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Short communication

A divergent synthesis of [1-^{14}C]-mono-*E* isomers of fatty acids

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Abstract

A convenient synthesis of [1-^{14}C]-mono-*trans* fatty acid using olefin inversion as a key-step is described. This methodology allows for a facile synthesis of [1-^{14}C]-labelled mono-*trans* analogues of oleic, linoleic and linolenic acids. As an example, only eleven steps were necessary to obtain the [1-^{14}C]-mono-*E* isomers of linolenic acid from its commercial all-*Z* form. In the first step, Barton's decarboxylation procedure yielded a bromo intermediate. Epoxidation of this compound resulted in the formation of three monoepoxides, which could be separated by HPLC. After identification by ^{1}H NMR and MS, the pure monoepoxides were then subjected to inversion consisting of a stereospecific deoxygenation followed by a β-elimination step. Finally, the labelling was introduced by substitution of the bromine by a [^{14}C]-cyano group followed by hydrolysis.

Keywords: [1-^{14}C]-*trans*-fatty acid; Inversion of olefin; Barton's bromodecarboxylation

1. Introduction

All-*Z* polyunsaturated fatty acids such as linolenic acid, linoleic and oleic acids are commonly found in many vegetable oils (Zöllner, 1986). During the industrial process of edible oils production, i.e deodorisation or deep frying processes, *E* fatty acids are formed (Ackman et al., 1974) as a result of heat treatments (245–250 °C) (Grandgirard et al., 1984) or partial hydrogenation steps (Ratnayake and Beare-Rogers, 1990). As a consequence, margarines, cooking oils and fried products contain significant amounts, up to 10%, of *E* polyunsaturated fatty acids (Wolff and Sebedio, 1991; Wolff, 1992). This finding has prompted researchers to explore the in vivo metabolism of these fatty acids isomers with the help of labelled compounds. For this purpose many *E* fatty acids were labelled in our laboratory with carbon-14 at the carboxylic position (Eynard et al., 1996; Berdeaux et al., 1996). The in vivo biological results showed different behaviours in the desaturation–elongation process as well as in the oxidative metabolism (Bretillon et al., 1998a,b) of the *E* isomers compared to their *Z* analogues, highlighting the interest of such biological studies. The main difficulty concerning the preparation of labelled *E* fatty acids was the synthesis of unlabelled starting products which were not commercially available. In the past, our synthetic strategy was based on a step-by-step construction of each *E* isomer precursor using Wittig or copper-catalysed coupling reactions. This strategy is time consuming and involves many steps for each isomer precursor preparation. For example, thirteen steps were necessary to obtain the [1-^{14}C]-(9*E*,12*Z*,15*Z*)-octadecatrienoic acid.

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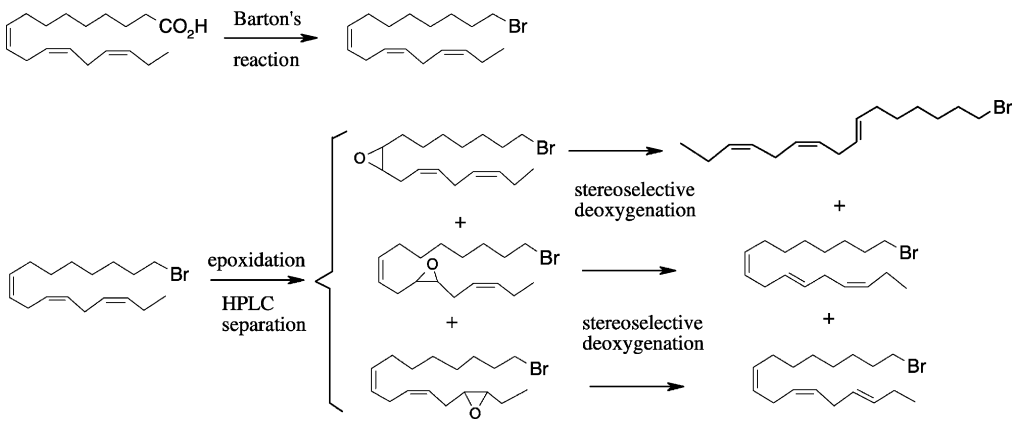
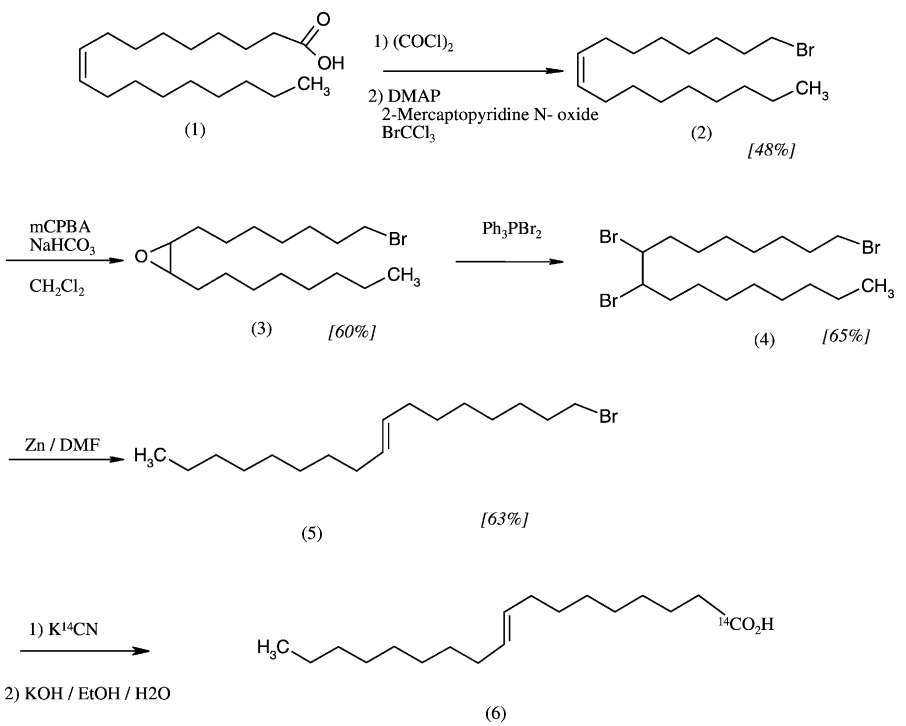
This communication describes a successful new approach to this chemical problem and provides a convenient methodology for the divergent synthesis of radiolabelled *E* fatty acids starting from natural all-*Z* isomers.

