Synthesis of *all-trans*-Parinaric Acid-d_8 Specifically Deuteriated at All Vinyl Positions†

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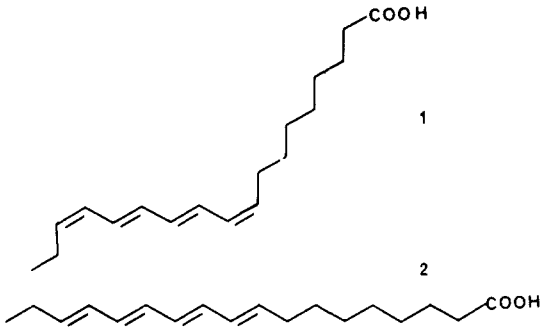
Parinaric acid is a widely used fluorescent probe of biological systems. The all-trans isomer (9(*E*),11(*E*),13(*E*),15(*E*)-octadecatetraenoic acid) specifically deuteriated at all vinyl positions was prepared by using the Wittig reaction to couple a diene phosphorane with an α,β-unsaturated aldehyde–ester. The preparation of each component included the stereoselective reduction of a substituted propynoic ester with lithium aluminum deuteride (LAD), introducing the trans double bond as well as most of the deuterium in one step, in high isotopic purity without unwanted hydrogen–deuterium exchange. By inclusion of deuterium, the probe can be used with other techniques, such as deuterium NMR and neutron diffraction, further increasing its utility. The synthesis can be used to prepare other tetraenes by correct choice of starting propynoic esters.

Introduction

Parinaric acid (9,11,13,15-octadecatetraenoic acid, PnA) is a naturally occuring tetraene fatty acid first isolated in 1933.[1] The naturally occurring form, designated α-parinaric acid (**1**), can be isomerized to a higher melting form called β-parinaric acid (**2**). PnA was shown to include the tetraene moiety as early as 1935[2] but the all-trans structure of **2** was not deduced until 1959.[3] It required an additional 6 years to determine the cis,trans,trans,cis structure of **1**.[4]



A recent NMR study of both isomers further confirmed the assigned structures.[5] During the preparation of this manuscript the first synthesis of **2** was reported.[6]

In 1975 Sklar, Hudson, and Simoni introduced the use of parinaric acid as a fluorescent membrane probe.[7] In that and following papers[8] they demonstrated that PnA could be used to detect phase transitions in bilayers as well as interactions between lipids and proteins. The probe was biosynthetically incorporated into phospholipids and a thorough spectroscopic characterization has been performed.[8]

Since then parinaric acid has become a widely used membrane probe and is now commercially available.[9] Both isomers are structurally similar to naturally occurring membrane lipids. As a result, the orientation and location of the chromophore relative to the membrane is known and disruptions due to the probe should be minor. Fluorescence techniques are useful for measuring events in the nanosecond time scale. By incorporating deuterium into the tetraene chromophore, it should be possible to use this probe in other experiments, such as deuterium NMR,[10] allowing the measurement of phenomena in the microsecond time scale, or in neutron diffraction studies where the concentration of deuterium in a known location can serve as a marker in such studies.[11] Additional information about the dynamics of membrane behavior as well as the interactions between proteins and lipids can be obtained by using other tetraenes, e.g. with the chromophore in the middle and near the head group. This synthesis provides a general method to prepare these isomers, both deuteriated and protonated as desired.

Results and Discussion

Bergelson[12] had shown that α,β-unsaturated phosphoranes gave predominantly the trans isomer when condensed with aldehydes in the Wittig reaction. Preliminary experiments showed that the aliphatic "tail" portion would be better to use as the Wittig salt since the salts were crystalline, in contrast to attempts to make the salt from the carboxylic "head" end which gave only oils. The general method used here for preparing tetraenes involved a diene phosphorane (eq 1). This use of a diene phosphorane was expected to give more of the desired trans double bond than the monoene phosphorane. It was also more stable in air than the triene Wittig salts (a = 0, 1) which rapidly turned oily black when exposed to air while

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(3) Kaufmann, H. P.; Sud, R. K. *Chem. Ber.* **1959**, *92*, 2797.
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(7) Sklar, L. A.; Hudson, B. S.; Simoni, R. D. *Proc. Natl. Acad. Sci. U.S.A.* **1975**, *72*, 1649.
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(9) Both α- and β-parinaric acid can be purchased from Molecular Probes, 4849 Pitchford Ave., Eugene, OR 97402.
(10) Paddy, M. R.; Dahlquist, F. W.; Davis, J. H.; Bloom, M. *Biochemistry* **1981**, *20*, 2657.
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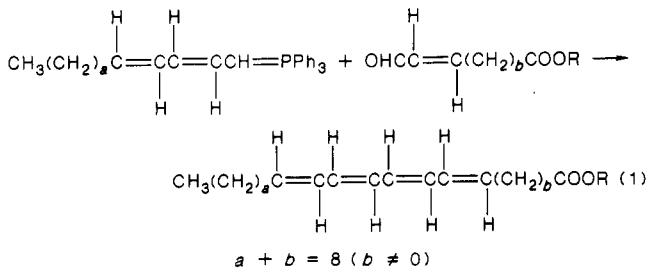
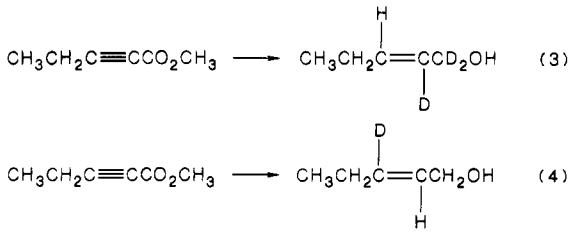


damp with various solvents (hexane, ether, benzene, DMF, acetone). The intermediate triene halides needed for the Wittig salts were also much more labile than the diene analogues. The preparations of each of the two reactants used in eq 1 are outlined in Schemes I and II.

