Syntheses of three metabolites of icosapentaenoic and docosahexaenoic acids

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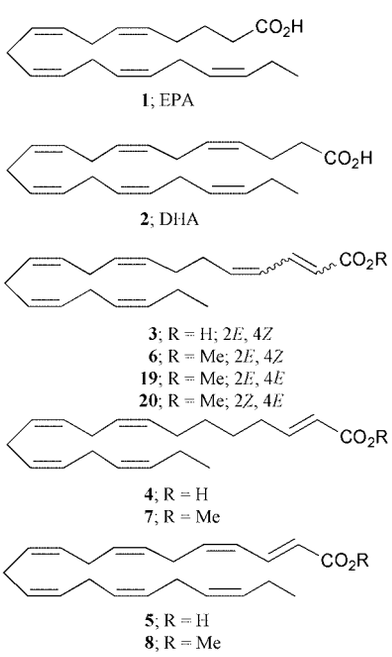
Received (in Cambridge, UK) 22nd May 2000, Accepted 28th July 2000
Published on the Web 25th August 2000

Starting from the $n-3$ polyunsaturated fatty acids icosapentaenoic acid (**1**, EPA) and docosahexaenoic acid (**2**, DHA), the syntheses of methyl 2*E*,4*Z*,8*Z*,11*Z*,14*Z*,17*Z*-icosa-2,4,8,11,14,17-hexaenoate (**6**), 2*E*,8*Z*,11*Z*,14*Z*,17*Z*-icosa-2,8,11,14,17-pentaenoate (**7**) and 2*E*,4*Z*,7*Z*,10*Z*,13*Z*,16*Z*,19*Z*-docosa-2,4,7,10,13,16,19-heptaenoate (**8**), in high stereochemical purity, have been accomplished. The corresponding carboxylic acids have been reported as metabolites in the bioconversion of EPA and DHA.

Introduction

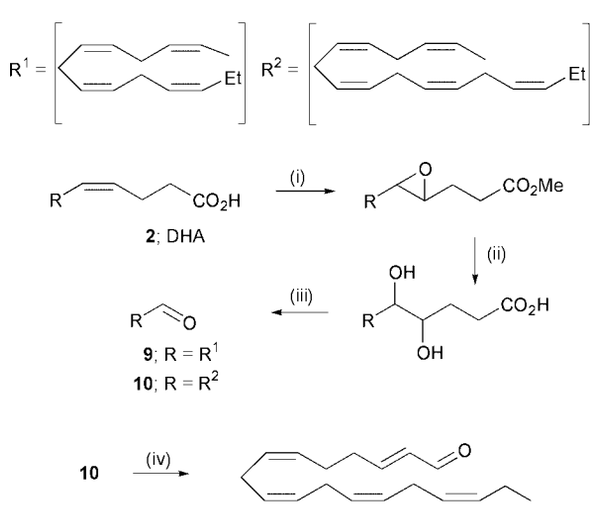
It is well established that the $n-3$ polyunsaturated fatty acids icosapentaenoic acid (**1**, EPA) and docosahexaenoic acid (**2**, DHA) are of dietary importance to humans; several beneficial biological effects have been reported for these acids particularly related to cardiovascular diseases, but also in connection with inflammatory problems.[1] Moreover, DHA is probably an essential fatty acid for infants in their first months of life.



The metabolism of EPA and DHA in mammals is known in considerable detail.[2] In a recent paper, Sprecher and coworkers[3] reported that EPA, when incubated with peroxisomes in the presence of NAD^+, was oxidized to a mixture of metabolites, two of which were assigned the structures (2*E*,4*Z*,8*Z*,11*Z*,14*Z*,17*Z*)-icosahexaenoic acid (**3**) and (2*E*,8*Z*,11*Z*,14*Z*,17*Z*)-icosapentaenoic acid (**4**). Under similar conditions, but in the presence of NAD^+ and NADH peroxisomal oxidation of DHA gave (2*E*,4*Z*,7*Z*,10*Z*,13*Z*,16*Z*,19*Z*)-docosaheptaenoic acid (**5**), which was suggested as the precursor of other metabolites, *viz.* stereoisomeric docosa-3,5,7,10,13,16,19-heptaenoic acids. The structural assignments of the metabolites were based only on the MS and UV spectra of the corresponding methyl esters. Considering the minute quantities available from the biochemical studies the structures are best established firmly by comparison with authentic samples; however, none of the above acids or their methyl esters had been synthesized previously. We have for some time been engaged in the chemical manipulation of EPA and DHA aiming at an enhancement of their beneficial biological activity.[4,5] As a result of these studies we were in the possession of compounds that seemed convenient as starting materials for the syntheses of these metabolites, and this paper describes our effort towards this goal.

Results and discussion

We chose to prepare the methyl esters **6**, **7** and **8**, while anticipating that the corresponding acids would be formed by hydrolysis with lithium hydroxide in aqueous methanol.[4] We have previously reported[5] the oxidative degradation of EPA and DHA by a three step procedure to the aldehydes **9** and **10**, respectively, in about 75% overall yields. Furthermore, DBU promoted isomerization of the latter provided the aldehyde **11** in nearly quantitative yield, with the α,β-unsaturated bond in an *E*-configuration.[4] These reactions are depicted in Scheme 1, and we contemplated using the readily available aldehydes as starting materials for the syntheses of the methyl esters **6**–**8**. Synthetic routes to the C:20-6 ester **6** could start from either

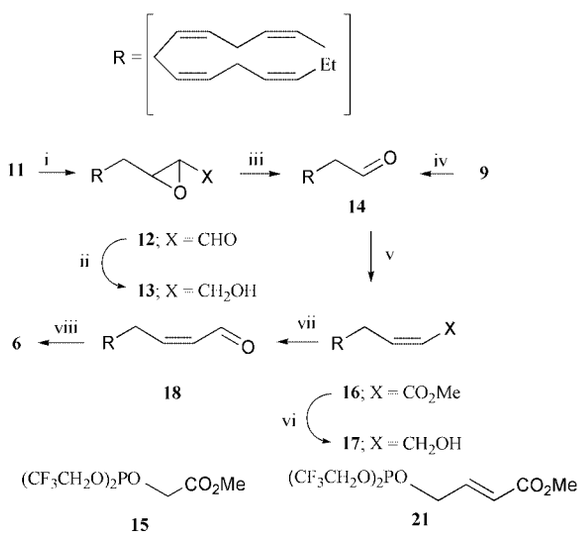


Scheme 1 *Reagents and conditions*: i, I_2, KI, $KHCO_3$, $THF–H_2O$, K_2CO_3, MeOH, rt; ii, HCO_2H, Ac_2O, rt; LiOH, $MeOH–H_2O$; iii, $NaIO_4$, $MeOH–H_2O$; iv, DBU, ether, rt.