2. Results and discussion

A valuable route to introduce a carbon-14 into the carboxylic position of fatty acids consists to synthesise a bromo derivative, obtained by the Barton



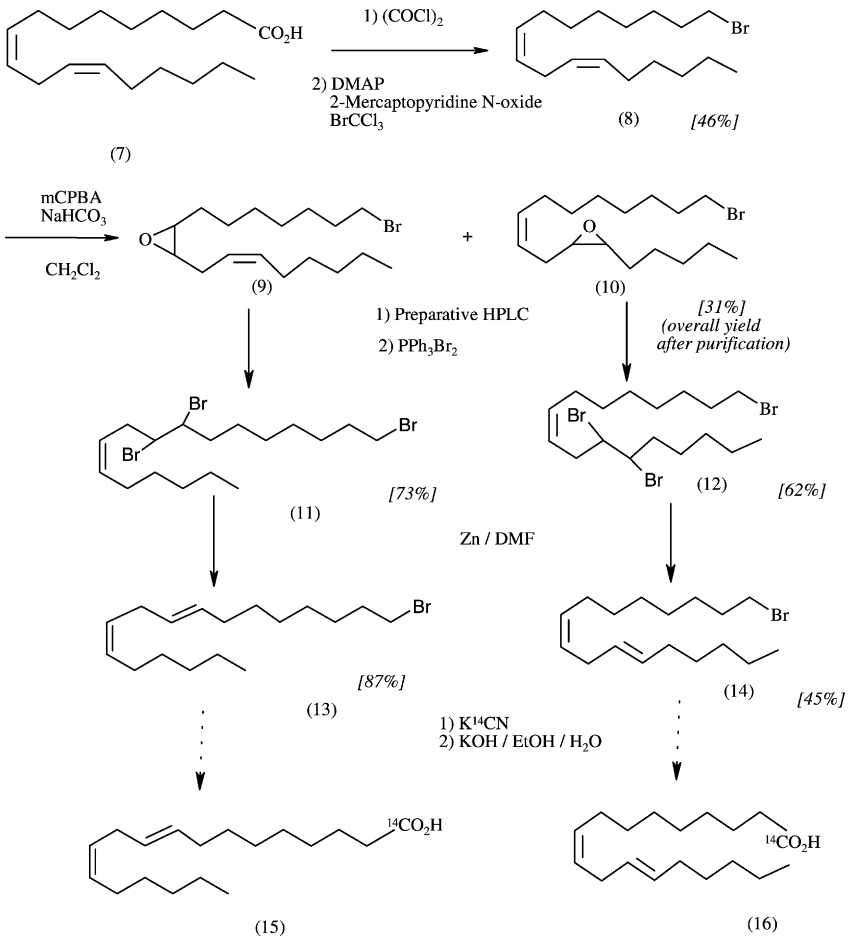
Scheme 1. Principle of the divergent synthesis.