Reduction of methyl 2-propynoate with LAD in tetrahydrofuran (THF) followed by D_2O quench cleanly[13] gave the tetradeuteriated trans alcohol **3** as shown by the absence of an ^1H NMR signal from 7 to 3 ppm, no ^2H NMR signal from 3 to 0 ppm (no label scrambling to the allylic methylene carbon), and a strong IR band at 720 cm^{-1} consistent with a trans CD=CD structure.[14] When the reaction was performed at −78 °C followed by H_2O quench, only the dideuteriated propynol was obtained (eq 2). This

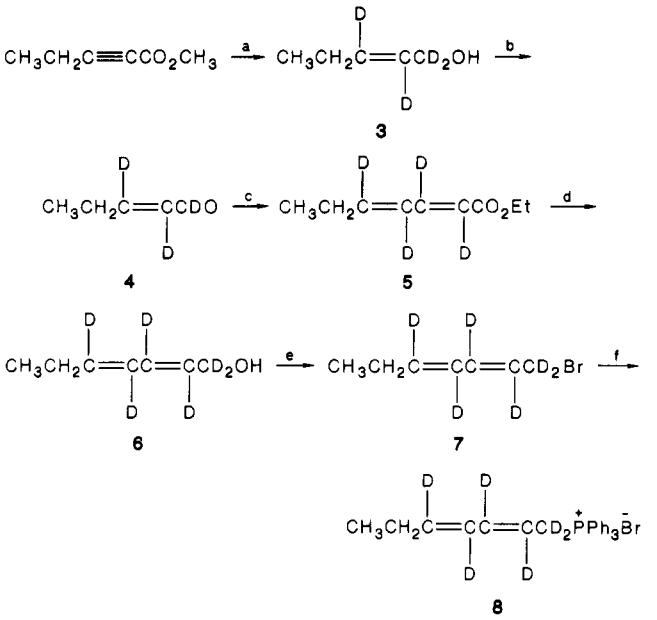
$$CH_3CH_2C{\equiv}CCO_2CH_3 \rightarrow CH_3CH_2C{\equiv}CCD_2OH \quad (2)$$

allows the deuteriation of double bonds at specific positions by the appropriate choice of reduction conditions. The possibility of deuteriating individual vinyl carbons as desired was also briefly examined. The reduction was repeated in refluxing THF using LAD with H_2O quench and lithium aluminum hydride (LAH) with D_2O quench (eq 3 and 4). Although the products shown were predominant,



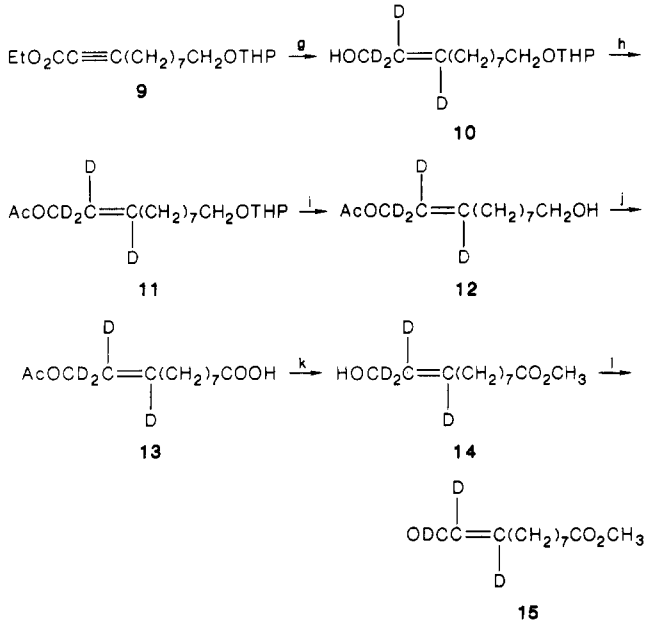
there were detectible amounts (by NMR) of product arising from hydride or deuteride delivery to C-3. In earlier studies on the reduction of substituted 2-propyn-1-ols, Corey[15] and co-workers encountered unexplained variations in the amount of C-2 vs C-3 hydride reduction products. They stated that the desired compounds could be cleanly obtained by addition of aluminum trichloride or sodium methoxide, depending upon which substance was wanted. These modifications were not tried but might lead to further control in the placement of deuterium within the tetraene.

Scheme I[a]



[a] (a) LAD, THF, 65 °C, 12 h; D_2O, 3 °C; H_2O; (b) PCC, CH_2Cl_2, room temperature, 3 h; (c) $(Et_2O)_2POCD_2CO_2Et$, NaH, THF, 0 °C, 1 h; (d) DIBAL-D, Et_2O, −70 → 0 °C, 2 h; (e) PBr_3, Et_2O, −70 °C → room temperature, 3.5 h; (f) Ph_3P, benzene, room temperature, 12 h.

Scheme II[a]



[a] (g) LAD, THF, 65 °C; D_2O; H_2O; (h) Ac_2O, Py, room temperature, 4 h; (i) PPTS, EtOH, 40–50 °C, 4–5 h; (j) CrO_3, H_2SO_4, acetone, 0 °C; 3 h; (k) 3% HCl, MeOH, 56 °C, 4 h; (l) PCC, CH_2Cl_2, room temperature, 1 h.

Oxidation of **3** to aldehyde **4** went in acceptable yield when using pyridinium chlorochromate (PCC). The protonated analogue was oxidized better by using activated manganese dioxide, but a large deuterium isotope effect[16] led to drastically reduced yields, probably due to the greatly increased amounts of reagent (and therefore sol-

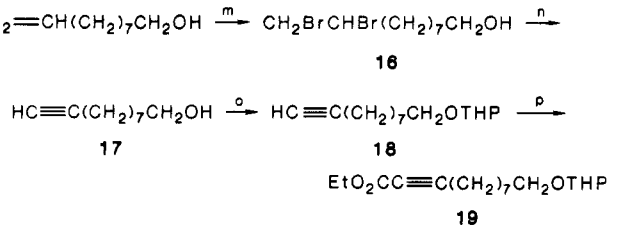
(13) Mass spectral fragmentation patterns have been used in many cases to determine isotopic purity of deuterium-labeled compounds. Due to the complexity of the polyene mass spectra this method was not employed. Both proton and deuterium NMR spectra were recorded for all intermediates as well as the final product. In all cases, the expected spectra were obtained, both in regards to peak shape and chemical shift and relative integrations. The absence of any proton signal in the regions associated with aldehyde, double bonds, or allylic alcohols, plus the presence of the above-mentioned signals in the deuterium NMR spectra, leads us to believe that deuterium purity approaches that of the reagents used to incorporate the deuterium, in this case at least 98% for LAD and LiD used to make DIBAL-D (from Aldrich), 99% for D_2O (from Cambridge Labs.). The absence of any signal in the vicinity of 2 ppm in the deuterium NMR spectra indicates that label scrambling (e.g., due to enolization during PCC oxidation) is not a significant problem.

(14) Johansen, J. E.; Liaaen-Jensen, S. *Acta. Chem. Scand., Ser. B* **1974**, *28*, 306.

(15) Corey, E. J.; Katzenellenbogen, J. A.; Posner, G. H. *J. Am. Chem. Soc.* **1967**, *89*, 4245.