DOI: 10.1039/b004101g

the C : 15-4 aldehyde **9** or the C : 18-5 aldehyde **11**, while selective reduction of the *trans* double bond of **11** followed by annexation of a C-2 fragment should lead to the C : 20-5 ester **7**. Finally, reaction of the aldehyde **10** with an appropriate C-4 fragment should provide the C : 22-7 ester **8**.

We expected the main synthetic problem to be control of the stereochemistry; the configuration of the $\Delta^{2,3}$ double bond in all the target esters is *trans*, while the remaining double bonds have the usual *Z*-configuration of naturally occurring fatty acids. A Wittig-type reaction seemed the first choice for the appropriate chain elongation of the aldehydes. The aldehyde **11** contains all but one of the double bonds of the target molecule **6** correctly arranged regiochemically as well as stereochemically; however, not surprisingly, a stereoselective isomerization of the $\Delta^{2,3}$ double bond to the required *Z*-configuration was not achieved. Hence, we attempted to degrade the aldehyde **11** by two carbon atoms. It was first transformed with alkaline hydrogen peroxide to the epoxide **12** (Scheme 2), but the subsequent reaction with



Scheme 2 *Reagents and conditions*: i, H_2O_2, NaOH; ii, $NaBH_4$, MeOH; iii, H_5IO_6, ether, rt, iv, (methoxymethyl)triphenylphosphonium chloride, BuLi, ether, 0 °C; v, phosphonate **15**, potassium hexamethyldisilazane, THF, −78 °C; vi, DIBAL-H, hexane, 0 °C; vii, Dess–Martin periodinane, CH_2Cl_2, 0 °C; viii, trimethyl phosphonoacetate, BuLi, DME, −78 °C.

periodic acid in ether gave a mixture of products. On the other hand, when the carbonyl group of **12** was selectively reduced with $NaBH_4$ in methanol to the epoxyalcohol **13**, the oxidation with periodic acid gave the relatively stable C : 16-4 aldehyde **14** in 37% overall yield. The compound was actually more conveniently prepared by a one-pot chain elongation of the aldehyde **9** with the ylide derived from commercially available (methoxymethyl)triphenylphosphonium bromide and butyllithium; subsequent acid hydrolysis provided the aldehyde **14**, albeit in only 38% yield. Horner–Wadsworth–Emmons reactions of aldehydes with stabilized phosphonate anions are thermodynamically controlled usually resulting in the new double bond being formed preferentially with the *E*-configuration. However, some years ago Still and Gennari reported[6] that methyl [bis(trifluoroethyl)phosphono]acetate (**15**) in the presence of potassium hexamethyldisilazane (KHMDS) and 18-crown-6 reacted with aldehydes yielding the corresponding α,β-unsaturated esters with excellent *Z*-stereoselectivity. Similar results were also recently obtained from reactions of aldehydes with diarylphosphonate anions.[7] When the aldehyde **14** was subjected to the phosphonate **15**, the ester **16** was formed stereoselectively (>98%) in 85% yield. Reduction of the ester with DIBAL-H at 0 °C afforded the allylic alcohol **17**, and first attempts to oxidize this were made with manganese dioxide in dichloromethane, however, the reaction time of 6 h at room temperature required for completion caused partial stereoisomerization yielding the aldehyde as a 3 : 2 mixture of the 2*Z*- and 2*E*-isomers, respectively. It appeared that the compound was sensitive to light as well. The stereochemical problem was largely alleviated by changing to the Dess–Martin oxidation reagent periodinane;[8] using a short reaction time at low temperature in the dark, the all-*Z* aldehyde **18** was obtained almost pure in high yield. It can be kept unchanged for several weeks in benzene at −20 °C under argon. Finally, the aldehyde reacted with the anion of trimethyl phosphonoacetate to afford the methyl ester **6** in 60% yield as the 2*E*,4*Z*-isomer in high stereochemical purity. A small amount of the 2*E*,4*E*-isomer (*vide infra*) was also present in the product, as indicated by the ^{1}H NMR spectrum. The ester **6** exhibited a UV absorption maximum in methanol solution at 264 nm, which is to be compared with the maximum at 266 nm reported by Sprecher and co-workers for the metabolite.[3] Two other stereoisomers of the ester **6** were prepared as well. Treatment of the aldehyde **11** with the trimethyl phosphonoacetate anion furnished the 2*E*,4*E*-isomer **19**, and the reaction of the same aldehyde with the phosphonate **15** by the published procedure afforded the 2*Z*,4*E*-isomer **20** in 80% yield with >98% stereochemical purity; the *J* values for the protons of the conjugated double bonds were 11.7 and 15.3 Hz. On the other hand, a similar reaction of **11** with ethyl diphenylphosphonoacetate in the presence of KHMDS–18-crown-6 gave the ester **20** in 30% yield, as a 77 : 23 mixture of the 2*Z*,4*E*- and 2*E*,4*E*-isomers, respectively. Hence, for our purpose the Still reagent was clearly superior.

An alternative route to **6** would be to introduce in one step the two double bonds with the required stereochemistry. This could possibly be achieved by reacting the aldehyde **14** with the anion of the phosphonate **21**, the vinyl analogue of the Still reagent with an *E* double bond. It was prepared from trimethyl phosphonocrotonate using a *trans*-esterification procedure described in the literature;[6] a mixture of products was obtained from which the phosphonate **21** was separated by flash chromatography, and a vicinal coupling constant of 15.6 Hz for the olefinic protons in the ^{1}H NMR spectrum showed that the *E*-isomer was formed. As a model experiment the phosphonate **21** was treated with octanal under the same conditions as those used with the Still reagent. Thus an approximately 1 : 1 mixture of the 2*Z*,4*E*- and 2*E*,4*E*-isomers of methyl deca-2,4-dienoate was obtained, as shown by comparison of the ^{1}H NMR spectra with those reported in the literature.[9] The apparent lack of selectivity discouraged us from further use of this vinylogous phosphonate reagent.

