1330, 1130, 1030 cm^{-1}; MS (EI) m/e (relative intensity) 189 (M^+ - $OCH(CH_3)_2$, 3), 147 (189 - C_3H_6, 3), 43 (C_3H_7, 100).

Methyl 8-(Triphenylphosphonio)nonanoate Bromide (11). Following the cited reference,[14] azelaic monomethyl ester (12.8 g, 63.3 mmol) afforded phosphonium salt 11 as a thick colorless oil (15 g, 29.2 mmol, 46%).

[14,14,15,15,17,18-$^2H_6$]-Linoleic Acid (12). Phosphonium salt 11 (8.77 g, 17.1 mmol, 1.8 equiv) was stirred and heated at 120–130 °C for 3 h under vacuum and then dissolved in THF (160 mL). To this solution at −40 °C was added a 1 M THF solution of sodium bis(trimethylsilyl)amide (15 mL, 15 mmol). The resulting orange solution was stirred at 0 °C for 1 h and at rt for 2 h. Simultaneously, acetal 9 (2.30 g, 9.30 mmol, 1 equiv) was hydrolyzed[13] to the corresponding crude aldehyde 10 which was dissolved in THF (3 mL) and added to the ylide solution at −100 °C. The pear-shaped flask was rinsed twice with THF (2 × 1 mL). After hydrolysis with 1 N HCl until pH 1 and workup as described in the first Wittig reaction, the mixture was chromatographed on silica gel (70–230 mesh, eluent ether/pentane = 1/10) giving crude methyl hexadeuterated linoleate (2.66 g, R_f 0.71, silica, ether/pentane = 1/4) which was dissolved in THF (58 mL). To this solution at 0 °C was added LiOH (0.5 M aqueous solution, 40 mL). The mixture was stirred for 15 h, acidified to pH 1, saturated with solid NaCl, and extracted with 1:1 ether/pentane (3 × 50 mL). Washing with brine (2 × 15 mL), drying, concentration, and chromatography on silica gel (230–400 mesh, eluent ether/pentane = 1/10–3/1) gave pure hexadeuterated linoleic acid 12 (1.92 g, 6.71 mmol, 72%) as a pale yellow oil: R_f 0.15 (silica, ether/pentane = 1/4).

Data for [5,5,6,6,8,9-$^2H_6$]-*cis*-3-nonenal (10): ^1H NMR δ 9.61 (s, 1 H, HCO), 5.69–5.37 (m, 2 H, CH=CH), 3.14 (d, J = 7.0 Hz, 2 H, CH_2CHO), 1.23 (br s, 3 H, $CHDCH_2$), 0.83 (br s, 2 H, CH_2D); ^{13}C NMR δ 199.61 (s, 1 C, CHO), 135.45 (s, 1 C, $CHCH_2$), 118.01 (s, 1 C, CD_2CH), 42.58 (s, 1 C, CH_2CHO), 31.21 (s, 1 C, CH_2CD_2), 22.01 (t, J = 19.1 Hz, 1 C, CHD), 13.62 (t, J = 19.1 Hz, 1 C, CH_2D); IR (film) 2990, 2930, 2860, 2200, 2190, 1735, 1700, 1440, 1390, 1355 cm^{-1}.

Data for hexadeuteriated linoleic acid 12: ^1H NMR δ 5.43–5.24 (m, 4 H, ethylenic), 2.75 (m, 2 H, malonic), 2.32 (t, J = 7.4 Hz, 2 H, CH_2COOH), 2.04–2.01 (m, 2 H, allylic), 1.64–1.57 (m, 2 H, CH_2CH_2COOH), 1.28 (br s, 8 H, $(CH_2)_4$), 1.24 (br s, 3 H, $CHDCH_2$), 0.84 (br s, 2 H, CH_2D); ^{13}C NMR δ 180.28 (s, 1 C, COOH), 130.17 (s, 1 C, ethylenic), 130.06 (s, 1 C, ethylenic), 128.24 (s, 1 C, ethylenic), 128.08 (s, 1 C, ethylenic), 34.19 (s, 1 C, CH_2), 31.26 (s, 1 C, CH_2), 29.68 (s, 1 C, CH_2), 29.16 (s, 3 C, 3 × CH_2), 27.28 (s, 1 C, CH_2), 25.77 (s, 1 C, CH_2), 24.77 (s, 1 C, CH_2), 22.11 (t, J = 19.0 Hz, 1 C, CHD), 13.64 (t, J = 19.1 Hz, 1 C, CH_2D); IR (film) 3400, 3050, 2915, 2830, 2220, 1740, 1650 cm^{-1}; MS (EI) m/e (relative intensity), 287 (M + 1, 28), 286 (M, 100), 285 (M − 1, 20); MS of natural linoleic acid 281 (M + 1, 26), 280 (M, 100), 279 (M − 1, 10).

Chemical Purification of Linoleic Acid-d_6 (12). A solution of linoleic acid 12 containing diene acid 14 (ratio 12/14 = 88/12, 3.86 g, 13.5 mmol, 1 equiv) and maleic anhydride (5.2 g, 54 mmol, 4 equiv) was refluxed in xylene (27 mL). Reaction progress was checked by the disappearance of the UV-active spot on TLC. After 2 h, the solution was cooled to rt and diluted with ether (40 mL). Hydrolysis with water (30 mL), addition of solid LiOH until basic pH, and acificiation to pH 3 with HCl (1 N) gave a mixture which was extracted with ether (3 × 60 mL) and washed with 50% saturated NaCl aqueous solution (2 × 10 mL). The crude product was purified by chromatography on silica gel (70–230 mesh, eluent: ether/pentane = 1/1–4/1) giving pure acid 12 (3.05 g, 10.6 mmol, 79%).

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