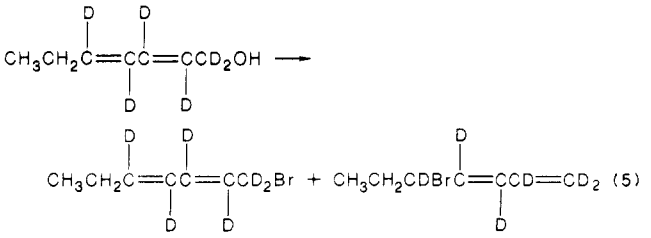
(16) For examples of unusually large isotope effects with MnO_2, see: Goldman, I. M. *J. Org. Chem.*s **1969**, *34*, 3289. Freeman, F.; Grant, J. B.; Hester, N. B.; Kamego, A. A.; Kasner, M. L.; Mc Laughlin, T. G.; Paull, E. W. *J. Org. Chem.* **1970**, *35*, 982.

Scheme III[a]



[a] (m) Br_2, CCl_4, −5 °C; (n) $NaNH_2$, DMSO, 0 °C → room temperature, 12 h; (o) DHP, PPTS, CH_2Cl_2, room temperature, 4 h; (p) *n*BuLi, THF, −78 °C; EtO_2CCl, THF, −78 °C → room temperature, 12 h.

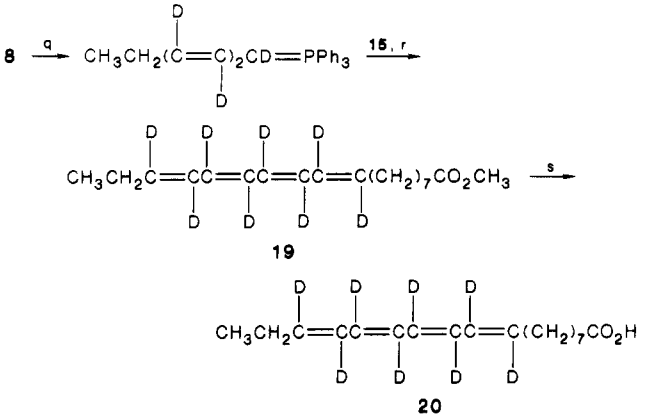
vent) needed for complete reaction of the starting alcohol, leading to losses of the volatile aldehyde during isolation. Deuteriated triethyl phosphonoacetate, prepared in 70% yield from deuteriated acetic acid, was condensed with **4** to give the all-trans diene ester **5**. The ester was reduced to the hexadeuteriated alcohol **6** in 85% yield with diisobutylaluminum deuteride (DIBAL-D).[17] Alternatively, LAD gave **6** in 74% yield.[18] Treatment of **6** with phosphorous tribromide (PBr_3) gave the bromide **7** along with the secondary isomer in a ratio of 3:1 (eq 5). Other bro-



minating agents (Ph_3PBr_2, CBr_4/Ph_3P, methanesulfonyl chloride (MsCl)/LiBr) gave results similar to or worse (MsCl/LiBr) than those of PBr_3. It was not necessary to separate the isomers as only the primary phosphonium salt **8** was formed when the mixture was treated with triphenylphosphine at room temperature. When the protonated analogue was used, the residual secondary bromide could be partially converted to the primary Wittig salt by heating at reflux overnight, probably via an S_N2' mechanism.[19] However, similar treatment of the deuteriated secondary bromide led only to decomposition of the substance.

Preparation of the "head" portion began with the LAD reduction of the substituted propynoic ester **9**, prepared as shown in Scheme III. Experiments with LAD and LAH gave results similar to those observed for methyl 2-propynoate. Reduction of **9** with LAD gave only the trans alcohol **10**, which was protected as the acetate **11**. The tetrahydropyranyloxy (THP) ethers **9–11** decomposed upon attempted distillation but could be purified in 5-g batches by using flash chromatography.[20] Removal of the

Scheme IV[a]



[a] (q) *n*BuLi, THF, room temperature, 1 h; (r) THF, 4 h; (s) 5% KOH, MeOH, 56 °C, 2.5 h.

THP group followed by Jones oxidation of the unmasked alcohol gave the acid–acetate **13**, which was esterified and deprotected in one step with acidic methanol. The ω-hydroxy ester **14** was oxidized with PCC to give the required α,β-unsaturated aldehyde.[16]

Due to the extreme sensitivity of the tetraene to light and air, the final coupling reaction and subsequent hydrolysis and purifications were performed under red light in an argon atmosphere. All solvents were thoroughly degassed with argon and all manipulations (including NMR, UV, and melting point sample preparations) were done in a glovebag. The preparation is outlined in Scheme IV.

Wittig salt **8**, suspended in THF, was treated with *n*-butyllithium to give a red solution of the phosphorane. This solution was then treated with a THF solution of the aldehyde **15** to give the tetraene ester **19** in 77% yield after flash chromatography. The ester was heated in refluxing methanol containing 5% KOH for 2.5 h, and then purified by extractive workup followed by flash chromatography to give the acid in 85% yield. Samples were recrystallized from hexane to give fine white needles (mp 96–97 °C). The acid was best stored as a dilute solution containing BHT at −20 °C.

Conclusions

The method described was used to prepare parinaric acid specifically deuteriated at all vinyl positions. By beginning with the proper substituted propynoic esters, tetraene fatty acids containing the chromophore in other parts of the lipid chain should also be accessible. Attractive features of this synthesis include the incorporation of deuterium with high isotopic purity without label scrambling as well as excellent stereoselectivity in the introduction of the double bonds. The method compares favorably with the other published synthesis of parinaric acid[6] in that no separation of regioisomers is required.

Experimental Section

Melting points were determined on a Mel-Temp apparatus and are uncorrected. Infrared spectra were run on Sargent-Welch SP-200 or Beckman 4240 instruments as thin films on KBr plates unless otherwise noted. Proton NMR spectra were obtained on a Varian XL-100 spectrophotometer in deuteriochloroform solutions. Chemical shifts are reported in ppm (δ) downfield from Me_4Si. In reporting the NMR spectra, the following notations are used: s = singlet, d = doublet, t = triplet, q = quartet, m =

(17) Prepared from diisobutylaluminum chloride and lithium deuteride in 92% yield by using the following methods: Ziegler, K.; Gellert, H. G.; Martin, H.; Nagel, K.; Schneider, J. *Liebigs Ann. Chem.* **1954**, *589*, 91. Ziegler, K.; Lehmkul, H. *Method. Org. Chem.* **1970**, *13/4*, 57.