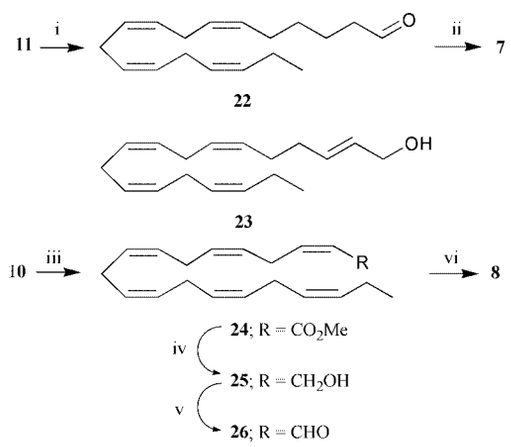
Provided we could reduce regioselectively the $\Delta^{2,3}$ double bond of the aldehyde **11,** the synthesis of the methyl ester **7** seemed quite straight forward. After several attempts with different reducing agents and reaction conditions[10,11] (Table 1), the aldehyde **11** was reduced with excellent selectivity to (all-*Z*)-octadecatetraenal (**22**) in 82% yield, using DIBAL-H in the presence of cuprous iodide and with HMPA–THF as solvent (Scheme 3).[12] The allylic alcohol **23**[4] was formed as a by-product. The subsequent reaction of **22** with the anion of trimethyl phosphonoacetate proceeded to give the ester **7** in 53% yield; the compound was >98% stereochemically pure according to the ^{1}H NMR spectrum, which also established the *E*-configuration by a vicinal coupling constant of 15.6 Hz for the olefinic protons of the $\Delta^{2,3}$ double bond. As expected the compound exhibited a UV absorption maximum at 206 nm in either methanol or cyclohexane as solvents.

The ester **8** contains the same conjugated diene moiety as **6** and a similar synthetic route was chosen, starting from the aldehyde **10**. Reaction of the latter with the phosphonate **15** furnished in good yield the (all-*Z*)-ester **24** with at least 95% stereoselectivity as determined by ^{1}H NMR. Reduction of the ester with DIBAL-H at 0 °C afforded the allylic alcohol **25**, which was oxidized to the aldehyde **26** in almost quantitative yield with the Dess–Martin reagent. The aldehyde was further

Table 1 Reductions of aldehyde **11** with hydride reagents

Entry	Reagents and conditions	Yield (%)		
		22[a]	**23**[a]	**11**[a]
1	$LiAlH_4$, THF, rt, 30 min	0	>90	0
2[10]	LiCl, CuI, Me_3SiCl, Bu_3SnH, THF, −60 °C to rt, 15 h	>20	0	>70
3[11]	$LiAlH_4$, CuI, THF, rt	0	>90	0
4[11]	$LiAlH_4$, CuI, HMPA, THF, −78 °C to rt, 15 h	<10	20	>60
5[12]	DIBAL-H, CuI, HMPA, THF, 0 °C, 1.5 h	55[b]	28[b]	Trace
6[12]	DIBAL-H, CuI, HMPA, THF, −50 °C, 1.5 h	82[b]	<10[b]	Trace

[a] Ratio based on ^{1}H NMR analysis of crude product, when not indicated otherwise. [b] % isolated yield.



Scheme 3 *Reagents and conditions*: i, DIBAL-H, CuI, HMPA, THF, −50 °C; ii, trimethyl phosphonoacetate, NaOMe, MeOH, 0 °C; iii, phosphonate **15**, potassium hexamethyldisilazane, THF, −78 °C; iv, DIBAL-H, hexane, 0 °C; v, Dess–Martin periodinane, CH_2Cl_2, 0 °C; vi, trimethyl phosphonoacetate, BuLi, DME, −78 °C.

transformed to the methyl ester **8** in 55% yield with the anion of trimethyl phosphonoacetate. The conjugated double bonds of the ester were firmly established as 2*E*,4*Z* by the *J* values of 15.3 and 11.6 Hz, respectively, for the olefinic protons in the ^{1}H NMR spectrum. Furthermore, the J_{34} of all the conjugated dienes is of the same magnitude as the coupling constant for *cis* olefinic protons, indicating that the diene moiety attains the preferred s-*trans* conformation.[13]

The UV absorption maximum of compound **8** at 265 nm, recorded in methanol solution, was only slightly shifted bathochromically as compared with that of the 2*E*,4*E*-isomer.

The assignment of double bond configurations based on UV spectra is certainly questionable; the difference between the absorption maxima of stereoisomeric dienoic esters may actually be smaller than that caused by a solvent effect.[14] It is therefore essential that the spectra being compared are recorded in the same solvent. Sprecher and co-workers[3] did not report the solvent used for their UV measurements (it was probably the HPLC eluent), and a comparison of their data with ours is therefore burdened with some uncertainty; however, with that reservation the UV maxima recorded for our compounds confirm the structures assigned to the metabolites.

In conclusion we have synthesized the methyl esters of three polyunsaturated fatty acid metabolites **3**, **4** and **5** starting from the naturally occurring fatty acids EPA and DHA. Since ^{14}C-labelled trimethyl phosphonoacetate is commercially available, specifically labelled samples of the acids can easily be prepared using our synthetic routes.

Experimental

General

The NMR spectra were recorded in $CDCl_3$ with a Varian Gemini 200, a Bruker Avance DPX 200 instrument, a Bruker Avance DPX 300 instrument or with a Bruker Avance DRX 500 instrument. IR spectra were obtained with a Perkin-Elmer 1310 infrared spectrophotometer or a Nicolet Magna-IR 550 spectrometer. Mass spectra were recorded at 70 eV with a Fisons VG Pro spectrometer. The UV spectra were recorded on a Shimadzu UV-260 spectrometer. Dry dichloromethane was obtained by distillation over calcium hydride. Methanol was dried over magnesium.

