Efficient Stereoselective Synthesis of Methyl Arachidonate via C3 Homologation

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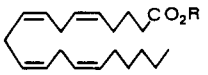
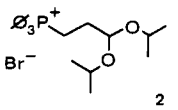
Arachidonic acid (**1a**), *all-cis*-5,8,11,14-eicosatetraenoic acid, plays a central role in a biological control system as a precursor molecule that is transformed into potent mediators with far ranging effects.[1] The classical procedure for the synthesis of arachidonic acid and other "skipped" cis polyenic compounds was introduced by Osbond.[2] It is the preparation of the corresponding tetraynoic acid followed by the selective hydrogenation of the acetylenic bonds to cis olefins. The polyacetylenic compounds were built up in successive condensations of acetylenic Grignard reagents with propargylic bromides. Some variations of this standard approach have been adopted by other authors for the synthesis of **1b**.[3]



1a R = H
b R = CH_3



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We report herein a new, efficient, and the shortest total synthesis of methyl arachidonate (**1b**) involving C3 homologations by Wittig reactions, giving the four cis double bonds and starting from hexanal. In a previous paper,[4] we developed an easy preparation of (3,3-diisopropoxypropyl)triphenylphosphonium bromide (**2**), which is an excellent 3-carbon homologating agent to provide in high yields pure *cis*-β,γ-ethylenic aldehydes via the Wittig reaction. With use of salt **2**, two or more successive condensations will allow a build up of "skipped" *all-Z*-polyene systems like we find in arachidonic cascade[5] and some pheromones.[6]

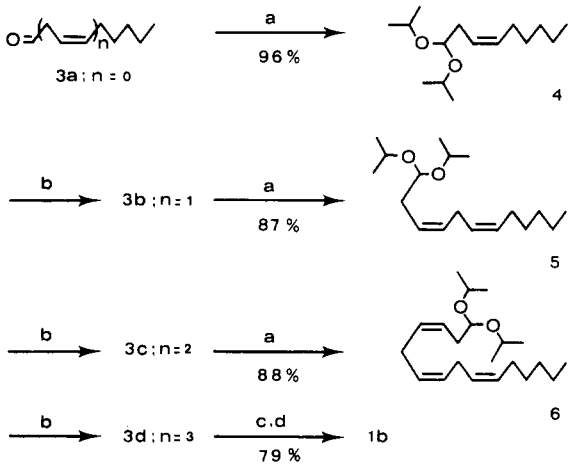
Therefore, the four steps of the synthesis (except for the first one) included two parts: (i) a fast and mild hydrolysis of *cis*-β,γ-ethylenic aldehyde diisopropyl acetals **4**, **5**, or **6**, providing pure aldehydes **3** without modification of the *Z* double bond systems, and (ii) immediate addition of **3** to the ready ylid solution at −100 °C (Scheme I).

Workup and two flash chromatographic separations gave the homologated compounds in very good yield (79–96%).

Due to the activation of methylene groups in α and δ positions to the carbonyl, aldehydes **3** were sensitive toward acidity, basicity, and oxidation.[7] They were only isolated for spectroscopic measurements. Cis Wittig reaction conditions[8] (lithium salt free, high dilution, low temperature) gave pure cis olefins, checked by ^{13}C NMR spectroscopy. Each ethylenic and allylic carbon gave a single chemical shift instead of two when *n*-BuLi was used to make the ylid (*Z*/*E* ratio, 95/5).

Thus our total synthesis is short (only four steps), inexpensive (cheap starting reagents), and easy to run in gram quantities (and to scale up); it provides **1b** in 58% overall yield from hexanal by successive C3 homologations. Such a strategy could potentially be employed to introduce a heteroatom in one location along the carbon chain by using in one step a modified phosphonium salt **2**. The ability to incorporate either a heteroatom or a radiolabel will be an important tool helpful for studying the biological properties of unsaturated fatty acids.