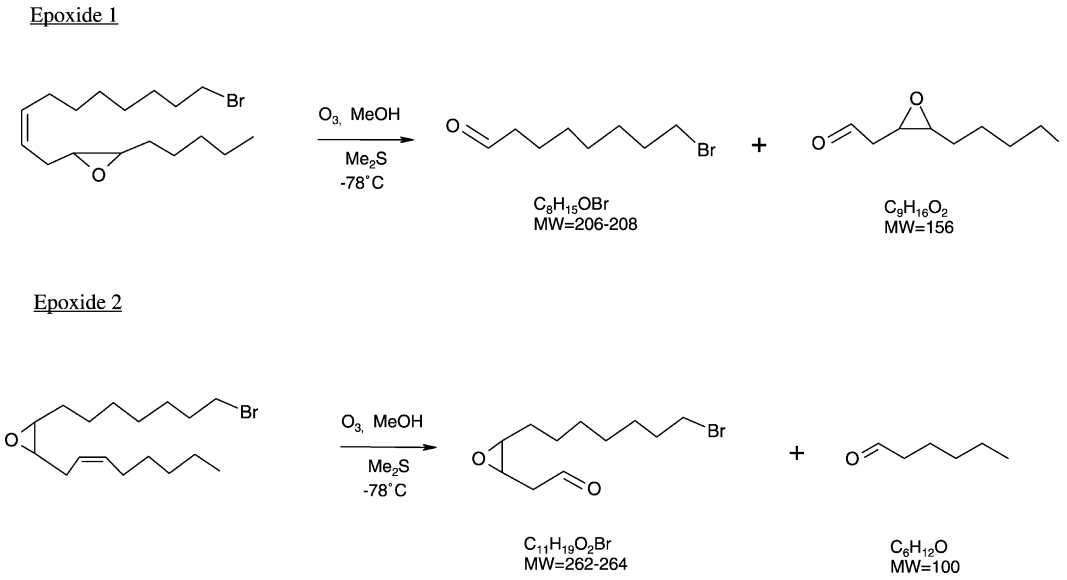
Scheme 2. Synthesis of the *trans* isomer of oleic acid.

bromodecarboxylation reaction (Barton et al., 1983) and to substitute the bromide atom by [^{14}C]-potassium cyanide followed by hydrolysis of the [^{14}C]-cyano group. Our main strategy, illustrated on Scheme 1, was to prepare such bromo precursor from commercially available all-*Z* fatty acids and to generate a mixture of isomers which will be separated and further functionalised. Oleic, linoleic and linolenic acids present an increasing complexity of their structure and were chosen to establish the procedure. Our synthetic route involved first to epoxidize the bromo all-*Z* derivative with one equivalent of metachloroperbenzoic acid (Imuta and Ziffer, 1979). After HPLC chromatography separation, each monoepoxyde can be submitted to stereospecific deoxygenation in order to form the corresponding double bound. This is the key-step which permits to reduce the length of the synthetic pathway. However, we had to face three difficulties. First, we had to set up a stereoselective inversion procedure compatible with the terminal bromine. Secondly, we had to establish a chromatographic separation of each monoepoxides. Thirdly, we had to elucidate each monoepoxide structure.

The first and main difficulty we had to face, was the stereospecific deoxygenation of the epoxides to the corresponding *E* olefins. Many reviews have considered the inversion of double bounds in olefins (Sonnet, 1980; Wong et al., 1987). In order to evaluate the known procedures, we used oleic acid as a reference compound. We studied some published techniques (Mena et al., 1984; Dervan and Shippey, 1976; Rosenblum et al., 1975) but they were not



Scheme 3. Synthesis of mono-*trans* isomers of linoleic acid.