(18) The high yield obtained by using LAD was unique to this polyene. In other experiments using LAH and LAD to reduce the methyl and ethyl esters of crotonic, 2-pentenoic, sorbic, and 2,4,6-octatrienoic acids yields did not exceed 50% and isolation was complicated by polymeric mixtures. For other examples of difficulties encountered with unbranched α,β-unsaturated systems, see: Bohlman, F.; Ahrens, H. L.; Kritzler, H. *Abh. Braunschw. Wiss. Ges.* **1957**, *9*, 173; *Chem. Abstr.* **1958**, *52*, 10874i. Muramatsu, H.; Tajima, K.; Iwahaski, M.; Masumoto, K.; Horiuchi, T. *J. Labelled Compds.* **1972**, *8*, 305. Martin, C. J.; Schepartz, A. I.; Daubert, B. F. *J. Am. Chem. Soc.* **1948**, *70*, 2601.

(19) March, J. *Advanced Organic Chemistry*; McGraw-Hill Inc.; New York, 1977; pp 303–7.

(20) Still, W. C.; Kahn, M.; Mita, A. *J. Org. Chem.* 1978, *43*, 2480.

multiplet, and b = broad. Deuterium NMR spectra were recorded on a Nicolet 360-MHz spectrophotometer by Dr. Charles Klopfenstein as solutions in chloroform with deuteriochloroform added as an internal reference at 7.27 ppm unless otherwise noted. Resolution was such that the coupling constants were not determined. As a result, ^{2}H NMR are reported as chemical shift followed by the number of deuterium. Mass spectra were recorded by Dr. Richard Wielesek of the University of Oregon.

All solvents used were commercial reagent grade. Diethyl ether (Et_2O) and THF were dried by distillation from either LAH or sodium benzophenone ketyl before use. Dichloromethane and CCl_4 were dried by standing over P_2O_5 followed by distillation from CaH_2 before use. Up to the point that the tetraene was made, all reactions were run under a nitrogen atmosphere as were the solvent distillations. At this point all solvents used were degassed by passing a stream of argon through them for at least 1 half-hour, except for THF and Et_2O which were refluxed over LAH under an argon atmosphere for at least 1 half-hour prior to distillation.

The reactions used to prepare the Wittig reagent were followed by recording NMR spectra of aliquots taken at different intervals. In the preparation of the head portion of the molecule, reactions were followed by thin layer chromatography on silica gel strips eluted with a solution of hexane–ethyl acetate (2:1) and developed by iodine vapor, unless otherwise noted. Flash chromatography was done using ca. 10 cm of Baker silica gel in columns with diameters which were dependent upon sample size. For samples greater than 1 g, a 50-mm diameter column was typical; while 25-mm was standard for smaller sample sizes. Samples were eluted with hexane–ethyl acetate (2:1) solutions unless otherwise noted. Pressure was applied by using nitrogen but was not carefully monitored since good separations were achieved over a range of flow rates at the solvent head of 1–3 in. per minute. Unless otherwise noted, solutions were concentrated by evaporation on a rotary evaporator at ca. 15–25-mm vacuum. None of the deuteriated compounds appear to have been made before. Literature references cited for physical constants are for the analogous protonated compounds.

2(*E*)-Penten-1-ol-*1,1,2,3-d*$_4$ (3). A 500-mL round-bottom flask equipped with a mechanical stirrer and addition funnel was charged with THF (200 mL) and LAD (Aldrich, 4.98 g, 0.119 mol). The flask was immersed in an ice bath and a solution of methyl 2-pentynoate (13.30 g, 0.119 mol) in THF (30 mL) was slowly added to the rapidly stirred suspension. When the addition was finished (ca. 20 min), the ice bath was removed and the flask allowed to warm to room temperature. A warm water bath (~50 °C) was applied and the solution was stirred for 22 h. An ice bath was then applied and 8 mL of D_2O was dripped in over 5 min. The solution was stirred for an additional 30 min and then 15 mL of a 15% sodium hydroxide solution was added to the flask with rapid stirring, which was continued until the precipitate became granular. The solution was filtered and the precipitate was washed with Et_2O (5 × 20 mL). The combined organic solutions were dried over $MgSO_4$ and the solvent was then removed by fractional distillation. The product was distilled to give **3** (6.87 g, 0.076 mol, 64%): bp 55–56 °C (26 mm) [lit.[21] bp 45–46 °C (15 mm)]; ^{1}H NMR 2.05 (b, q, J = 7 Hz, 2 H), 1.62 (b s, 1 H), 1.01 (t, J = 7 Hz, 3 H); ^{2}H NMR 5.70 (1 ^{2}H), 5.58 (1 ^{2}H), 3.97 (2 ^{2}H); MS, m/e 90; IR 3615, 3315, 2960, 2915, 2875, 2845, 2460, 2205, 2080, 1640, 1465, 1380, 1320, 1270, 1180, 1130, 1075, 1015, 965, 950, 910, 855, 800, 775, 715 cm^{-1}.

2(*E*)-Pentenal-*1,2,3-d*$_3$ (4). A 250-mL round-bottom flask was charged with PCC (14.8 g, 68.6 mmol), anhydrous sodium acetate (.562 g, 6.86 mmol), Celite (14 g), and CH_2Cl_2 (50 mL). The flask was cooled with an ice bath while a solution of **3** (3.09 g, 34.3 mmol) in CH_2Cl_2 (30 mL) was added over 15 min. The solution was stirred for an additional 3 h, while warming to room temperature, until the reaction was finished (GC). The sample was then filtered, the flask was rinsed with Et_2O (4 × 15 mL), and the rinses were poured over the collected precipitate. The combined organic solutions were passed thru a short silica column to give a water-clear liquid. The solvent was carefully distilled off at atmospheric pressure and then the product was distilled to give **4** (1.26 g, 14.5 mmol, 95% pure by GC, 40% yield): bp 66–67 °C (100 mm) [lit.[21] bp 54 °C (60 mm)]; ^{1}H NMR 2.37 (b q, J = 7 Hz, 2 H), 1.13, (t, J = 7 Hz, 3 H); ^{2}H NMR 9.48 (1 ^{2}H), 6.92 (1 ^{2}H), 5.11 (1 ^{2}H), IR ($CHCl_3$) 2970, 2935, 2875, 2085, 1665, 1600, 1455, 1410, 1375, 1315, 1180, 1130, 1080, 905, 860, 700, 645 cm^{-1}.

Preparation of Triethyl Phosphonoacetate-*d*$_2$. A flask was charged with technical grade red phosphorous (4.0 g, 0.13 mol) and acetic acid-d_4 (20 g, 0.31 mol). It was then cooled in an ice bath while Br_2 (30 mL, d = 3.12, 0.58 mol) was slowly added. When the addition was finished, the ice bath was removed, the addition funnel was replaced with a condenser, and an oil bath was applied. The bath was heated to 73 °C and held there for 6 h. The sample was then fractionally distilled to give bromoacetyl bromide-d_2 (51.50 g, 0.253 mol, 81% based on acetic acid-d_4), bp 147–149 °C [lit.[22] bp 147–150 °C]. An additional 3 g of material was collected from 150–170 °C.