Scheme I[a]



[a] (a) **2** + $NaN(SiMe_3)_2$, THF–HMPA or THF–toluene; (b) H_2O + TsOH; (c) $Ph_3P^+(CH_2)_4CO_2H$, Br^- + $NaN(SiMe_3)_2$, THF–HMPA; (d) CH_2N_2.

Experimental Section

General Methods. ^1H and ^{13}C NMR spectra in $CDCl_3$ solution were recorded on a Varian XL 200 spectrometer with Me_4Si as the internal standard. Mass spectra were obtained on a Varian MAT 311 mass spectrometer. All reactions were performed in flame-dried glassware under positive pressure of argon. Reaction mixtures were stirred magnetically. Reagents and solvents were introduced via syringes through rubber septa. All phosphonium salts were dried twice in the reaction flask by azeotropic distillation under vacuum with anhydrous benzene. All solvents (THF, toluene, HMPA) were distilled from calcium hydride before being used. Reactions run at −100 °C were cooled in 95% ethanol with liquid nitrogen and slowly warmed up to 0 °C over 4 h. Reaction solutions were dried over anhydrous magnesium sulfate for 5 min and concentrated with a Büchi rotary evaporator at 15–20 mmHg. Toluene was removed in the same manner with a bath temperature of 35 °C. After Wittig reactions, two flash chromatographic separations were successively performed by using E. Merck silica gel (70–230 mesh) with 1/10 ether–pentane as eluent and then with 230–400 mesh silica gel with 1/80 ether–pentane as eluent. All compounds were stored frozen in anhydrous benzene at −20 °C. The purity of all title compounds was shown to be >95% by ^1H NMR and ^{13}C NMR analyses. Microanalyses were performed by the Department of Chemistry for **4**, **5**, **6**, and **1b**; carbon and hydrogen results were within ±0.4% of theory.

Materials. Hexanal (from Aldrich Chemical Co.) was distilled from molecular sieves (4 Å) before being used. Phosphonium salt **2** was prepared by using the method reported previously.[4] Sodium bis(trimethylsilyl)amide 1 M in THF and (carboxybutyl)tri-

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(7) For example, in one reaction, we isolated the unreacted aldehyde as isomer of **3c**, *E*,*Z*-2,6-dodecadienal: ^1H NMR δ 9.46 (1, d, J = 8.0 Hz), 6.80 (1, d, J = 16.0 Hz, t, J = 6.4 Hz), 6.10 (1, d, J = 16.0 Hz, d, J = 8.0 Hz), 5.49–5.25 (2, m), 2.39 (2, t, J = 6.6 Hz, d, J = 6.4 Hz), 2.20 (2, t, J = 6.6 Hz, d, J = 6.4 Hz), 2.07–1.97 (2, m), 1.24–1.19 (6, br s), 0.88 (3, t).
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Table I. ^{13}C NMR Chemical Shifts[a] of Intermediates[b] and 1b[c,d]

carbon atoms	4	3b	5	3c	6	3d	1b
20	14.10	14.05	14.10	14.08	14.10	14.09	14.11
19	22.86	22.59	22.63	22.60	22.65	22.62	22.66
18	31.63	31.50	31.57	31.54	31.60	31.56	31.59
17	29.36	29.07	29.39	29.28	29.42	29.35	29.40
16	27.54	27.65	27.28	27.29	27.28	27.28	27.28
15	123.39	118.11	130.24	131.13	129.92	130.66	130.48
14	132.07	135.47	127.61	126.49	127.65	126.83	127.56
13	33.83	42.61	25.94	25.99	25.96	25.69	25.67
12	100.19	199.56	124.00	118.36	128.48	129.24	128.58
11			130.40	133.56	127.92	127.28	127.87
10			33.81	42.53	25.71	26.00	25.67
9			99.98	199.37	124.86	118.64	128.21
8					130.33	133.24	128.16
7					33.85	42.52	25.67
6					99.96	199.19	128.90
5							128.90
4							26.59
3							24.82
2							33.41
1							173.92