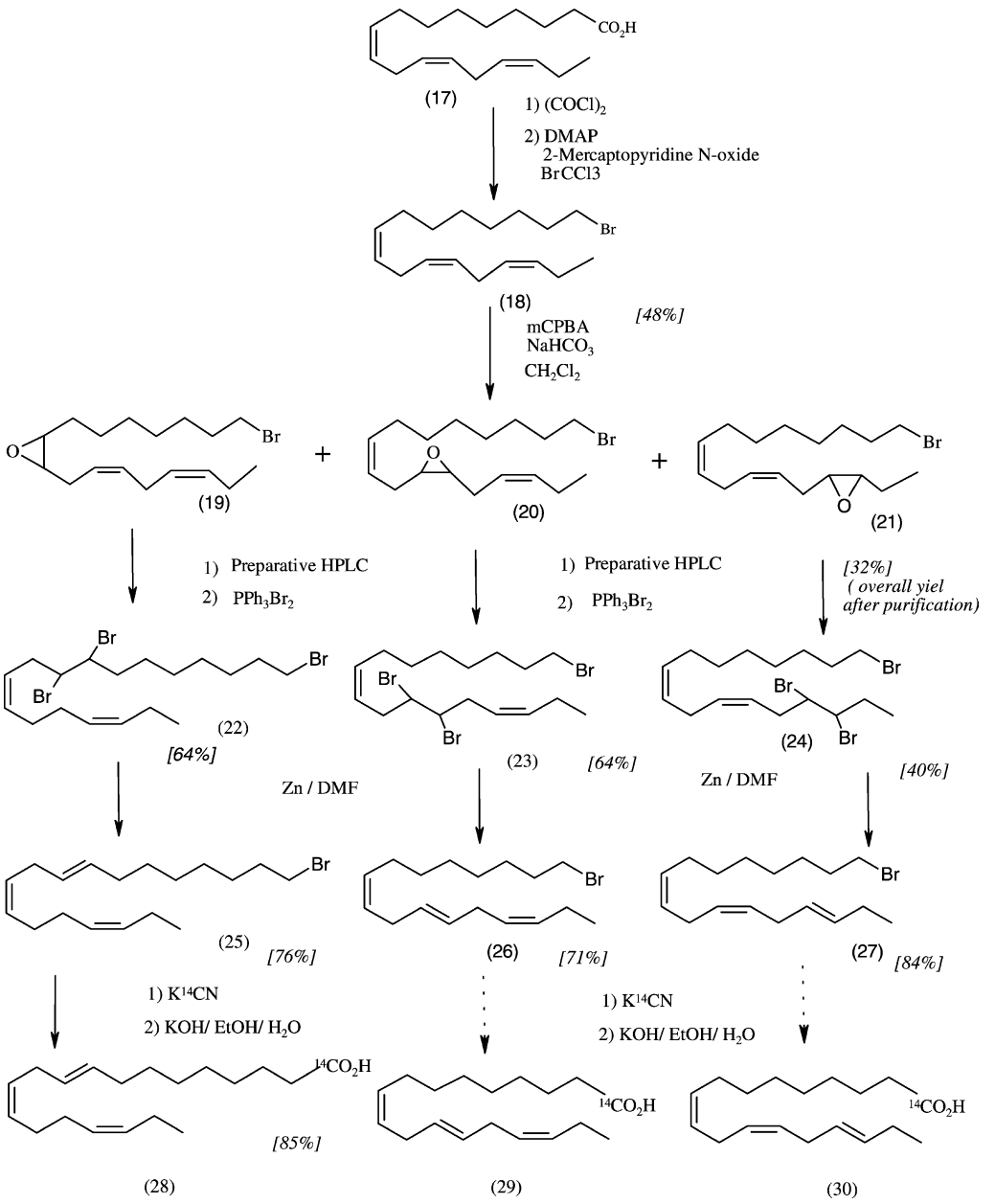
Scheme 4. Linoleic acid: differentiation of the epoxides.

compatible with the presence of a bromine on the terminal carbon of the chain. Finally, we adapted with success a high stereospecific method using triphenylphosphine dibromide and zinc (Sonnet and Oliver, 1976). The corresponding *E* bromo precursor was obtained in 41% global yield with a stereochemical purity greater than 99% (Scheme 2).

The one-carbon homologation was then accomplished by reaction of this *E* bromoprecursor with [1-^{14}C] potassium cyanide in DMSO to give the nitrile derivative affording after hydrolysis, the [1-^{14}C] corresponding labelled *E* oleic acid in good yield.