Bromoacetyl bromide-d_2 (51.5 g, 0.253 mol) was added to ice-cold EtOD (45 mL, 0.75 mol) over 1.5 h. After the addition was finished the ice bath was removed and the solution was allowed to stir overnight while being warmed to room temperature. Most of the EtOD was then distilled off at aspirator vacuum and the residue was washed with D_2O and D_2O saturated with sodium chloride and then distilled to give ethyl bromoacetate-d_2 (35.75 g, 0.212 mol, 84% based on bromoacetyl bromide). The 3 g of crude bromoacetyl bromide-d_2 (bp 150–170 °C) was mixed with EtOD to rinse the flasks and then worked up and distilled to give an additional 2.70 g of product for a total of 0.228 mol (90%).

A 250-mL round-bottom flask with addition funnel and condenser was charged with triethyl phosphite (65 mL, 0.31 mol). The combined samples of ethyl bromoacetate d_2 were poured into the addition funnel, the flask was rinsed with 7 mL of triethyl phosphite, and the ester was then added in three portions to the reaction flask. Each addition caused the solution to reflux vigorously. Once the solution had stopped boiling after the final addition, the addition funnel and condenser were replaced by a distillation head. The sample was heated up to 80 °C at atmospheric pressure and then distilled at reduced pressure to give the desired phosphonoacetate **12** (48.73 g, 0.215 mol, 93% yield), bp 112 °C (2.8 mm) [lit.[22] 142–145 °C (9 mm)]. This gave a total yield of 69% for the three steps based on the starting acetic acid-d_4.

Ethyl 2(*E*)-Heptadienoate-*2,3,4,5-d*$_4$ (5). A 50-mL round-bottom flask was charged with sodium hydride (60% in mineral oil, 1.61 g, 40 mmol) and THF (40 mL). The flask was immersed in an ice bath and a solution of **12** (9.32 g, 42 mmol) in THF (40 mL) was added over 40 min. The resulting transparent, light brown solution was stirred for an additional 50 min. A solution of **4** (3.25 g, 37.3 mmol) in THF (50 mL) was added over 15 min. After being stirred an additional hour, the water-clear solution, containing a small amount of brown gel, was worked up by adding 30 mL of ice water to the solution, followed by separation of the two phases. The aqueous layer was extracted with pentane (2 × 25 mL) and the combined organic layers were dried with Na_2SO_4. Solvent was removed and the residue was distilled to give **5** (4.11 g, 26 mmol, 72%): bp 107 °C; ^{1}H NMR 4.19 (q, J = 7 Hz, 2 H), 2.18 (b q, J = 7 Hz, 2 H), 1.29 (t, J = 7 Hz, 3 H), 1.05 (t, J = 7 Hz, 3 H); ^{2}H NMR (acetone-d_6, internal standard 2.17 ppm) 7.33 (1 ^{2}H), 6.24 (2 ^{2}H), 5.86 (1 ^{2}H); MS, m/e 158; UV λ_{max} 258 nm (log ϵ 4.3); IR 2970, 2930, 2875, 2220, 1700, 1605, 1580, 1460, 1390, 1370, 1300, 1250, 1170, 1115, 1090, 1040, 885, 855 cm^{-1}.

Preparation of Diisobutylaluminum Deuteride (DIBAL-D). A flame-dried 500-mL flask filled with argon was charged with 150 mL of Et_2O and 87.5 g (0.50 mol) of diisobutylaluminum chloride (pyrophoric liquid!) while being cooled in an ice bath. Lithium deuteride (5.0 g, 0.56 mol) was added in ca. 1-g portions with rapid stirring. The solution was then allowed to warm to room temperature during which time the gray particles were slowly replaced by a white precipitate. The solution, after centrifugation to remove particles, gave a positive test for chloride. It was then refluxed overnight while stirring. The chloride test was repeated and only a faint trace was detected. The sample was filtered under argon to remove lithium chloride and then stripped of solvent, first at room temperature with an aspirator vacuum and then at

(21) Jaenicke, L.; Seferiadis, K. *Chem. Ber.* **1975**, *108*, 225.

(22) Aldrich Chemical Company catalogue.

40 °C. Additional solid formed during this process but was not removed from the flask. The sample was then distilled to give DIBAL-D (64.47 g, 0.45 mol, 90%), bp 120 °C (1 mm) [lit.[23] bp 140 °C (4 mm)].

2(*E*),4(*E*)-Heptadien-1-ol-*1,1,2,3,4,5-d*$_6$ (6). A 250-mL round-bottom flask was charged with a solution of **5** (3.11 g, 26 mmol) in Et_2O (50 mL) and then cooled with a dry ice–acetone bath. A 50-mL solution of 1.5 M DIBAL-D in Et_2O was added over 30 min and then the solution was allowed to warm to 0 °C over 2 h. The reaction was quenched by slowly adding 2 M NH_4Cl (aq) solution until gas evolution stopped. The reaction mixture became thick with gelatinous material. This was separated from the Et_2O, dissolved with 2 M H_2SO_4, and then extracted with Et_2O (4 × 20 mL). Ether layers were combined, then washed (H_2O, 5% $NaHCO_3$, brine), and dried with $MgSO_4$, and solvent was removed without using a water bath. The sample was then distilled (bulb to bulb) to give **6** (2.48 g, 22 mmol, 81%): bp 105 °C (22 mm) [lit.[24] bp 94–95 °C (15 mm); ^{1}H NMR 2.10 (b q, J = 7 Hz, 2 H), 1.52 (b s, 1 H), 1.01 (t, J = 7 Hz, 3 H); ^{2}H NMR 6.20 (1 ^{2}H), 6.03 (1 ^{2}H), 5.75 (2 ^{2}H), 4.07 (2 ^{2}H).

1-Bromo-2(*E*),4(*E*)-heptadiene-*1,1,2,3,4,5-d*$_6$ (7). A 25-mL round-bottom flask was charged with PBr_3 (1.23 g, 4.52 mmol) and then cooled with dry ice–acetone. A solution of **6** (1.28 g, 10.8 mmol) in Et_2O (10 mL) was added over 20 min. The solution was allowed to gradually warm to −25 °C during the next 30 min, at which point the cooling bath was removed. It was stirred an additional 3.5 h and then poured into separatory funnel containing ice and solid $NaHCO_3$ (2 g). The flask was rinsed with Et_2O (2 × 15 mL) and the rinses were added to the separatory funnel. The mixture was shaken until the ice had melted and then separated. The aqueous layer was extracted with Et_2O (2 × 15 mL), and the organic layers were combined, washed with brine, and dried with $NaSO_4$. A small aliquot was removed and concentrated. The resulting pale yellow liquid was taken up in deuteriochloroform for ^{1}H NMR (2.07, br quint; 1.0, t; 1.2, d; 1.5–1.8, m).