[a] 100 to 120 mg of sample in 2 mL of $CHCl_3$. In ppm from TMS. [b] Chemical shifts of the diisopropoxy moiety of **4**, **5**, and **6**: 67.76 for α carbon atoms; 23.43 and 22.61 for β carbon atoms. [c] Chemical shift of the methoxy group: 51.41. [d] The ^{13}C NMR chemical shifts of ethylenic carbon atoms of **1b** were determined by using the upfield shift reagent, $Pr(fod)_3$, at several concentrations.

phenylphosphonium bromide were provided by Aldrich.

Table I lists the ^{13}C NMR chemical shifts of the various intermediates prepared. They are numbered as arachidonic acid from C_{20} (CH_3) to C_1 (carboxyl). The values listed are consistent with the assigned structures and the literature data.[9]

(*Z*)-3-Nonenal Diisopropyl Acetal 4. Method A. This was obtained from hexanal according to the procedure reported previously[4] in 96% yield.

Method B. To a suspension of phosphonium salt **2** (6.45 g, 12.87 mmol, 1.54 equiv) in THF–toluene (20/120 mL) at 0 °C was added a 1 M THF solution of sodium bis(trimethylsilyl)amide (12.2 mL, 12.2 mmol, 1.46 equiv) in 2 min. The orange solution was stirred at room temperature for 1 h. After the mixture was cooled to −100 °C, hexanal **3a** (1 mL, 8.34 mmol, 1 equiv) was added dropwise. The reaction mixture was allowed to warm up to 0 °C and then hydrolyzed with saturated NH_4Cl (30 mL). After dissolution of mineral salts by addition of H_2O (10 mL), the mixture was extracted with ether (2 × 150 mL). Combined organic layers were washed with brine (40 mL), dried, and concentrated. The crude material was flash chromatographed twice to give pure **4** (1.905 g, 94%) as a colorless oil: ^1H NMR δ 5.58–5.36 (2, m), 4.54 (1, t, J = 5.6 Hz), 3.88 (2, sept, J = 6.2 Hz), 2.36 (2, d, J = 6.2 Hz, d, J = 5.6 Hz), 2.10–1.99 (2, m), 1.34–1.28 (6, br s), 1.20 (6, d, J = 6.2 Hz), 1.14 (6, d, J = 6.2 Hz), 0.90 (3, t); HRMS calcd for $C_{12}H_{22}O$ (M − iPrOH) 182.1670, found 182.1661.