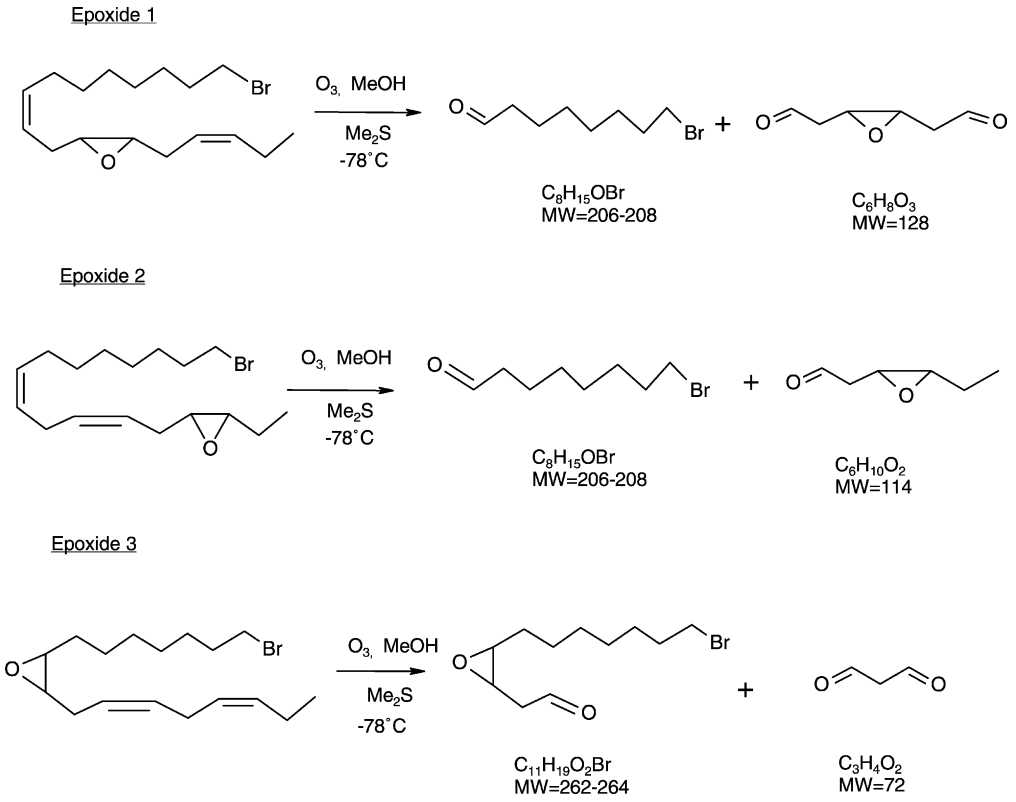
Encouraged by this result, we evaluated the procedure for the synthesis of mono-*E* linoleic acids isomers. After bromodecarboxylation of linoleic acid, the bromoderivative was oxidised with one equivalent of metachloroperbenzoic acid yielding a mixture of two monoepoxides, starting materials and by-products such as diepoxides. A flash chromatography was therefore necessary to isolate both monoepoxides which were successfully separated via a preparative Zorbax Sil 21.2 mm i.d. × 25 cm HPLC column using a mixture of 2,2,4-trimethylpentane–petroleum ether–diisopropyl ether (95–2.5–2.5) as eluant. Two purified fractions were obtained and first named epoxide 1 and epoxide 2 according to their order of elution (Schemes 3 and 4). Each fraction was subjected to an ozonolysis treatment (Pappas et al., 1966) followed by mass spectrometry analysis (DCI/NH_3) leading to the following results: *m*/*z*: 224, 226 for epoxide 1 and 280, 282 for epoxide 2. These major peak corresponded to the predicted molecular mass increased of 18 (+NH_3). The elucidation of the structure of both epoxides was therefore unambiguous.

These results validated our synthetic approach and motivated us to go through a more difficult task. Oxidation of the bromo derivative of all-*Z* linolenic acid generated three monoepoxides, first named epoxide 1, 2, 3 according to their elution order. We succeed to identify them with the help of NMR spectrometry combined with an ozonolysis followed by mass spectrometry analysis. Mass spectrometry of the ozonolysis residues showed the following results: *m*/*z*: 224–226 for epoxide 1; 224–226 for epoxide 2 and 280–282 for epoxide 3 (Scheme 5). We compared these experimental results with the expected fragmentations obtained from the three possible structures (Scheme 6). Two structures should give the theoretical values *m*/*z*: 224, 226; and the third one 280, 282. (Other fragments were too volatile to be observed.) So, mass spectrometry was enough to determinate the identity of epoxide 3. We investigate the structure of epoxide 1 using its NMR spectrum which showed a specific NMR signal (proton type 4, q.d, 2H) at



Scheme 5. Synthesis of the mono-*trans* isomers of linolenic acid.

2.15–2.45 ppm. As only one structure presented this feature, it was possible to assign the structure of epoxide 1. Furthermore, we identified epoxide 2, whose NMR spectrum showed two characteristic type 3 protons (m) at 1.55 ppm. (the different types of protons are described in Fig. 1.) As the ozonolysis-mass spectrum method did not differentiate epoxide 1 and epoxide 2, NMR spectrum was not enough to lift the ambiguity between epoxide 2 and epoxide 3. The combination of the two processes was necessary in this case.



Scheme 6. Linolenic acid: differentiation of the epoxides.

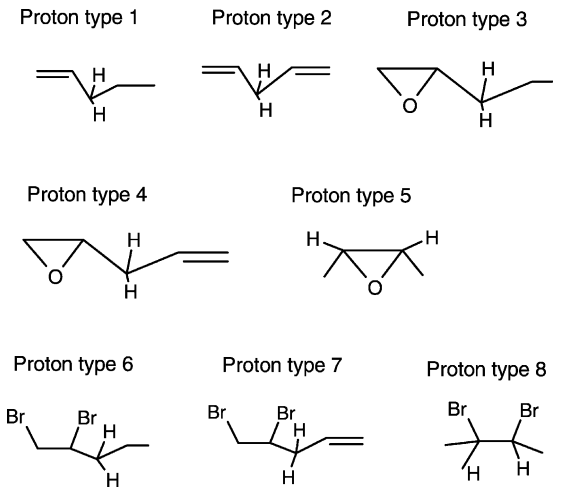


Fig. 1. Types of protons present in ^{1}H NMR($CDCl_3$) spectra.

