Combined analysis of C-18 unsaturated fatty acids using Natural Abundance Deuterium 2D NMR Spectroscopy in Chiral Oriented solvents

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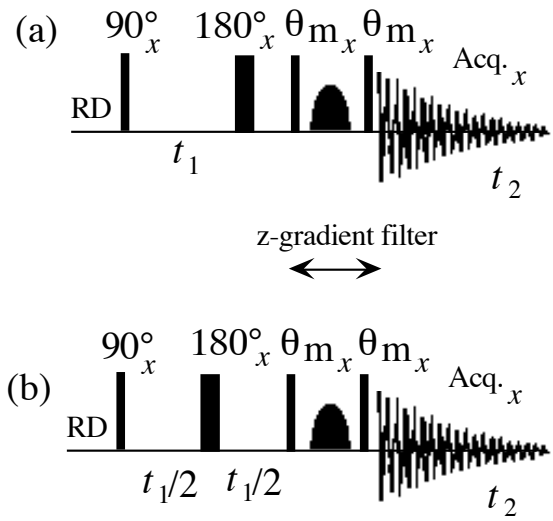

Figure SI-1. Pulse sequence of (a) the *Q*-COSY Fz and (b) the *Q*-resolved Fz 2D experiment. Note the presence of z-gradient filter (θ_m = 54.7°) inserted prior to the t_2 acquisition period. The protons are decoupled during all the duration of the experiment using the WALTZ-16 sequence.

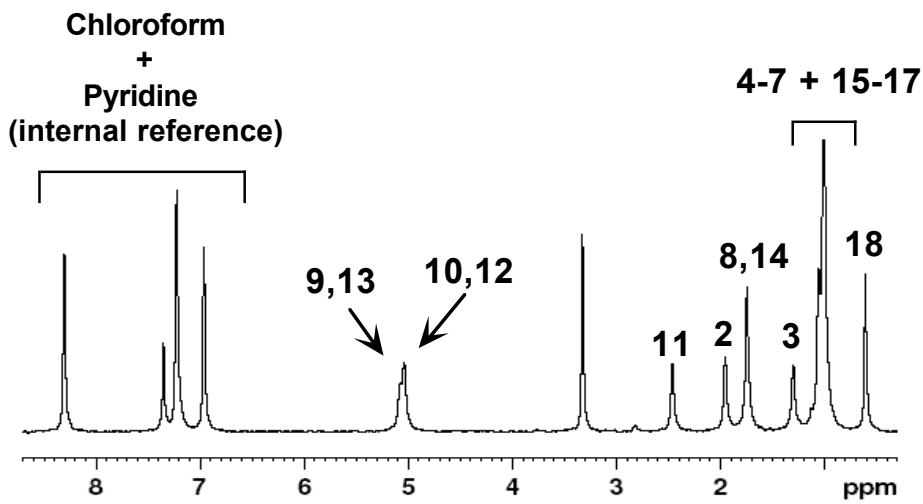

Figure SI-2. 92.1 MHz ^2H-{^1H} isotropic 1D spectrum of methyl linoleate **2** recorded in quantitative conditions. Note the superposition of numerous deuterium signals in the aliphatic region, and the presence of pyridine peak used as internal reference for the $(^2H/^1H)_i$ measurement. This 1D spectrum was recorded in five hours (NS = 30000 scans) with an ^2H cryoprobe. Exponential filtering (LB = 1 Hz) is applied prior to Fourier transformation.