2(*E*),4(*E*)-Heptadienyltriphenylphosphonium Bromide-*1,1,2,3,4,5-d*$_6$ (8). The remaining sample was stripped of most solvent, then dissolved in a solution of triphenylphosphine (5.2 g, 29 mmol) in benzene (10 mL), and stirred under nitrogen at room temperature. Precipitate formed, though noticably slower than when the hydrogen analogue was used. The sample was stirred for 2 days and then filtered. The solid was washed successively with benzene and Et_2O to give a white powder (2.49 g, 5.6 mmol, 52% crude yield from **6**) which had the expected NMR, IR, and UV spectra. A small portion was recrystallized to give white needles (CH_3CN:ethyl acetate): mp 189–190 °C; ^{1}H NMR 7.74 (m, 15 H), 2.02 (dq, J = 7, 3 Hz, 2 H), 0.93 (t, J = 7 Hz, 3 H); ^{2}H NMR m, 6.4–4.2; IR 3350, 3040, 2415, 2200, 2090, 1960, 1890, 1810, 1760, 1705, 1610, 1585, 1480, 1440, 1430, 1370, 1330, 1310, 1105, 990, 810, 670, 650; UV λ_{max} 225 nm (log ϵ 4.5), 250 sh (4.2).

9,10-Dibromodecan-1-ol (16). A 500-mL round-bottom flask was charged with CCl_4 (245 mL) and 9-decen-1-ol (76.5 g, 0.490 mol) and then cooled with an ice–salt bath. A solution of Br_2 (25 mL, 0.49 mol) in CCl_4 (45 mL) was added slowly so that the pot temperature was less than −5 °C throughout the addition. The sample was stirred for an additional hour and then the Br_2 and CCl_4 were distilled off with the aid of an aspirator vacuum. The residue was then distilled to give **16** (147 g, 0.466 mol, 95%): bp 158 °C (1.2 mm); ^{1}H NMR 4.32–4.02 (m, 1 H), 3.96–3.44 (m, 4 H), 2.30–1.14 (m, 15 H); IR 3340, 2930, 2850, 1465, 1435, 1230, 1145, 1055, 725, 645 cm^{-1}.

9-Decyn-1-ol (17).[25] A 2-L three-neck flask, equipped with thermometer, mechanical stirrer, and addition funnel, was charged with DMSO (1300 mL) and sodium amide (90 g, 2.3 mol). A solution of **16** (92.0 g, 0.291 mol) in DMSO (150 mL) was added to the ice bath cooled flask at a rate such that the temperature remained below +20 °C throughout the addition. The bath was then removed and the reaction was stirred overnight. The mixture was worked up in four portions by pouring each portion into a 2-L separatory funnel containing 1 L of ice water, extracting the aqueous layer with 3 × 100 mL of Et_2O, and then combining all organic layers. The mixture was washed with 200 mL of water and 200 mL of brine and then dried with $MgSO_4$. The solution was concentrated and the residue was fractionally distilled to give **17** (30.6 g, 0.198 mol, 68%), one spot by TLC, with no evidence of alkene, allene, or internal triple bonds by IR or NMR: bp 91–92 °C (0.9 mm); ^{1}H NMR 3.58 (t, J = 7 Hz, 2 H), 2.12 (bdt, J = 2 Hz, 7 Hz, 2 H), 1.98 (s, 1 H), 1.87 (t, J = 7 Hz, 1 H), 1.67–1.08 (m, 12 H); IR 3300, 2940, 2850, 2110, 1480, 1450, 1345, 1155, 1070, 895, 735, 640 cm$^{2-1}$.

10-(2-Tetrahydropyranyloxy)dec-1-yne (18). A 100-mL Erlenmeyer flask was charged with **17** (8.80 g, 57 mmol) dissolved in CH_2Cl_2 (20 mL). Pyridinium *p*-toluenesulfonate (PPTS)[26] (1.43 g, 5.7 mmol) was added to the flask, which was then swirled to dissolve the catalyst. Dihydropyran (5.75 g, 68.4 mmol) was then added to the flask followed by a CH_2Cl_2 (3 mL) rinse of the weighing vessel. It was then stoppered and allowed to sit, with occasional swirling, for 4 h. It gradually warmed during the first half hour before cooling back to ambient temperature. The solution was then filtered into a separatory funnel to remove precipitated catalyst. The reaction flask was rinsed with hexane (50 mL), the rinse was poured over the filtered catalyst, and the combined organic liquid was washed with 5% $NaHCO_3$ (2 × 10 mL) and brine (10 mL) and then dried with K_2CO_3. The solution was concentrated and the sample was distilled to give **18** (13.61 g, 100%, one spot by TLC): bp 95–98 °C (0.10 mm) [lit.[27] bp 95–96 °C (0.05 mm)]; ^{1}H NMR 4.58 (bs, 1 H), 4.06–3.24 (m, 4 H), 1.99 (bdt, J = 2 Hz, 7 Hz, 2 H), 1.93 (t, J = 2 Hz, 1 H), 1.90–1.14 (m, 18 H); IR 3310, 2930, 2850, 2120, 1465, 1455, 1445, 1355, 1325, 1265, 1205, 1140, 1120, 1080, 1035, 990, 910, 870, 815, 725, 625 cm^{-1}.

Ethyl 11-(2-Tetrahydropyranyloxy)undec-2-ynoate (9). A 250-mL round-bottom flask was charged with **18** (13.6 g, 57 mmol) and THF (60 mL) and then cooled with a dry ice/acetone bath. A solution of *n*-butyllithium in hexane (28.5 mL, 2.1 M) was added to the flask via addition funnel over 15 min. A second 250-mL round-bottom flask was charged with ethyl chloroformate (7.88 g, 1.3 equiv) and cooled with dry ice/acetone. The lithium alkynide solution was added via canula to the ethyl chloroformate over ca. 40 min. The alkynide flask was rinsed with THF (10 mL, introduced via syringe) which was then added to the ethyl chloroformate solution. The reaction was stirred under nitrogen overnight during which time it was allowed to warm to ambient temperature. The solution was then concentrated and rinsed into a separatory funnel with hexane (2 × 50 mL). The organic solution was washed with water (20 mL), 5% $NaHCO_3$ (10 mL), and brine (10 mL) and then dried with Na_2SO_4. It was then concentrated to give 18.8 g of yellow oil. The oil was flash chromatographed in three portions to give **9** (16.8 g, 95%, one spot on TLC): ^{1}H NMR 4.56 (bs, 1 H), 4.19 (q, J = 7 Hz, 2 H), 4.00–3.20 (m, 4 H), 2.30 (t, J = 7 Hz, 2 H), 1.94–1.12 (m, 21 H); MS, m/e 310.215; IR 2930, 2860, 2230, 1715, 1465, 1455, 1445, 1365, 1355, 1325, 1300, 1255, 1205, 1140, 1105, 1080, 1035, 990, 910, 870, 815, 755, 725 cm^{-1}.

