Supplemental Data

FIGURE SD1

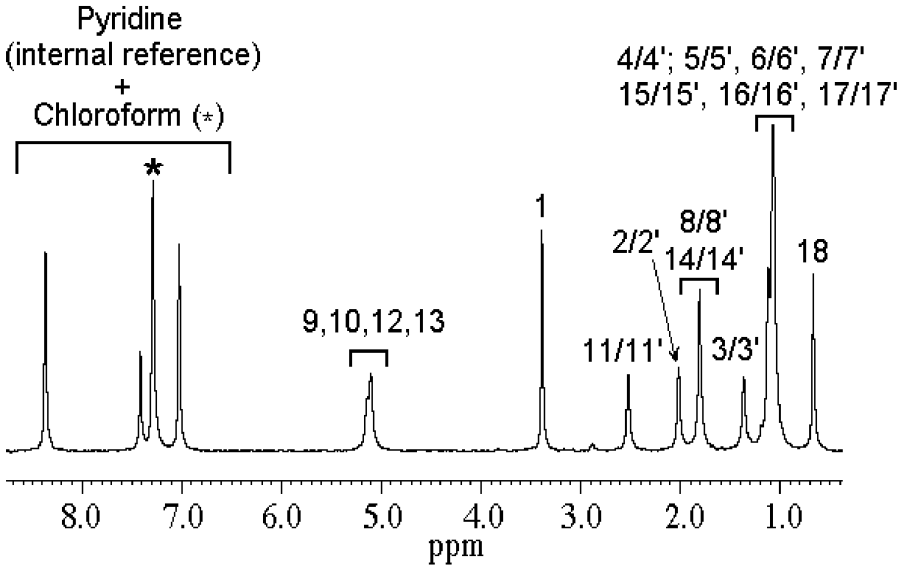

Figure SD1: 92.1 MHz one-dimensional ^{2}H-{^{1}H} NMR spectrum of methyl linoleate recorded in quantitative conditions in isotropic media at 310 K. Note the superposition of numerous deuterium signals of CH_2 groups in the aliphatic region, and the presence of the pyridine peaks used as internal reference for the $(^{2}H/^{1}H)_i$ measurement. The deuterium signal of chloroform (marked by an asterisk) is calibrated at $\delta = 7.23$ ppm. The spectrum was recorded in five hours (NS = 3200 scans) with a selective 5-mm ^{2}H NMR cryoprobe.

FIGURE SD2

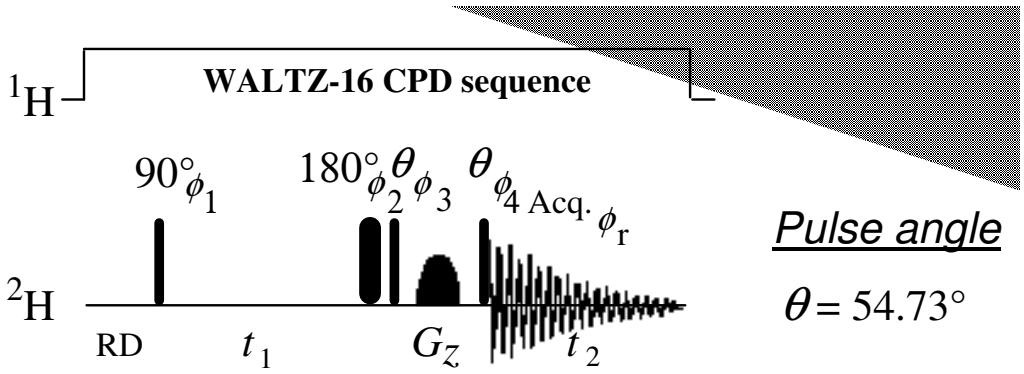

Figure SD2: Schematic description of the *Q*-COSY Fz 2D pulse sequence. (1) The basic phase cycling is $\phi_1 = 4(x)$; ϕ_2= x,y,-x,-y; $\phi_3 = \phi_4 = 4(x)$; $\phi_r = 2(x,-x)$. In this 2D sequence (derived from the *Q*-COSY 2D experiment), a modified z-filter (adapted for spin I = 1) is added. Here the gradient pulse, G_Z, suppresses all the coherences of order 1 and above, and keeps spin polarization and spin order terms unchanged. With this sequence, a phased map in both spectral dimensions is obtained after two Fourier transformations. Proton decoupling is achieved using classical CPD sequences, such as WALTZ-16, with a decoupling power identical to that applied in isotropic NMR.