After the labelling step, confirmed the structure of (9*E*, 12*Z*, 15*Z*)-[1-^{14}C]-octadeca-9,12,15-trienoic acid by coelution on a HP-23 *cis*/*trans* FAME gas chromatography column with an authentic sample obtained previously through a total synthesis (Eynard et al., 1994).

3. Conclusion

We carried out, via an efficient and concise procedure, the stereospecific synthesis of mono-*E* fatty acid isomers starting from all-*Z* isomer. Only eleven steps were necessary to obtain the three mono-*E* isomers of linolenic acid therefore showing the competitivity of the methodology toward more classical approaches (previous pathway afforded only one isomer in twelve steps). We are therefore convinced that this approach represents an advance and is of practical utility in the area of labelled fatty acids synthesis. This procedure

is applicable to a large variety of lipids allowing a time-saving synthesis of their *trans* analogues.

4. Experimental

A typical procedure for the inversion of a double bond of linolenic acid is described.

4.1. (3Z,6Z,9Z)-17-Bromoheptadeca-3,6,9-triene

Oxalyl chloride (10 g, 78.8 mmol) was added to a mixture of (9*E*,12*Z*,15*Z*)-octadeca-9,12,15-trienoic acid (Aldrich Chemicals) (5 g, 17.95 mmol) in 40 ml of dry toluene stirred under argon. The mixture was stirred at room temperature for 15 h under an argon atmosphere, concentrated under vacuum, and diluted three times with 30 ml of dry toluene before concentration to remove the oxalyl chloride residue, which could be very harmful for the second part of the reaction. The product was used without further purification. 2-Mercaptopyridine-*N*-oxide, sodium salt hydrate (3.5 g, 23.4 mmol) and 4-dimethylaminopyridine (244 mg, 2 mmol) were dried under vacuum overnight and introduced with 75 ml of bromotrichloromethane into a three-necked flask in an anhydrous glove box. Under the ventilated hood the flask was fitted with a reflux condenser and a system providing a slow flow of argon. The slurry was stirred and heated under reflux (105 °C).

In an anhydrous glove box the raw acyl chloride was diluted with 25 ml of bromotrichloromethane and stored in a syringe. This mixture was added through a septum to the reaction flask over 30 min. Bubbles and an orange coloration were observed and the reaction was stirred under reflux for 2 h.

A brown product appeared as a side product of the reaction. After cooling at room temperature the reaction mixture was filtered in order to eliminate the brown residue. The crude product was then concentrated under vacuum and flash chromatographed on silicagel with heptane elution to give (3*Z*,6*Z*,9*Z*)-17-bromoheptadeca-3,6,9-triene (2.71 g, 8.6 mmol, yield = 48%). IR (film): $\upsilon = 3011\,cm^{-1}$(s), 2963(s), 2930(s), 1652(w), 1460(m), 1395(w), 1252(m), 1067(w), 916(w), 793(w), 721(m), 648(w), 564(w). ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 0.95$ (t, $J = 7.5$ Hz, 3H, $-CH_2-C\underline{H}_3$), 1.30–1.40 (m, 8H), 1.85 (q, $J =$ 6.8 Hz, 2H, $-C\underline{H}_2-CH_2-Br$), 2.0–2.15 (m, 4H, proton type 1), 2.80 (t, $J = 5.8$ Hz, 4H, proton type 2), 3.40 (t, $J = 6.8$ Hz, 2H, $-\underline{CH}_2-Br$, 5.25–5.45 (m, 6H, olefinic H). MS (DCI/NH_3): *m*/*z* (%): 333 (15), 332[$M^+ + 18$] (63), 331 (20), 330[$M^+ + 18$] (87), 314[M^+] (20), 313 (22), 312[M^+] (26), 311 (18), 305 (34), 256 (40), 121 (15), 109 (21), 108 (100), 95 (40), 93 (20).