(all-*Z*)-2,3-Epoxyoctadeca-6,9,12,15-tetraenal 12

The pH of a solution of 30% hydrogen peroxide (2.3 g, 20.3 mmol) in MeOH (100 ml) was adjusted to 9 by addition of 0.1 M NaOH. Then a solution of aldehyde **11** (4.1 g, 15.9 mmol) in MeOH (80 ml) was added dropwise at 0 °C. The pH was maintained at 8–9 by addition of 0.1 M NaOH. After 1 h at 0 °C, water was added and the solution extracted with hexane–ether (1 : 1). The combined organic phases were washed with water, brine and dried ($MgSO_4$). Evaporation of solvents followed by flash chromatography (silica gel, 95 : 5 hexane–EtOAc) gave the epoxyaldehyde **12** (3.0 g, 69%). ν_{max}(film)/cm^{-1} 3012, 2964, 2933, 1731, 1437. δ_H(300 MHz): 0.93 (t, *J* 7.5 Hz, 3H, CH_3), 1.59–1.78 (m, 2H, CH_2), 2.05 (m, 2H, CH_2), 2,17–2.28 (m, 2H, CH_2), 2.69–2.87 (m, 6H, 3 × CH_2), 3.10 (dd, *J* 1.9, 6.2 Hz, 1H, C-2), 3.16–3.24 (m, 1H, C-3), 5.20–5.45 (m, 8H, 8 × CH=), 8.97 (d, *J* 6.2 Hz, 1H, CHO). δ_C(75 MHz): 14.1 (CH_3), 20.4 (C-17), 23.4, 25.4, 25.5, 25.6 (4 × CH_2), 31.0 (C-4), 56.1 (C-3), 59.0 (C-2), 126.9, 127.6, 127.7, 127.8, 128.3, 128.5, 129.4, 131.9 (8 × CH=), 198.0 (C=O). MS (EI): *m*/*z* 274 (M^+, 1.4%), 218, 79 (100). HRMS (EI): found: 274.1923; calc. for $C_{18}H_{26}O_2$: 274.1933.

(all-*Z*)-2,3-Epoxyoctadeca-6,9,12,15-tetraenol 13

A solution of $NaBH_4$ (1.4 g, 37.0 mmol) in MeOH (50 ml) was added to an ice-cooled solution of the epoxyaldehyde **12** (2.9 g, 10.6 mmol) in MeOH (50 ml). After 30 min at 0 °C, 1.4 M HCl (30 ml) was added and the mixture extracted with 1 : 1 of hexane–ether. The combined organic extracts were washed with water until neutral, then with brine and dried ($MgSO_4$). Evaporation of solvents followed by flash chromatography (silica gel, 7 : 3 hexane–EtOAc) gave the epoxyalcohol **13** (2.5 g, 86%), as an oil. ν_{max}(film)/cm^{-1} 3423 (br), 3012, 2963, 2932, 1430. δ_H(300 MHz): 0.93 (t, *J* 7.5 Hz, 3H, CH_3), 1.54–1.67 (m, 2H, CH_2), 2.03 (m, 2H, CH_2), 2.12–2.23 (m, 2H, CH_2), 2.38 (br s, 1H, OH), 2.69–2.85 (m, 6H, 3 × CH_2), 2.85–2.92 (m, 2H, CH_2), 3.54 (ddd, *J* 2.1, 4.6, 12.6 Hz, 1H, C-3), 3.84 (ddd, *J* 2.4, 3.2, 12.6 Hz, 1H, C-2), 5.20–5.45 (m, 8H, 8 × CH=). δ_C(75 MHz): 14.2 (CH_3), 20.4 (C-17), 23.6, 25.4, 25.5, 25.6 (4 × CH_2), 31.4 (C-4), 55.4 (C-3), 58.6 (C-2), 61.7 (C-1), 126.9, 127.7, 127.9, 128.2, 128.4, 128.5, 128.8, 131.9 (8 × CH=). MS (EI): *m*/*z* 276 (M^+, 0.4%), 79 (100). HRMS (EI): found: 276.2066; calc. for $C_{18}H_{28}O_2$: 276.2089.

(all-*Z*)-Hexadeca-4,7,10,13-tetraenal 14

Procedure 1. Periodic acid (5.5 g, 24.1 mmol) was added to a solution of the epoxyalcohol **13** (2.4 g, 8.7 mmol) in dry ether

(150 ml). The mixture was left stirring at room temperature for 4 h. The mixture was filtered and the filtrate washed with water until neutral, then with brine and dried ($MgSO_4$). Evaporation of solvents followed by flash chromatography (silica gel, 95:5 hexane–EtOAc) gave aldehyde **14** (1.2 g, 61%), as an oil.

Procedure 2. A solution of BuLi (1.4 M in hexane, 0.41 ml, 0.57 mmol) was added dropwise to an ice-cooled mixture of (methoxymethyl)triphenylphosphonium chloride (240 mg, 0.69 mmol) in dry ether (8 ml). After stirring for 5 min at 0 °C, a solution of the aldehyde **9** (100 mg, 0.46 mmol) in dry ether (1 ml) was added. The mixture was left stirring at ambient temperature for 2.5 h. Water was added and the mixture extracted with ether. The combined extracts were concentrated, the residue dissolved in dioxane (7 ml) and cooled to 0 °C. A solution of 80% aqueous formic acid (7 ml) was added. After stirring for 1 h at room temperature, water was added and the mixture extracted with hexane. The combined organic extracts were neutralized with saturated, aqueous $NaHCO_3$, washed with water and brine, and dried ($MgSO_4$). Evaporation of solvents followed by flash chromatography (silica gel, 95:5 hexane–EtOAc) gave the aldehyde **14** (40 mg, 38%) as an oil. ν_{max}(film)/cm^{-1} 3011, 2963, 2932, 1725, 1390. δ_H(300 MHz): 0.94 (t, *J* 7.5 Hz, 3H, CH_3), 2.04 (m, 2H, CH_2), 2.32–2.43 (m, 2H, CH_2), 2.44–2.53 (m, 2H, CH_2), 2.72–2.89 (m, 6H, 3 × CH_2), 5.21–5.48 (m, 8H, 8 × CH=), 9.74 (t, *J* 1.4 Hz, 1H, CHO). δ_C(75 MHz): 14.2 (CH_3), 20.5 (C-15), 20.0, 25.4, 25.5, 25.6 (4 × CH_2), 43.6 (C-2), 126.9, 127.5, 127.6, 127.7, 128.3, 128.5, 129.3, 131.9 (8 × CH=), 201.7 (C=O). MS (EI): *m*/*z* 232 (M^+, 0.5%), 188, 79 (100). HRMS (EI): found: 232.1812; calc. for $C_{16}H_{24}O$: 232.1827.