FIGURE SD3

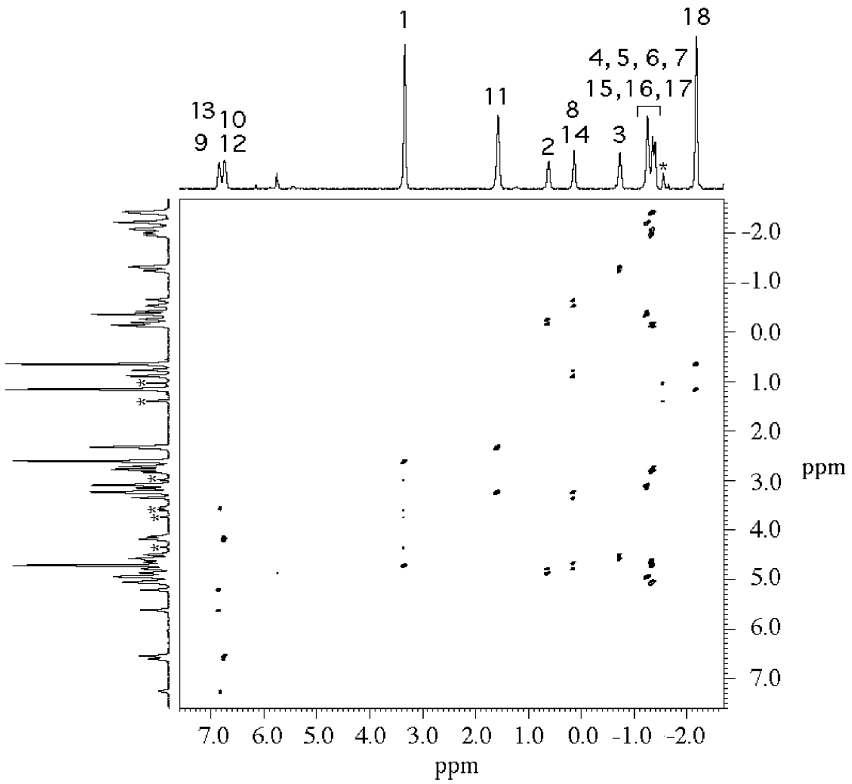

Figure SD3: 92.1 MHz *Q*-COSY Fz MHz two-dimensional ^{2}H-{^{1}H} NMR spectrum of methyl linoleate (culture D, enriched with [1-^{2}H]glucose) dissolved in PBLG/$CHCl_3$ mesophase and recorded at 300 K. The recycling delay was of 1 s repetition time and the number of scans for each t_1 increment is 96 FIDs. The 2D map is symmetrized, and then a tilt procedure is applied. Peaks marked with an asterisk correspond to the methyl and methylene signals of ethanol used for stabilizing chloroform. The scale (in ppm) in the F_2 dimension has no chemical meaning.