4.2. 2-(7-Bromoheptyl)-3-(2Z,5Z)-octa-2,5-dienyl-oxirane

A mixture of (3*Z*,6*Z*,9*Z*)-17-bromoheptadeca-3,6,9-triene (2.55 g, 8.14 mmol), 20 ml of dichloromethane and 20 ml of a saturated aqueous solution of sodium hydrogen carbonate was stirred at 0 °C using an ice-bath. To this mixture was added *meta*-chloroperbenzoic acid (2.10 g, 8.14 mmol, value obtained after titration). The reaction mixture was cooled all day at 0 °C and was allowed to warm to room temperature overnight. The two layers were separated and the aqueous layer was extracted three times with 20 ml of dichloromethane. The combined organic layers were washed with a saturated aqueous solution of sodium thiosulphate in order to reduce a probable excess of metachloroperbenzoic acid. The organic layer was then concentrated under vacuum. Flash chromatography on silica gel with hexane–ether (99–1) afforded a purified mixture of 2-((*Z*)-10-bromodec-2-enyl)-3-(*Z*)-pent-2-enyl oxirane (epoxide 1), 2-((2*Z*, 5*Z*)-13-bromotrideca-2,5-dienyl)-3-ethyl oxirane (epoxide 2) and 2-(7-bromoheptyl)-3-(2*Z*,5*Z*)-octa-2,5-dienyl-oxirane (epoxide 3) (1.250 g, 3.80 mmol, yield = 47%). A preparative HPLC separation was conducted using a preparative silica column (21.2 mm i.d. × 25 cm) made of high performance chromatographic Zorbax packings (average particle diameter = 8 μm). A mixture of isooctane–diisopropyl ether–petroleum ether (95–2.5–2.5) was used as eluant. The flow rate was set at 15 ml/min. These conditions allowed to load 50 mg of sample on the column and afforded 258 mg of isomer 1, 306 mg of isomer 2 and 302 mg of isomer 3. Yield after purification = 32%. 2-(7-bromoheptyl)-3-(2*Z*,5*Z*)-octa-2,5-dienyl-oxirane. IR (film): $\upsilon = 3012\,cm^{-1}$(m), 2962(s), 2930(s), 2856(s), 1461(m), 1382(w), 868(w) and 562(w). ^{1}H NMR($CDCl_3$): $\delta = 0.95$ (t, $J = 7.5$ Hz, 3H, $-CH_2-C\underline{H}_3$), 1.2–1.4 (m, 8H), 1.5 (m, 2H, proton

type 3), 1.85 (q, $J = 6.8$ Hz, 2H, –C$\underline{H}_2$–CH_2–Br), 2.05 (q, $J = 7.3$ Hz, 2H, proton type 1), 2.15–2.45 (m, 2H, proton type 4), 2.8 (t, $J = 6.7$, 2H, proton type 2), 2.95 (m, 2H, proton type 5), 3.40 (t, $J = 6.8$ Hz, –$\underline{CH}_2$–Br), 5.25–5.55 (m, 4H, olefinic H). MS (DCI/NH_3): *m/z* (%): 349 (14), 348[$M^+ + 18$] (87), 347 (24) 346[$M^+ + 18$] (100), 331 (12), 329 (10), 313 (12), 311 (13), 123 (5).

4.3. Erythro (3Z,6Z)-9,10,17-tribromoheptadeca-3,6-diene

A mixture of 2-(7-bromoheptyl)-3-(2*Z*,5*Z*)-octa-2,5-dienyl-oxirane (0.190 g, 0.58 mmol) in 5 ml of toluene was added to triphenylphosphine dibromide (0.244 g, 0.58 mmol) in 5 ml of toluene. The mixture was vigorously stirred at room temperature overnight, and the crude product was then flash chromatographed on silica gel. The elution with hexane–diethyl ether (99–1) afforded erythro (3*Z*,6*Z*)-9,10,17-tribromo-heptadeca-3,6-diene (0.178 g, 0.37 mmol, yield = 64%). IR (film): $\upsilon = 3011\ cm^{-1}$(m), 2931(s), 2857(s), 1458(m), 1433(m), 1217(w), 1148(w), 538(w) and 513(w). ^{1}H NMR ($CDCl_3$): $\delta = 0.95$ (t, $J = 7.5$ Hz, 3H, –CH_2–C$\underline{H}_3$), 1.2–1.45 (m, 8H), 1.85 (q, $J = 6.8$, 2H, –C$\underline{H}_2$–CH_2–Br), 1.95–2.15 (m, 4H, protons type 1 and type 6), 2.70–2.85 (m, 4H, protons type 2 and type7), 3.40 (t, $J = 6.8$ Hz, 2H, –$\underline{CH}_2$–Br), 5.25–5.5 (m, 6H, olefinic H + proton type 8). MS (DCI/NH_3): *m/z* (%): 494 (29), 493 (24), 492 (95), 491 (30), 490 (100), 489 (11), 488[$M^+ + 18$] (32), 330 (20), 328 (12), 314 (21), 313 (92), 312 (28), 311 (95).