General Procedure for the Preparation of 5 and 6: (*Z*,*Z*)-3,6-Dodecadienal Diisopropyl Acetal (5). To a suspension of phosphonium salt **2** (5.34 g, 10.65 mmol, 1.75 equiv) in THF–toluene (14/90 mL) at 0 °C was added a 1 M THF solution of sodium bistrimethylsilylamide (9 mL, 9 mmol, 1.48 equiv) in 2 min. The orange solution of ylid was stirred at room temperature for 1 h while compound **4** (1.467 g, 6.06 mmol, 1 equiv) was hydrolyzed in refluxing THF (120 mL) with 0.1 M aqueous solution of *p*-toluenesulfonic acid (3 mL, 0.3 mmol, 0.05 equiv) for 0.3 h. Then, this mixture was cooled to 0 °C, diluted in pentane (80 mL), and washed with water (10 mL) and brine (2 × 10 mL). The combined aqueous layers were extracted once with pentane (20 mL). All organic layers were dried over $MgSO_4$, concentrated, transfered into a pear-shaped flask, and then thoroughly dried three times by azeotropic distillation on a rotatory evaporator with anhydrous benzene. The residual aldehyde **3b** was diluted in THF (3 mL) and added dropwise to the ylid solution cooled to −100 °C. The pear-shaped flask was rinsed twice with THF (1 mL), and the reaction mixture was allowed to warm up to 0 °C. The hydrolysis with saturated NH_4Cl (20 mL), homogenization of the aqueous layer with water (5 mL), extraction with ether (3 × 60 mL), washing with brine (20 mL), drying, and concentration provided crude material, which was flash chromatographed twice to give pure **5** (1.48 g, 87%) as a colorless oil. **3b**: ^1H NMR δ 9.2 (1, t, J = 1.85 Hz), 5.72–5.24 (2, m), 3.07 (2, d, J = 6.4 Hz, d, J = 1.85 Hz), 2.15–1.82 (2, m), 1.38–1.16 (6, br s), 0.82 (3, t); HRMS calcd for $C_9H_{16}O$ 140.1201, found 140.1208. **5**: ^1H NMR δ 5.50–5.32 (4, m), 4.56 (1, t, J = 5.6 Hz), 3.88 (2, sept, J = 6.2 Hz), 2.81 (2, m), 2.38 (2, d, J = 6.2 Hz, d, j = 5.6 Hz), 2.11–2.00 (2, m), 1.34–1.29 (6, br s), 1.20 (6, d, J = 6.2 Hz), 1.14 (6, d, J = 6.2 Hz), 0.90 (3, t); HRMS calcd for $C_{15}H_{26}O$ (M − iPrOH) 222.1977, found 222.1983.

(*Z*,*Z*,*Z*)-3,6,9-Pentadecatrienal Diisopropyl Acetal (6). Homologation of compound **5** (1.218 g, 4.31 mmol) with the same procedure led to **6** (1.23 g, 3.82 mmol, 88%). **3c**: ^1H NMR δ 9.2 (1, t, J = 1.85 Hz), 5.51–5.24 (2, m), 5.24–4.97 (2, m), 3.08 (2, d, J = 6.4 Hz, d, J = 1.85 Hz), 2.67 (2, m), 2.00–1.91 (2, m), 1.34–1.18 (6, br s), 0.77 (3, t). **6**: ^1H NMR δ 5.40–5.20 (6, m), 4.49 (1, t, J = 5.6 Hz), 3.88 (2, sept, J = 6.2 Hz), 2.78–2.70 (4, m), 2.31 (2, d, J = 6.2 Hz, d, J = 5.6 Hz), 1.29–1.20 (6, br s), 1.20 (6, d, J = 6.2 Hz), 1.14 (6, d, J = 6.2 Hz), 0.82 (3, t); HRMS calcd for $C_{18}H_{30}O$ (M − iPrOH) 262.2296, found 262.2291.

Methyl Arachidonate (1b). To a suspension of (carboxybutyl)triphenylphosphonium bromide (1.813 g, 4.09 mmol, 2.06 equiv) in THF–HMPA (3/1.5 mL) was added a 1 M THF solution of sodium bis(trimethylsilyl)amide (8 mL, 8 mmol, 4.04 equiv) at 0 °C in 2 min. The orange mixture was stirred at room temperature for 2 h while compound **6** (0.64 g, 1.98 mmol, 1 equiv) was hydrolyzed for 0.3 h in refluxing THF (40 mL) with a 0.1 M aqueous solution of *p*-toluenesulfonic acid (1 mL, 0.1 mmol, 0.05 equiv). The work up described above gave the anhydrous residual aldehyde **3d**, which was diluted in THF (2 mL) and added dropwise to the deep orange mixture of ylid cooled to −100 °C. The pear-shaped flask was rinsed twice with THF (1 mL), and the reaction mixture was allowed to warm up to 0 °C. The hydrolysis with saturated NH_4Cl (10 mL), acidification to pH 1 with 2 N HCl (4 mL), extraction with ether (3 × 20 mL), washing with H_2O (3 mL) and brine (3 mL), drying, and concentration provided crude material, which was esterified at 0 °C with freshly distilled diazomethane solution in ether. Two flash chromatographic separations of the final residue gave pure methyl arachidonate (**1b**) (0.496 g, 79%) as a colorless oil. **3d**: ^1H NMR δ 9.2 (1, t, J = 1.85 Hz), 5.51–5.25 (2, m), 5.25–5.03 (4, m), 3.07 (2, d, J = 6.4 Hz, d, J = 1.85 Hz), 2.74–2.59 (4, m), 2.00–1.88 (2, m), 1.37–1.17 (6, br s), 0.85 (3, t). **1b**: ^1H NMR δ 5.48–5.30 (8, m), 3.66 (3, s), 2.90–2.77 (6, m), 2.33 (2, t, J = 7.2 Hz), 2.17–2.00 (2, m), 1.79–1.64 (2, m), 1.38–1.28 (6, br s), 0.91 (3, t); HRMS calcd for $C_{21}H_{34}O_2$ 318.2558, found 318.2552.