11-(2-Tetrahydropyranyloxy)undec-2(*E*)-en-1-ol-*1,1,2,3-d*$_4$ (10). A 250-mL round-bottom flask was charged with LAD (2.52 g, 60 mmol) and THF (60 mL). The flask was cooled with dry ice/acetone and a solution of **9** (12.5 g, 40.3 mmol) in THF (40 mL) was added over 30 min. The cooling bath was replaced by a 50 °C water bath and the solution was stirred overnight. It was then cooled and the reaction was quenched by slowly adding D_2O (4 mL). The reaction was stirred an additional 2 h and then treated with aqueous NaOH (8 mL, 15%) and rapidly stirred until the aluminum salts became granular. The solution was then filtered, the flask and precipitate were washed with Et_2O (4 ×

(23) Texas Alkyls Company brochure.
(24) Bestmann, H. J.; Suss, J.; Vasterowsky, O. *Tetrahedron Lett.* **1979**, 2467.
(25) Prepared via a modification of the method of Klein and Gurfinkel (Klein, J.; Gurfinkel, E. *Tetrahedron* **1970**, *26*, 2127) used to make terminal hydrocarbon alkynes. In contrast to their findings, treatment with methylsulfinylcarbanion in DMSO at room temperature led to formation of some allene as well as metalation of the intermediate vinyl bromide to give alkene upon workup. Also, when $NaNH_2$ in DMSO was heated to 60 °C, as required for good yield of hydrocarbon 1-alkyne, without allene or internal alkyne formation, the hydroxy compound gave allene as well as alkyne.
(26) Miyashita, N.; Yoshikoshi, A.; Grieco, P. A. *J. Org. Chem.* **1977**, *42*, 3772.
(27) Rossi, R. *Synthesis* **1981**, 359.

20 mL), and the combined filtrates were dried with Na_2SO_4 followed by K_2CO_3. The sample was concentrated to give **10** (10.5 g, 96% crude). The alcohol was flash chromatographed in two portions to give 9.85 g of pure compound (one spot on TLC): ^1H NMR 4.58 (bs, 1 H), 4.02–3.22 (m, 4 H), 2.20–1.10 (m, 19 H); ^2H NMR 5.69, 5.63 (unres) (2 ^2H), 4.03 (2 ^2H), MS, *m/e* 274; IR 3410, 2930, 2855, 2210, 2080, 1635, 1465, 1450, 1440, 1380, 1365, 1350, 1325, 1265, 1205, 1140, 1120, 1080, 1035, 1025, 970, 905, 870, 815, 720 cm^{-1}.

11-(2-Tetrahydropyranyloxy)undec-2(*E*)-en-1-yl Acetate-*1,1,2,3,-d*$_4$ (11). A solution of acetic anhydride (2.04 g, 20 mmol) in pyridine (10 mL) was cooled in an ice bath. A solution of **10** (3.54 g, 12.9 mmol) in pyridine (5 mL) was added in one portion and the mixture was stirred for 4 h while being warmed to room temperature. It was then poured onto a mixture of ice (ca. 50 g) plus hexane (30 mL), shaken, and then separated. The aqueous layer was extracted with hexane (3 × 15 mL) and then the combined organic layers were washed with water and brine and then dried with $MgSO_4$. The sample was concentrated and the residual oil was flash chromatographed to give **11** (3.8 g, 12.0 mmol, 93% yield): ^1H NMR 4.57 (bs, 1 H), 4.02–3.20 (m, 4 H), 2.10–1.95 (s on m, 5 H), 1.95–1.10 (m, 18 H); ^2H NMR 5.78, 5.57 (unres) (2 ^2H), 4.47 (2 ^2H); MS, *m/e* 315.249 ($M^+ - 1$); mass spectra of **11** and the analogous protonated compound both show $M^+ - 1$ as the highest weight peak; IR 2935, 2860, 2230, 1745, 1640, 1470, 1455, 1445, 1380, 1325, 1260, 1205, 1140, 1125, 1080, 1035, 910, 870, 725 cm^{-1}.

11-Hydroxyundec-2(*E*)-en-1-yl Acetate-*1,1,2,3-d*$_4$ (12). A solution of **11** (3.23 g, 10.2 mmol), PPTS (0.251 g, 1.0 mmol), and absolute ethanol (20 mL) was heated for 5 h at 40–50 °C. The sample was concentrated and the residue was taken up in Et_2O (30 mL) to give a milky white solution which was transferred to a separatory funnel. The reaction flask was rinsed with water (10 mL) and Et_2O (10 mL), and then the washes were added to the separatory funnel and shaken to give two clear phases, which were separated. The organic layer was washed with water (5 mL), 1% $NaHCO_3$ (5 mL), and brine and then dried with Na_2SO_4. The solvent was removed and the residue was flash chromatographed to give **12** (1.69 g, 7.2 mmol, 71%), one spot by TLC: ^1H NMR 3.62 (t, J = 6 Hz, 2 H), 2.30–1.90 (m, 6 H), 1.80–1.10 (m, 12 H); ^2H NMR 5.63, 5.42 (unres), (2 ^2H), 4.43 (2 ^2H); MS, *m/e* 315 ($M^+ - 1$), both the deuteriated and analogous protonated compounds show this effect; IR 3420, 2930, 2860, 2260, 1740, 1640, 1465, 1435, 1370, 1260, 1200, 1030, 935, 720, 600 cm^{-1}.