FIGURE SD4

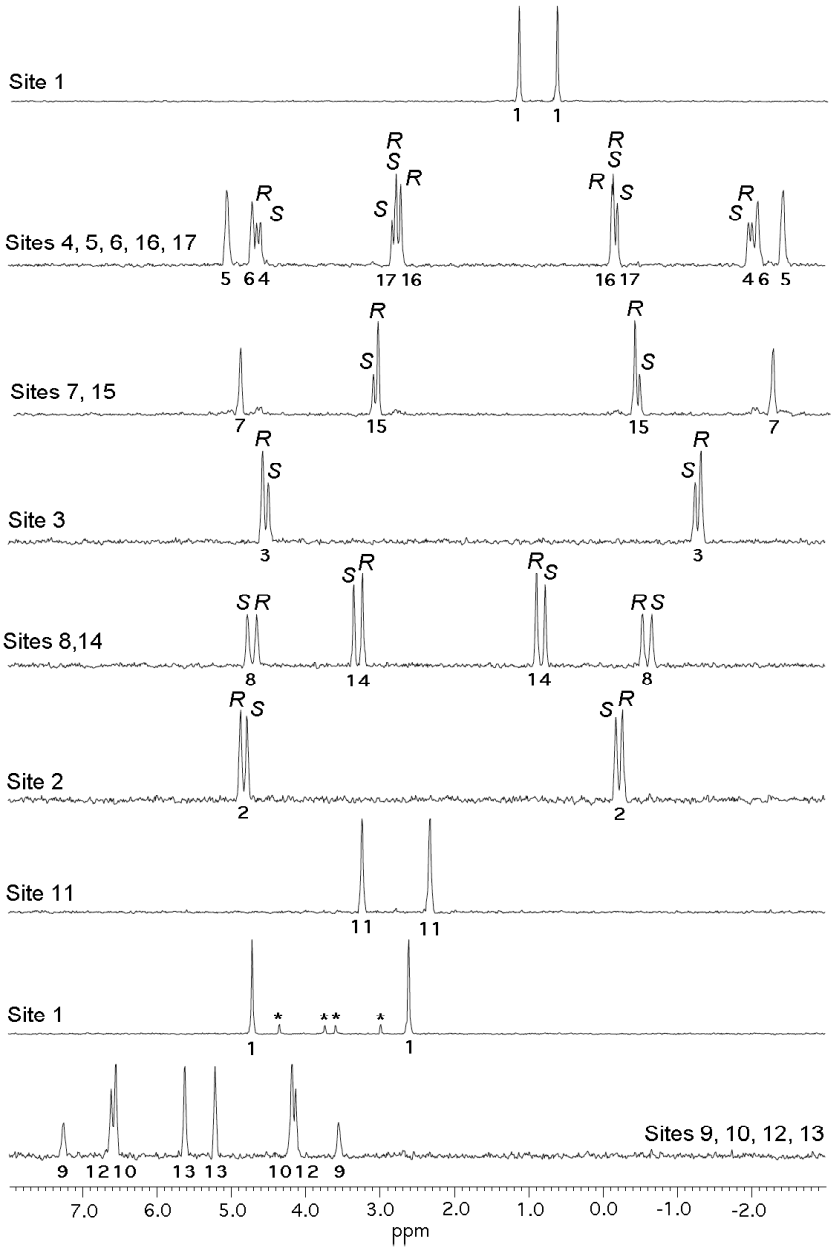

Figure SD4: Series of one-dimensional ^{2}H-{^{1}H} NMR subspectra extracted from the tilted *Q*-COSY Fz map shown in Figure SD2. The various non-equivalent methyl or methylene groups are noted from sites 1 to 18 (according to Figure 2c). When spectral enantiodiscrimination is possible and occurs, the *R*/*S* assignment of each quadrupolar doublet is given in agreement with results reported in reference 1. The quadrupolar doublets labeled with an asterisk (second subspectrum from bottom) correspond to the signals of the methylene group of ethanol. Note the discrimination of pro-*R* and pro-*S* sites of ethanol (two doublets visible).

Assignment of quadrupolar doublets associated with each methylene group of methyl linoleate dissolved in chiral anisotropic solvents—The ^{2}H quadrupolar doublets visible in the series of one-dimensional sub-spectra (Fig. SD4) have been assigned on the basis of (i) their ^{2}H chemical shifts, (ii) isotopic data obtained from isotropic quantitative natural abundance ^{2}H NMR and (iii) an inter-spectral comparison of the anisotropic natural abundance ^{2}H spectra of four fatty acids (methyl oleate, methyl linoleate, methyl linolenate and methyl

vernoleate) based on the analysis of molecular orientation of these four FAMEs in the PBLG/$CHCl_3$ mesophase (2).

Assignment of R/S stereochemical descriptors in chiral anisotropic NAD spectra of methyl linoleate—In a previous paper, Billault *et al.* (3) clearly showed that the $(^2H/^1H)_{pro\text{-}S}$ ratios were lower than the $(^2H/^1H)_{pro\text{-}R}$ ratios at even and odd methylene sites (sites 4 and 5) of bis(phenylthiohexane) (BPTH) derived by chemical degradation of methyl linoleate. As the BPTH used in this investigation had the same botanical origin (safflower) as the methyl linoleate analyzed in 2008 by Lesot *et al.* (2), the isotopic fractionation relative to sites 4 and 5 in BPTH was therefore the same for sites 16 and 17 in the corresponding methyl linoleate. Consequently, we can confidently predict that the $(^2H/^1H)_{pro\text{-}S}$ ratio is always smaller than the $(^2H/^1H)_{pro\text{-}R}$ ratio at each odd and even methylene (15 to 2) of methyl linoleate isolated from safflower. The *R*/*S* assignments of quadrupolar doublets associated with enantiotopic directions at each enantio-discriminated methylene group of methyl linoleate are reported in Figure SD4.