Acknowledgment. We thank Prof. J. R. Falck (University of Texas, Dallas, TX) for his interest and

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helpful discussion. We are indebted to Mr. Guênot (Université de Rennes, France) for high-resolution mass spectra.

Registry No. **1a**, 506-32-1; **1b**, 2566-89-4; **2**, 117203-40-4; **3a**, 66-25-1; **3b**, 31823-43-5; **3c**, 13553-09-8; **3d**, 13552-98-2; **4**, 117203-37-9; **5**, 117203-38-0; **6**, 117203-39-1; (carboxybutyl)triphenylphosphonium bromide, 17814-85-6; (*E,Z*)-2,6-dodecadienal, 21662-13-5.

Reduction of Aryl-Nitroso Compounds by 1,4-Dihydronicotinamides

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Nitroso compounds are of considerable current interest since their similarities to aldehydes are well established. The aryl–nitroso group is isosteric and isoelectronic with the aromatic aldehyde group,[1] but much more reactive.[2] In view of using the aryl–nitroso group as an analogue of NADH-dependent reduction of carbonyl compounds in enzymatic and in model systems, we have investigated the mechanism of reduction of aryl–nitroso compounds by NADH and N_1-(2,6-dichlorobenzyl)-1,4-dihydronicotinamide (DBDN-4H_2) in model systems. For this purpose, we have distinguished two types of aryl–nitroso compounds: unsubstituted (representative: nitrosobenzene) and ring substituted inthe ortho or para positions by OH, NH_2, NHR, NR_2, and similar functional groups providing a keto–enol tautomerism (representative: 1-nitroso-2-naphthol).

Nitrosobenzene and 1-nitroso-2-naphthol were readily reduced by NADH and DBDN-4H_2 in the absence of oxygen, in neutral and weakly alkaline aqueous buffers and in dry methanol; the stoichiometry with NADH was

$$\mathrm{ArNO} + \mathrm{NADH} + \mathrm{H^+} \rightarrow \mathrm{ArNHOH} + \mathrm{NAD^+} \quad (1)$$

$$\mathrm{HOArNO} + 2\ \mathrm{NADH} + 2\ \mathrm{H^+} \rightarrow \mathrm{HOArNH_2} + 2\ \mathrm{NAD^+} + \mathrm{H_2O} \quad (2)$$

Under the pseudo-first-order conditions ([nitroso compounds] = (0.5–2) × 10^{-4} M; [NADH] = 2 × 10^{-3} M) in a buffer of pH 7.5, the disappearance of the nitroso group was found to follow the first-order rate law for at least 3 half-times; also, at pH 7.5, reactions 1 and 2 were found to be second order overall.