4.4. (3Z,6Z,9E)-17-Bromoheptadeca-3,6,9-triene

A solution of erythro (3*Z*,6*Z*)-9,10,17-tribromoheptadeca-3,6-diene (0.109 g, 0.23 mmol) in *N*,*N*-dimethylformamide (2 ml) was added to an ice-cooled slurry of activated zinc (0.109 mg), acetic acid (three drops) and *N*,*N*-dimethylformamide (5 ml). Stirring was continued at 0 °C for 2 h before the reaction mixture was diluted with water and filtered. After extraction with pentane and concentration in vacuum, a flash chromatography on silica gel with pure heptane afforded purified (3*Z*,6*Z*,9*E*)-17-bromoheptadeca-3,6,9-triene (0.055 g, 0.17 mmol, yield = 76%). IR (film): $\upsilon = 3012\ cm^{-1}$(m), 2963(s), 2929(s), 2855(s), 1655(w), 1463(m), 1438(m), 1393(w), 1255(m), 1070(w), 967(m), 792(w), 723(m), 646(w), 564(w). ^{1}H NMR($CDCl_3$): $\delta = 0.95$ (t, $J = 7.5$ Hz, 3H, –CH_2–C$\underline{H}_3$), 1.2–1.45 (m, 8H), 1.85 (q, $J = 7.1$, 2H, –C$\underline{H}_2$–CH_2–Br), 1.9–2.10 (m, 4H, proton type 1), 2.70–2.85 (m, 4H, proton type 2), 3.40 (t, $J = 6.8$, 2H, –$\underline{CH}_2$–Br), 5.25–5.45 (m, 6H, olefinic H). MS (DCI/NH_3): *m/z* (%): 333 (25), 332[$M^+ + 18$] (100), 331 (32), 330[$M^+ + 18$] (100), 314[M^+] (13), 313 (11), 312[M^+] (15), 311 (6), 256 (16), 108 (21).

4.5. (9E,12Z,15Z)-[1-^{14}C]-Octadeca-9,12,15-trienenitrile

In a 25 ml-flask, 49 mg of (3*Z*,6*Z*,9*E*)-17-bromoheptadeca-3,6,9-triene (0.15 mmol), 24 mg of ^{14}C potassium cyanide (55 mCi/mmol, 0.375 mmol, 20.6 mCi) and 3 ml of dimethylsulphoxide were stirred for 20 min at 80 °C. Ten milliliter of water were added and the aqueous layer was extracted three times with 20 ml diethyl ether. The mixture was dried under vacuum and purified using flash chromatography on silica gel with pentane–diethyl ether (95–5) as eluant. The purified product was submitted to radioactive counting and mass spectrometry. 7.9 mCi (55 mCi/mmol) (0.14 mmol) were obtained (96% yield). ^{1}H NMR($CDCl_3$): $\delta = 0.95$ (t, $J = 7.5$ Hz, 3H, –CH_2–C$\underline{H}_3$), 1.2–1.7 (m, 8H), 1.9–2.1 (m, 4H, proton type 1), 2.35 (t, $J = 7.1$, 2H, –C$\underline{H}_2$–CN), 2.7–2.8 (m, 4H, proton type 2), 5.25–5.45 (m, 6H, olefinic H). MS (DCI/NH_3): *m/z* (%): 281 (6), 280 (33), 279[$M^+ + 18$] (100), 278 (9), 277 (20).

4.6. (9E,12Z,15Z)-[1-^{14}C]-Octadeca-9,12,15-trienoic acid

In a 25 ml-flask, 7.9 mCi of (9*E*,12*Z*,15*Z*)-[1-^{14}C]-octadeca-9,12,15-trienenitrile: (0.14 mmol), 5 ml of a 40% aqueous solution of potassium hydroxide and 5 ml of absolute ethanol were stirred for 12 h at 85 °C. TLC control on silica gel with pentane–diethyl ether–acetic acid (90–10–1) showed the end of the reaction. The mixture was then acidified using 10 ml 5N hydrochloric acid and extracted with diethyl ether. The purification by flash chromatography using the same system as the TLC afforded 6.85 mCi of (9E,12Z,15Z)-[1-^{14}C]-octadeca-9,12,15-trienoic acid (55 mCi/mmol, 0.12 mmol, yield = 89%).

^{1}H NMR($CDCl_3$): $\delta = 0.95$ (t, $J = 7.5$ Hz, 3H, $-CH_2-C\underline{H}_3$), 1.2–1.4 (m, 8H), 1.65 (q, $J = 7.2$, 2H, $-C\underline{H}_2-CH_2-COOH$), 1.9–2.1 (m, 4H, proton type 1), 2.35 (t, $J = 7.4$, 2H, $-C\underline{H}_2-COOH$), 3.7–2.8 (m, 4H, proton type 2), 5.25–5.45 (m, 6H, olefinic H). MS (DCI/NH_3): *m/z* (%): 300 (5), 299 (32), 298[$M^+ + 18$] (100), 297 (9), 296 (15).

An aliquot of the corresponding methyl ester was obtained using oxalyl chloride and absolute methanol. Gas chromatography using a HP6890 GC system coupled with a 5973 mass selective detector and helium as carrier gas was used to compare with an authentic sample by a coelution on a HP-23 *cis*/*trans* FAME (60 m × 0.25 mm × 0.25 μm) capillary column using the following isocratic conditions: temperature, 200 °C; injector temperature, 250 °C. A single peak was detected: retention time; 11.09 min. All-*Z* linolenic acid methyl ester was not detected: retention time; 11.24 min. Its stereochemical and chemical purities were better than 99%.

The other products were synthesised following a similar procedure (yields are given under the corresponding product on the path scheme).

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