The formal assignment is problematical. With the methylene sites 6, 7 and 8 of methyl linoleate, the stereochemistry of deuterium sites located in front of the plane of symmetry is inverted compared with methyl stearate according to the CIP rules due to the presence of the double bond at position 9-10. Thus, except for the methylene site 8 in methyl linoleate, we have assigned the stereodescriptors, *R/S,* to the lowest- or highest-intensity doublets displayed in the natural abundance ^2H NMR sub-spectra (Fig. SD4). For methylene 8, the situation is inverted compared with other methylene sites as the CIP rules are applied.

As the composition of chiral anisotropic samples (in PBLG, in solute and in chloroform) and the experimental NMR conditions (temperature) in this study are identical to those used in the study of Lesot *et al.* (2), the relative position of quadrupolar doublets (centered on the same chemical shift) corresponding to the *R* and *S* enantioisotopomer is the same in both sets of NAD *Q*-COSY Fz 2D NMR spectra.

References for supplementary data

(1) Lafon, O., Lesot, P., Merlet, D., and Courtieu, J. (2004) *J. Magn. Reson.* 171, 135-142
(2) Lesot, P., Baillif, V., and Billault, I. (2008) *Anal. Chem.* 80, 2963-2972.
(3) Baillif, V., Robins, R. J., Billault, I. and Lesot, P. (2006) *J. Am. Chem. Soc.* 128, 11180-11187.

TABLE SD 1: ***$(^2H/^1H)_i^{iso}$ ratios (in ppm) of methyl linoleate extracted from cultures A-E measured by ^2H NMR results in liquid media***

Culture	Site	2	3	4 5 6 7	8	9 10	11	12 13	14	15 16,17	18
NA A	$(^2H/^1H)_i$[a]	123.3	112.3	117.9[b]	116.6[c]	102.8[d]	110.8	102.8[d]	116.6[c]	117.9[b]	119.2
	S.D.	2.2	0.6	1.5	1.1	0.6	2.4	0.6	1.1	1.5	1.8
D_2O B	$(^2H/^1H)_i$[a]	263.2	156.0	217.2[b]	242.8[c]	193.8[d]	172.0	193.8[d]	242.8[c]	217.2[b]	219.2
	S.D.	0.2	0.2	0.6	0.3	0.1	0.1	0.1	0.3	0.6	0.3
[6,6-$^2H_2$]glucose C	$(^2H/^1H)_i$[a]	139.6	113.3	132.7[b]	130.0[c]	96.1[d]	108.7	96.1[d]	130.0[c]	132.7[b]	191.4
	S.D.	0.8	0.2	0.1	0.4	0.1	0.8	0.1	0.4	0.1	0.7
[1-^2H]glucose D	$(^2H/^1H)_i$[a]	129.1	150.4	144.7[b]	118.7[c]	108.6[d]	139.3	108.6[d]	118.7[c]	144.7[b]	142.9
	S.D.	2.2	1.4	0.2	1.1	0.3	1.4	0.3	1.1	0.2	0.5
[2-$^2H_3$]acetate.Na E	$(^2H/^1H)_i$[a]	729.7	158.8	485.3[b]	1008.7[c]	130.2[d]	145.6	130.2[d]	1008.7[c]	485.3[b]	2987.8
	S.D.	3.2	7.6	5.6	0.4	0.8	2.3	0.8	0.4	5.6	5.2

[a] **$(^2H/^1H)_i^{iso}$ values were calculated from ^2H NMR spectra recorded in liquid media A to E as described in the text. Three acquisitions were made for each sample.** [b] **Sites 4-7 and sites 15, 16 and 17: all ^2H signals resonate at the same frequency.** [c] **Sites 8 and 14: all ^2H signals resonate at the same frequency.** [d] **Sites 9, 10, 12 and 13: all ^2H signals resonate at the same frequency.**