The product identification of reactions 1 and 2 was performed kinetically and analytically. Both reactions strictly obeyed the stoichiometry kinetically (eq 1, 2). Under the second-order rate conditions ([nitroso compounds] = (0.75–1.5) × 10^{-4} M; [NADH] = 1.5 × 10^{-4} M) at pH 7.5, NAD^+ was identified analytically as the sole product of NADH oxidation, appearing in the product proportionally to the disappearance of nitroso groups according to the stoichiometry (eq 1, 2). In addition, the product of reaction 2, 1-amino-2-naphthol, after acetylation with acetic anhydride was shown to be identical by TLC to an acetylated authentic sample of 1-amino-2-naphthol.

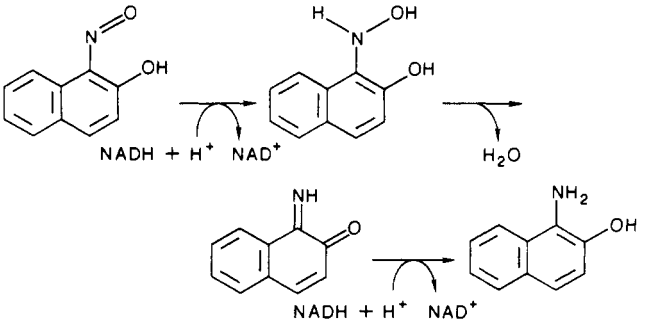
The phenolic group of 1-nitroso-2-naphthol was characterized by a pK of 8.2, which was estimated from the disappearance, in acid, of the alkaline maximum (415 nm) in the absorption spectra (maximum at 380 nm remained pH-unshifted). The change of the alkaline maximum at 415 nm was directly proportional to the pH dependence of the rate constant for the reduction of 1-nitroso-2-naphthol by NADH, with minimal rates in alkali; this indicated that only the phenol form of 1-nitroso-2-naphthol was reduced with NADH and the phenolate form was completely unreactive. Taken together, the above data and the data on the electrochemical reduction of para- and ortho-substituted aryl–nitroso compounds[3,4] supported the following mechanism of reaction 2:

Table I. Second-Order Rate Constants (k_2) for the Reduction of Nitroso Compounds with 1,4-Dihydronicotinamides[a]

	NADH		DBDN-4H_2	
k_2, M^{-1} min^{-1}	buffer[b]	methanol[c]	buffer	methanol
nitrosobenzene	11.350	50	$>10^5$	280
1-nitroso-2-naphthol	95	<1	925	<1

[a] [Nitroso compounds] = (5–8) × 10^{-5} M; [1,4-dihydronicotinamides] = (1–2) × 10^{-4} M; 30 °C, anaerobic conditions. [b] Sodium phosphate buffer, 0.1 M, pH 7.5. [c] Dry methanol.

Scheme I



The above mechanism was supported by the stoichiometry of reaction (NADH:oxidant = 2:1), which was observed for at least 2 half-times ([1-nitroso-2-naphthol] = 7 × 10^{-5} M; [NADH] = 1.6 × 10^{-4} M; pH 7.5 at 30 °C). Since the radical mechanism was excluded (see below), the first redox reaction of Scheme I must be regarded as rate-limiting for the overall process.

The rate constants of reactions 1 and 2 and related reactions were estimated by employing the second-order rate conditions (Table I).

Since both types of nitroso compounds were initially reduced to hydroxylamines, the primary kinetic hydrogen isotope effect of this redox reaction was measured in the temperature range 16.0–45.8 °C (Table II).

The kinetic data of Table II fitted the following Arrhenius equations:

$$\ln k_{HD} = (16.21 \pm 0.01) - (28.05 \pm 0.54\ \mathrm{kJ/mol})/RT \quad (3)$$

$$r = 0.999$$

$$\ln k_{HH} = (16.81 \pm 0.01) - (28.17 \pm 1.04\ \mathrm{kJ/mol})/RT \quad (4)$$

$$r = 0.994$$

From eq 3 and 4, the difference in activation energies ($[E_a]^{HH}_{HD}$) and, after a correction for the isotopic impurity

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