Methyl (all-*Z*)-octadeca-2,6,9,12,15-pentaenoate 16

A solution of potassium hexamethyldisilazane (0.66 M in toluene, 4.50 ml, 3.0 mmol) was added to a mixture of methyl [bis(trifluoroethyl)phosphono]acetate (**15**, 0.88 g, 2.8 mmol) and 18-crown-6 (3.30 g, 12.5 mmol) in THF (50 ml) at −78 °C, and the mixture was left stirring for 10 min. A solution of the aldehyde **14** (0.58 g, 2.5 mmol) in THF (5 ml) was then added. After 45 min at −78 °C, the mixture was allowed to attain room temperature. Saturated aqueous NH_4Cl was added and the aqueous phase extracted with ether. The combined organic extracts were washed with water, brine and dried ($MgSO_4$). The solution was concentrated under reduced pressure and the residue purified by flash chromatography (silica gel, 95:5 hexane–EtOAc) to give the ester **16** (0.60 g, 84%) (2*Z*:2*E* = 98:2). ν_{max}(film)/cm^{-1} 3012, 2964, 2932, 1725, 1639, 1438, 1176. δ_H(300 MHz): 0.94 (t, *J* 7.5 Hz, 3H, CH_3), 2.04 (m, 2H, CH_2), 2.14–2.30 (m, 2H, CH_2), 2.71 (dq, *J* 1.6, 7.4 Hz, 2H, CH_2), 2.74–2.88 (m, 6H, 3 × CH_2), 3.67 (s, 3H, OCH_3), 5.23–5.46 (m, 8H, 8 × CH=), 5.77 (dt, *J* 1.6, 11.5 Hz, 1H, C2-CH), 6.20 (dt, *J* 7.4, 11.5 Hz, 1H, C3-CH). δ_C(75 MHz): 14.2 (CH_3), 20.5 (C-17), 25.4, 25.5, 25.6, 26.5, 28.8 (5 × CH_2), 50.9 (OCH_3), 119.6, 126.9, 127.8, 128.0, 128.1, 128.5, 128.7, 128.8, 131.9 (9 × CH=), 149.7 (C-3), 166.6 (C=O). MS (EI): *m*/*z* 288 (M^+, 1%), 257, 79 (100). HRMS (EI): found: 288.2090; calc. for $C_{19}H_{28}O_2$: 288.2089.

(all-*Z*)-Octadeca-2,6,9,12,15-pentaenol 17

A solution of DIBAL-H (1 M hexane solution, 4.9 ml, 4.9 mmol) was added to a stirred solution of the ester **16** (0.70 g, 2.4 mmol) in dry hexane (40 ml) at 0 °C. After 2 h at 0 °C, HCl (1.4 M, 35 ml) was added and the aqueous phase extracted with ether. The combined organic extracts were washed with water, brine and dried ($MgSO_4$). Evaporation of solvents followed by flash chromatography (silica gel, 8:2 hexane–EtOAc) gave the alcohol **17** (0.48 g, 75%), as an oil. ν_{max}(film)/cm^{-1} 3324 (br), 3012, 2963, 2932, 1654, 1456. δ_H(300 MHz): 0.95 (t, *J* 7.5 Hz, 3H, CH_3), 1.45 (br s, 1H, OH), 2.05 (m, 2H, CH_2), 2.07–2.24 (m, 4H, 2 × CH_2), 2.72–2.88 (m, 6H, 3 × CH_2), 4.15 (d, *J* 6.3 Hz, 2H, CH_2OH), 5.22–5.43 (m, 8H, 8 × CH=), 5.44–5.73 (m, 2H, 2 × CH=). δ_C(75 MHz): 14.2 (CH_3), 20.5 (C-17), 25.4, 25.5, 25.6, 27.1, 27.3 (5 × CH_2), 58.4 (C-1), 127.0, 127.8, 127.9, 128.1, 128.5, 128.6, 129.0, 129.1, 132.0, 132.1 (10 × CH=). MS (EI): *m*/*z* 260 (M^+, 0.1%), 242, 79 (100). HRMS (EI): found: 260.2137; calc. for $C_{18}H_{28}O$: 260.2140.

(all-*Z*)-Octadeca-2,6,9,12,15-pentaenal 18

A solution of the alcohol **17** (150 mg, 0.58 mmol) in dry CH_2Cl_2 (1.5 ml) was added to an ice-cooled solution of periodinane (0.32 g, 0.75 mmol) in dry CH_2Cl_2 (10 ml) kept in the dark. After stirring at 0 °C for 1 h, the mixture was diluted with ether (15 ml) and poured into saturated aqueous $NaHCO_3$ containing 1.9 g $Na_2S_2O_3$ (10 ml). The mixture was stirred in the dark for 10–15 min. The layers were separated and the aqueous phase extracted with ether. The combined organic extracts were washed with water until neutral, then with brine and dried ($MgSO_4$). The solution was concentrated in the dark under reduced pressure to give the crude aldehyde **18** (0.14 g, 95%). ν_{max}(film)/cm^{-1} 3012, 2963, 2930, 1682, 1455. δ_H(300 MHz): 0.92 (t, *J* 7.5 Hz, 3H, CH_3), 2.02 (m, 2H, CH_2), 2.19–2.32 (m, 2H, CH_2), 2.58–2.69 (m, 2H, CH_2), 2.70–2.85 (m, 6H, 3 × CH_2), 5.18–5.50 (m, 8H, 8 × CH=), 5.96 (ddt, *J* 1.5, 8.0, 11.2 Hz, 1H, C2-CH), 6.57 (dt, *J* 8.1, 11.2 Hz, 1H, C3-CH), 10.00 (d, *J* 8.0 Hz, 1H, CHO); δ_C(75 MHz): 14.1 (CH_3), 20.4 (C-17), 25.4, 25.5, 25.6, 26.5, 27.8 (5 × CH_2), 126.8, 127.5, 127.6, 127.7, 128.3, 128.5, 129.6, 130.4, 131.9 (9 × CH=), 152.0 (C-3), 190.7 (C=O).