11-Acetoxyundec-9(*E*)-enoic Acid-*9,10,11,11-d*$_4$ (13). A 250-mL round-bottom flask was charged with Jones reagent (8 mL, 2.67 M $Na_2Cr_2O_7$ in 4 M H_2SO_4) and acetone (40 mL). A solution of **12** (1.22 g, 5.25 mmol) in acetone (40 mL) was added at room temperature over 2.5 h. The solution was stirred an additional 30 min and then decanted from the blue-green residue. The residue was dissolved in water (30 mL) and then extracted with Et_2O (2 × 30 mL). The Et_2O extracts were added to the decanted solution which was then concentrated to give a "slushy" residue. This was dissolved in water (30 mL) and extracted with Et_2O (2 × 30 mL). The solution was washed with water (20 mL) to remove residual chromium species and then extracted with 10% NaOH (20 mL). The aqueous phase was acidified with 6 M HCl to pH 1–2 and then extracted with Et_2O (4 × 10 mL). The combined organic extracts were washed with water (10 mL) and brine (10 mL) and then dried with Na_2SO_4. Flash chromatography gave the acid **13** (0.836 g, 3.41 mmol, 65%). Samples kept at −20 °C solidified to an amorphous white powder which would melt if transferred to the refrigerator (+10 °C): ^1H NMR 10.06 (bs, 1 H), 2.34 (t, J = 7 Hz, 2 H), 2.20–1.85 (m superimposed on a singlet, 5 H), 1.85–1.10 (m, 10 H); ^2H NMR 5.63, 5.42 (unres) (2 ^2H); IR 3500–2400 (broad band), 2930, 2860, 2205, 1740, 1705, 1410, 1370, 1070, 1025, 930, 720 cm^{-1}.

10-Carbomethoxydec-2(*E*)-en-1-ol-*1,1,2,3-d*$_4$ (14). A 25-mL round-bottom flask containing **13** (0.836 g, 3.41 mmol), methanol (15 mL), and acetyl chloride (0.45 mL, 3% v/v) was heated to reflux for 4 h and then mixed with water (30 mL) and Et_2O (30 mL) in a separatory funnel. After shaking, the aqueous layer was separated and extracted with Et_2O (2 × 15 mL). The combined organic layers were washed with 5% $NaHCO_3$ (10 mL), water (10 mL), and brine (10 mL) and then dried with Na_2SO_4. After concentrating the sample, it was flash chromatographed to give **14** (.565 g, 2.59 mmol, 77%): ^1H NMR 3.70 (s, 3 H), 2.30 (t, J = 7 Hz, 2 H), 2.04 (m, 2 H), 1.80–1.10 (m, 11 H); ^2H NMR 5.64, 5.58 (unres) (2 ^2H), 3.94 (2 ^2H).

10-Carbomethoxydec-2(*E*)-enal-*1,2,3-d*$_3$ (14). A 100-mL round-bottom flask was charged with PCC (1.08 g, 5.02 mmol) and CH_2Cl_2 (15 mL). A solution of **14** (0.565 g, 2.59 mmol) in CH_2Cl_2 (10 mL) was added in one portion. The solution was stirred at room temperature until the reaction was finished by TLC (H:EA 2:1, ca. 1 h), and the solution was then slowly diluted with Et_2O (50 mL). The solution was poured through a short column of Florisil and the flask was rinsed with Et_2O (2 × 15 mL), with the rinses also poured through the column. The clear solution was concentrated and then flash chromatographed to give the aldehyde **15** (0.514 g, 2.39 mmol, 92%): ^1H NMR 3.66 (s, 3 H), 2.30 (m, 4 H), 1.80–1.10 (m, 10 H); ^2H NMR 9.51 (1 ^2H), 6.87 (1 ^2H), 6.12 (1 ^2H).

Methyl 9(*E*),11(*E*),13(*E*),15(*E*)-Octadecatetraenoate-*9,10,11,12,13,14,15,16-d*$_8$ (19). A 100-mL round-bottom flask was charged with Wittig salt (**8**) (2.171 g, 4.91 mmol) and THF (10 mL) and then degassed by bubbling argon through the suspension for ca. 20 min. A solution of **15** (1.033 g, 4.50 mmol) in THF (10 mL) was placed in an addition funnel and bubbled with argon for ca. 20 min. A solution of *n*-butyllithium in hexane (2.4 mL, 2.1 M, 5.05 mmol) was slowly added via syringe to the Wittig suspension over 10 min, during which the solution turned blood red. The reaction was stirred an additional hour and then the aldehyde solution was added over 5 min. The solution was stirred for 4 h and then hexane (20 mL, degassed) was slowly dripped into the flask. The solution was stirred an additional 10 min, and then the stirrer was turned off and the suspension allowed to settle, giving a transparent supernatant over granular precipitate. At this point, the reaction vessel was transferred to a glovebag.

The sample was allowed to sit overnight, under argon, in the dark, and then was filtered to remove triphenylphosphine oxide. The flask was rinsed with hexane (3 × 10 mL) and each rinse was poured over the precipitate. The combined filtrates were passed through a short column of silica, the column was washed with hexane (4 × 10 mL), and then most of the solvent was removed on the rotary evaporator. The residual yellow oil was flash chromatographed (hexane–ethyl acetate, 4:1) to give the ester **19** as a faintly yellow oil (1.08 g, 3.46 mmol, 77%): ^1H NMR 3.68 (s, 3 H), 2.40–1.98 (m, 6 H), 1.80–1.15 (m, 10 H), 1.02 (t, J = 7 Hz, 3 H); ^2H NMR 6.26, 5.84, 5.59, 5.40 (unres); UV 284, 298, 313 nm.

9(*E*),11(*E*),13(*E*),15(*E*)-Octadecatetraenoic Acid-*9,10,11,12,13,14,15,16-d*$_8$ (20). After small samples were removed for NMR analysis, the remaining ester was treated with 10 mL of 5% KOH in methanol. Within 1 min a large amount of fine white needles had formed in the solution. TLC showed that the starting ester was the only species present and the UV looked normal for the tetraene, implying that the chromatographed oil had crystallized when stirred in the methanol solution. The reaction mixture was heated on a steambath, causing the solid to dissolve, leaving a faintly hazy yellow solution. Monitoring the reaction of TLC showed the disappearance of the ester with the formation of the acid with little (if any) contamination. After ca. 2.5 h the reaction was finished. The flask was allowed to cool overnight during which time a large amount of flaky material deposited. Both TLC and UV implied that large amounts of the tetraene were still present. The methanol suspension was partially concentrated and then treated with 30 mL of water (degassed!), which caused most of the solid to dissolve. The aqueous solution was acidified with 4 N HCl (degassed!) (pH 1–2) and then extracted with Et_2O (3 × 10 mL). The combined Et_2O extracts were washed with water (10 mL) and brine (10 mL) and then dried with Na_2SO_4. The solution was concentrated and then flash chromatographed (hexane–Et_2O, 9:1) to give a clear solution of **20** (one spot on TLC, UV analysis implied 85% yield). After concentrating the solution the residue was recrystallized (3×) from hexane to give very fine white needles with a constant melting point of 96–97 °C (lit.[3] mp 95–96 °C, 79 mg).

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