Methyl (2*E*,4*Z*,8*Z*,11*Z*,14*Z*,17*Z*)-icosa-2,4,8,11,14,17-hexaenoate 6

A solution of BuLi (1.4 M in hexane, 0.30 ml, 0.42 mmol) was added dropwise to a solution of trimethyl phosphonoacetate (0.075 ml, 0.46 mmol) in dry DME (4 ml) at 0 °C. After stirring for 10 min at 0 °C, the solution was cooled to −78 °C and the aldehyde **18** (100 mg, 0.39 mmol) in dry DME (1 ml) was added dropwise. After 2 h at −78 °C, the cold bath was removed and water was added. The mixture was extracted with ether. The combined organic extracts were washed with water, brine and dried ($MgSO_4$). Evaporation of solvents followed by flash chromatography (silica gel, 98:2 hexane–EtOAc) gave the ester **6** (69 mg, 57%). UV (MeOH): λ_{max} = 264 nm, log (ε/dm^3 mol^{-1} cm^{-1}) = 4.340. ν_{max}(film)/cm^{-1} 3012, 2963, 2933, 1720, 1639, 1435, 1270, 1160. δ_H(300 MHz): 0.94 (t, *J* 7.5 Hz, 3H, CH_3), 2.04 (m, 2H, CH_2), 2.12–2.24 (m, 2H, C7-CH_2), 2.28–2.42 (m, 2H, C6-CH_2), 2.71–2.87 (m, 6H, 3 × CH_2), 3.71 (s, 3H, OCH_3), 5.27–5.50 (m, 8H, 8 × CH=), 5.84 (d, *J* 15.2 Hz, 1H, C2-CH), 5.75–5.86 (m, 1H, C5-CH), 6.05–6.18 (m, 1H, C4-CH), 7.57 (ddd, *J* 0.9, 11.7, 15.3 Hz, 1H, C3-CH). δ_C(75 MHz): 14.2 (CH_3), 20.5 (C-19), 25.4, 25.5, 25.6 (3 × CH_2), 26.8 (C-7), 28.1 (C-6), 51.4 (OCH_3), 121.0 (C-2), 126.8 (C-4), 127.0, 127.8, 128.0, 128.1, 128.4, 128.5, 128.9, 131.9 (8 × CH=), 139.5 (C-3), 140.5 (C-4), 167.5 (C=O). MS (EI): 314 (M^+, 1.6%), 299, 283, 79 (100). HRMS (EI): found: 314.2246; calc. for $C_{21}H_{30}O_2$: 314.2246.

Methyl (2*E*,4*E*,8*Z*,11*Z*,14*Z*,17*Z*)-icosa-2,4,8,11,14,17-hexaenoate 19

A solution of LDA was prepared from BuLi (1.4 M in hexane, 1.94 ml, 2.72 mmol) and diisopropylamine (0.38 ml, 2.69 mmol) in dry THF (30 ml) at −20 °C. The solution was cooled to −78 °C and trimethyl phosphonoacetate (560 mg, 3.08 mmol) in dry THF (5 ml) was added. After an additional 30 min with stirring at −78 °C, the temperature was raised to −50 °C and a solution of the aldehyde **11** (500 mg, 1.94 mmol) in dry THF (5 ml) was added dropwise. The mixture was left stirring for 2 h. The mixture was poured into saturated aqueous NH_4Cl (25 ml), the organic phase was separated, and the aqueous phase extracted with ether. The combined organic phases were

washed with brine and dried ($MgSO_4$). Evaporation of solvents followed by flash chromatography (silica gel, 95:5 hexane–EtOAc) gave the ester **19** (430 mg, 70%), (2*E*:2*Z* = 95:5). UV (MeOH): λ_{max} = 260 nm, log (ε/dm^3 mol^{-1} cm^{-1}) = 4.445. ν_{max}(film)/cm^{-1} 3012, 2963, 2933, 1721, 1645, 1435, 1261, 1135. δ_H(300 MHz): 0.94 (t, *J* 7.5 Hz, 3H, CH_3), 2.04 (m, 2H, C19-CH_2), 2.11–2.31 (m, 4H, C6-, C7-CH_2), 2.71–2.88 (m, 6H, 3 × CH_2), 3.69 (s, 3H, OCH_3), 5.22–5.41 (m, 8H, 8 × CH=), 5.76 (d, *J* 15.4 Hz, 1H, C2–CH), 5.97–6.01 (m, 2H, C4-, C5-CH), 7.22 (dd, *J* 9.9, 15.4 Hz, 1H, C3-CH). δ_C(75 MHz): 14.2 (CH_3), 20.5 (C-19), 25.4, 25.5, 25.6, 26.3 (4 × CH_2), 32.8 (C-6), 51.3 (OCH_3), 119.0 (C-2), 126.9, 127.7, 127.9, 128.1, 128.4, 128.5, 128.6, 128.7, 131.9 (9 × CH=), 143.6 (C-3), 145.0 (C-5), 167.5 (C=O). MS (EI): *m*/*z* 314 (M$^+$, 0.8%), 283, 79 (100). HRMS (EI): found: 314.2241; calc. for $C_{21}H_{30}O_2$: 314.2246.

Methyl (2*Z*,4*E*,8*Z*,11*Z*,14*Z*,17*Z*)-icosa-2,4,8,11,14,17-hexaenoate 20

Reaction of the aldehyde **11** with the phosphonoacetate **15** as described for **16** afforded the ester **20** in 80% yield and 98% stereochemically pure. UV (MeOH): λ_{max} = 262 nm, log (ε/dm^3 mol^{-1} cm^{-1}) = 4.345. ν_{max}(film)/cm^{-1} 3012, 2964, 2933, 1720, 1640, 1438, 1195, 1177. δ_H(300 MHz): 0.94 (t, *J* 7.5 Hz, 3H, CH_3), 2.04 (m, 2H, C19–CH_2), 2.14–2.30 (m, 4H, C6-, C7-CH_2), 2.70–2.88 (m, 6H, 3 × CH_2), 3.68 (s, 3H, OCH_3), 5.22–5.42 (m, 8H, 8 × CH=), 5.55 (d, *J* 11.3 Hz, 1H, C2-CH), 6.03 (dt, *J* 6.6, 15.2 Hz, 1H, C5-CH), 6.51 (t, *J* 11.3 Hz, 1H, C3-CH), 7.36 (dd, *J* 11.3, 15.2 Hz, 1H, C4-CH). δ_C(75 MHz): 14.2 (CH_3), 20.5 (C-19), 25.4, 25.5, 25.6 (3 × CH_2), 26.4 (C-7), 32.9 (C-6), 50.9 (OCH_3), 115.3 (C-2), 126.8 (CH=), 127.8 (C-4), 127.9, 128.0, 128.4, 128.6, 128.7, 128.8, 131.9 (7 × CH=), 144.5 (C-5), 145.2 (C-3), 166.7 (C=O). MS (EI): *m*/*z* 314 (M$^+$, 2%), 283, 93 (100), 79. HRMS (EI): found: 314.2246; calc. for $C_{21}H_{30}O_2$: 314.2246.

Methyl (2*E*)-[bis(trifluoroethyl)phosphono]butenoate 21

The compound was prepared from trimethyl phosphonocrotonate in 26% yield, essentially as described[6] for the preparation of the phosphonoacetate **15**. ν_{max}(film)/cm^{-1} 3008, 2973, 2961, 1727, 1661, 1290, 1263, 1074, 964. δ_H(200 MHz): 2.86 (ddd, *J* 1.2, 7.8, 23.9 Hz, 2H), 3.68 (s, 3H), 4.34 (m, 4H), 5.98 (ddt, *J* 1.2, 5.5, 15.6 Hz, 1H), 6.76 (m, 1H). δ_C(50 MHz): 29.9 (d, J_{CP} 141.0 Hz, CH_2), 51.1 (CH_3), 62.3 (dq, J_{COP} 6.2 Hz, J_{CCF} 37.8 Hz, CH_2), 122.7 (dq, J_{CCOP} 7.5 Hz, J_{CF} 275.8 Hz, CF_3), 127.1 (d, J_{CCCP} 14.6 Hz, CH), 134.5 (d, J_{CCP} 11.8 Hz, CH), 165.5 (C=O). δ_F(188 MHz): −75.9 (t, J_{CF} 8.1 Hz). MS (EI): *m*/*z* 344 (M$^+$, 8%), 313, 312, 293, 285, 284 (100), 201, 163, 68. HRMS (EI): found: 344.0231; calc. for $C_9F_6H_{11}O_5P$: 344.0248.

(all-*Z*)-Octadeca-6,9,12,15-tetraenal 22

To a stirred suspension of CuI (50 mg, 0.26 mmol) in THF (15 ml), kept at −50 °C, HMPA (2.3 ml, 13.7 mmol) and DIBAL-H (1 M hexane solution, 2.6 ml, 2.6 mmol) were added successively. The mixture was left stirring at −50 °C for 20 min, and then a solution of the aldehyde **11** (500 mg, 1.9 mmol) in THF (5 ml) was added. After 1.5 h at −50 °C, 5% aq. HCl (20 ml) was added and the reaction mixture extracted with hexane. The combined organic extracts were washed with water until neutral, then with brine and dried ($MgSO_4$). Evaporation of solvents followed by flash chromatography (silica gel, 95:5 hexane–EtOAc) gave the aldehyde **22** (410 mg, 82%). ν_{max}(film)/cm^{-1} 3011, 2962, 2932, 1727, 1453. δ_H(300 MHz): 0.94 (t, *J* 7.5 Hz, 3H, CH_3), 1.32–1.42 (m, 2H, CH_2), 1.53–1.68 (m, 2H, CH_2), 1.98–2.12 (m, 4H, 2 × CH_2), 2.40 (dt, *J* 1.7, 7.3 Hz, 2H, C2-CH_2), 2.71–2.87 (m, 6H, 3 × CH_2), 5.19–5.41 (m, 8H, 8 × CH=), 9.72 (t, *J* 1.7 Hz, 1H, CHO). δ_C(75 MHz): 14.2 (CH_3), 20.4 (C-17), 25.5 (2 × CH_2), 21.6, 25.4, 26.8, 29.0 (4 × CH_2), 43.6 (C-2), 126.9, 127.8, 128.0, 128.1, 128.2, 128.4, 129.4, 131.9 (8 × CH=), 202.2 (C=O). MS (EI): *m*/*z* 260 (M$^+$, 1.5%), 232, 79 (100). HRMS (EI): found: 260.2128; calc. for $C_{18}H_{22}O$: 260.2140.

Methyl (2*E*,8*Z*,11*Z*,14*Z*,17*Z*)-icosa-2,8,11,14,17-pentaenoate 7

Trimethyl phosphonoacetate (84 mg, 0.46 mmol) was added to an ice-cooled solution of sodium methoxide (0.43 mmol) in MeOH (4 ml). After stirring for 10 min at 0 °C, a solution of the aldehyde **22** (100 mg, 0.39 mmol) in dry MeOH (2 ml) was added. After stirring for 2 h at ambient temperature, water was added and the mixture extracted with ether. The combined organic extracts were washed with water until neutral, then with brine and dried ($MgSO_4$). Evaporation of solvents followed by flash chromatography (silica gel, 98:2 hexane–EtOAc) gave the ester **7** (65 mg, 53%). UV (MeOH): λ_{max} = 206 nm, log (ε/dm^3 mol^{-1} cm^{-1}) = 4.459. ν_{max}(film)/cm^{-1} 3012, 2963, 2932, 1727, 1663, 1463, 1270, 1198. δ_H(300 MHz): 0.94 (t, *J* 7.5 Hz, 3H, CH_3), 1.22–1.50 (m, 4H, C5-, C6- CH_2), 1.95–2.12 (m, 4H, C7-, C19-CH_2), 2.13–2.25 (m, 2H, C4-CH_2), 2.70–2.86 (m, 6H, C10-, C13-, C16-CH_2), 3.69 (s, 3H, OCH_3), 5.19–5.42 (m, 8H, 8 × CH=), 5.79 (dt, *J* 1.5, 15.6 Hz, 1H, C2-CH), 6.94 (dt, *J* 7.0, 15.6 Hz, 1H, C3-CH). δ_C(50 MHz): 14.2 (CH_3), 20.5 (C-19), 25.5 (CH_2), 25.6 (2 × CH_2), 26.9 (C-7), 27.6 (C-6), 29.0 (C-5), 32.1 (C-4), 51.3 (OCH_3), 128.0 (2 × CH=), 120.9, 127.0, 127.9, 128.2, 128.5, 129.7, 132.0 (7 × CH=), 149.4 (C-3), 167.1 (C=O). MS (EI): *m*/*z* 316 (M$^+$, 4%), 285, 79 (100). HRMS (EI): found: 316.2394; calc. for $C_{18}H_{22}O$: 316.2402.

Methyl (all-*Z*)-icosa-2,5,8,11,14,17-hexaenoate 24

Using the method described for compound **16**, reaction of the aldehyde **10** with the phosphonoacetate **15** gave the ester **23** (70%), >95% stereochemically pure. ν_{max}(film)/cm^{-1} 3013, 2964, 2932, 1724, 1644, 1437, 1203, 1169. δ_H(300 MHz): 0.95 (t, *J* 7.5 Hz, 3H, CH_3), 2.05 (m, 2H, CH_2), 2.71–2.90 (m, 8H, 4 × CH_2), 3.45 (m, 2H, CH_2), 3.69 (s, 3H, OCH_3), 5.23–5.50 (m, 10 H, 10 × CH=), 5.77 (dt, *J* 1.8, 11.4 Hz, 1H, C2-CH), 6.16 (dt, *J* 7.4, 11.4 Hz, 1H, C3-CH). δ_C(75 MHz): 14.2 (CH_3), 20.5 (C-19), 25.5, 25.6, 25.7, 26.9, 27.4 (5 × CH_2), 51.0 (OCH_3), 119.1, 126.1, 127.0, 127.8, 127.9, 128.0, 128.2, 128.3, 128.5, 129.8, 131.9 (11 × CH=), 148.1 (C-3), 166.6 (C=O). MS (EI): *m*/*z* 314 (M$^+$, 0.8%), 283, 79 (100). HRMS (EI): found: 314.2250; calc. for $C_{21}H_{30}O_2$: 314.2246.

(all-*Z*)-Icosa-2,5,8,11,14,17-hexaenol 25

Reduction of the ester **23** with DIBAL-H as described for the preparation of **17** gave the alcohol **24** in 76% yield. ν_{max}(film)/cm^{-1} 3331 (br), 3014, 2964, 2932, 1435. δ_H(300 MHz): 0.95 (t, *J* 7.5 Hz, 3H, CH_3), 1.53 (br s, 1H, OH), 2.05 (m, 2H, CH_2), 2.72–2.93 (m, 10H, 5 × CH_2), 4.20 (d, *J* 6.2 Hz, 2H, CH_2), 5.23–5.47 (m, 10 H, 10 × CH=), 5.48–5.67 (m, 2H, CH=). δ_C(75 MHz): 14.2 (CH_3), 20.5 (C-19), 25.5, 25.6, 25.8 (4 × CH_2), 58.4 (C-1), 127.0, 127.5, 127.8, 127.9, 128.0, 128.2, 128.3, 128.5, 128.6, 128.8, 130.7, 132.0 (12 × CH=). MS (EI): *m*/*z* 286 (M$^+$, 0.1%), 268, 79 (100). HRMS (EI): found: 286.2296; calc. for $C_{20}H_{30}O$: 286.2297.

(all-*Z*)-Icosa-2,5,8,11,14,17-hexaenal 26

Oxidation of the alcohol **24** with periodinane as described for the preparation of **18** gave in 96% yield the crude aldehyde **25**, which was used in the next step without further purification. δ_H(300 MHz): 0.93 (t, *J* 7.5 Hz, 3H, CH_3), 2.04 (m, 2H), 2.70–2.88 (m, 8H, 4 × CH_2), 3.35 (m, 2H, CH_2), 5.20–5.45 (m, 9H, 9 × CH=), 5.46–5.58 (m, 1H, CH=), 5.94 (ddt, *J* 1.6, 7.8, 11.1 Hz, 1H, C2-CH), 6.52 (dt, *J* 8.1, 11.1 Hz, 1H, C3-CH), 10.08 (d, *J* 7.8 Hz, 1H, CHO). δ_C(75 MHz): 14.2 (CH_3), 20.5 (C-19), 25.4, 25.5, 25.6, 25.7, 26.4 (5 × CH_2), 125.0, 126.9, 127.2, 127.6, 127.7, 128.4, 128.5, 128.7, 129.6, 130.6, 131.9 (11 × CH=), 150.2 (C-3), 190.5 (C=O).

Methyl (2*E*,4*Z*,7*Z*,10*Z*,13*Z*,16*Z*,19*Z*)-docosa-2,4,7,10,13,16,19-heptaenoate 8

Following the procedure described for the preparation of the ester **6**, reaction of the aldehyde **25** and trimethyl phosphonoacetate gave the ester **8** in 55% yield. UV (MeOH): λ_{max} = 265 nm, log (ε/dm^3 mol^{-1} cm^{-1}) = 4.362. ν_{max}(film)/cm^{-1} 3014, 2963, 2932, 1719, 1637, 1436, 1265, 1165. δ_H(500 MHz): 0.95 (t, *J* 7.5 Hz, 3H, CH_3), 2.05 (m, 2H, C21-CH_2), 2.75–2.87 (m, 8H, 4 × CH_2), 3.06 (m, 2H, C6-CH_2), 3.72 (s, 3H, OCH_3), 5.23–5.47 (m, 10H, 10 × CH=), 5.71–5.86 (m, 1H, C5-CH), 5.87 (d, *J* 15.3 Hz, 1H, C2-CH), 6.07–6.16 (m, 1H, C4-CH), 7.61 (ddd, *J* 0.9, 11.7, 15.3 Hz, 1H, C3-CH). δ_C(75 MHz): 14.2 (CH_3), 20.5 (C-21), 25.4, 25.5, 25.6, 25.7 (4 × CH_2), 26.5 (C-6), 51.5 (OCH_3), 121.3 (C-2), 126.4 (C-4), 126.5, 127.0, 127.7, 127.8, 127.9, 128.3, 128.4, 128.5, 129.5, 132.0 (10 × CH=), 138.8 (C-5), 139.2 (C-3), 167.5 (C=O). MS (EI): *m*/*z* 340 (M^+, 1%), 309, 79 (100). HRMS (EI): found: 340.2422; calc. for $C_{23}H_{32}O_2$: 340.2402).

Acknowledgements

We thank Norsk Hydro for generous gifts of the ethyl esters of EPA and DHA.

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