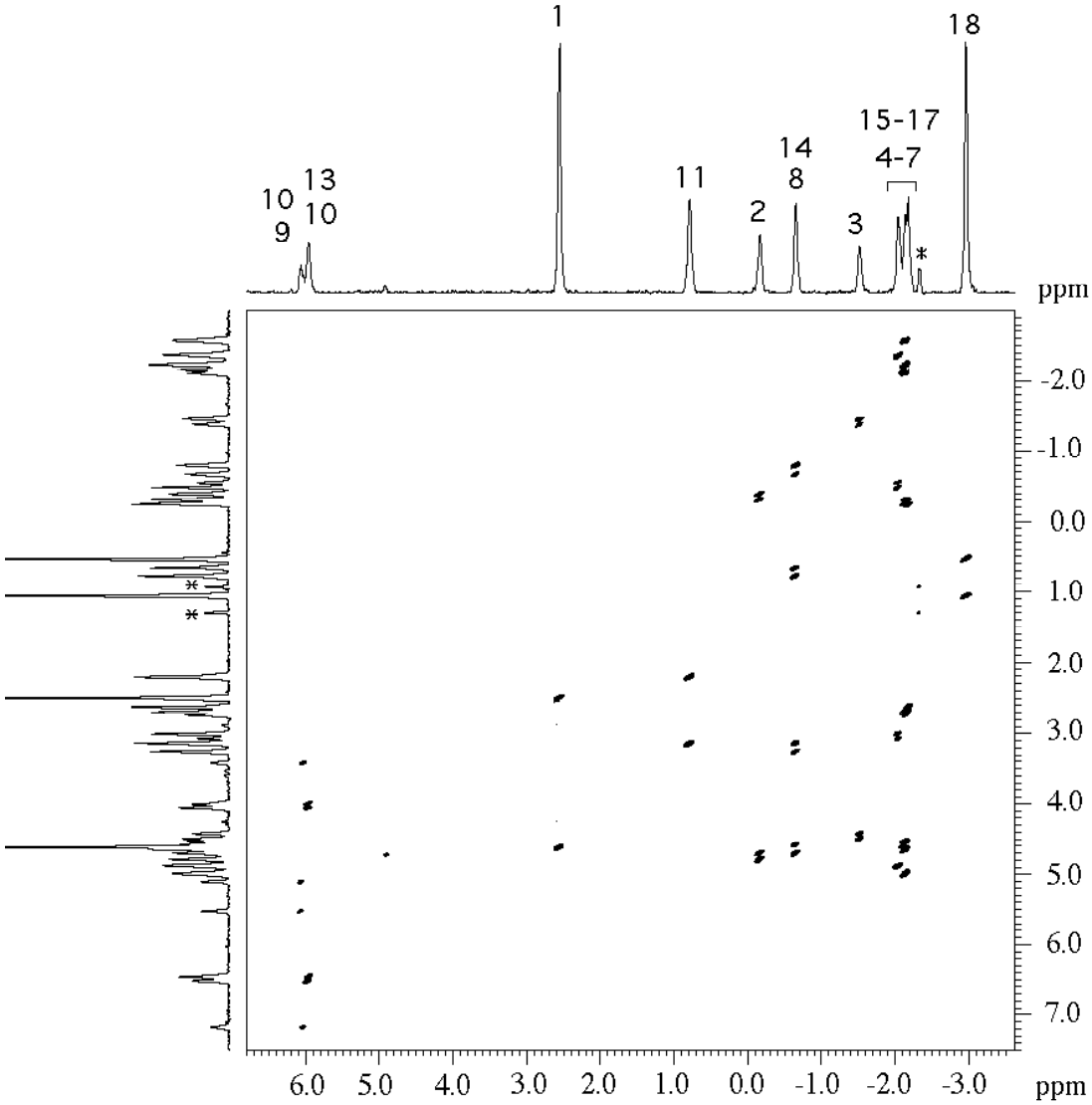

Figure SI-3. 92.1 MHz NAD *Q*-COSY Fz 2D spectrum of methyl linoleate **2** dissolved in PBLG/$CHCl_3$ phase and recorded at 300 K. The number of scans for each t_1 increment is 96. The recycling delay was of 1 s repetition time. The 2D map is symmetrized and a tilt procedure then applied. Peaks labeled with an asterisk correspond to the methyl signal of ethanol using for stabilizing chloroform. The scale (in ppm) in the F_2 dimension has no chemical meaning (see ref. 23).

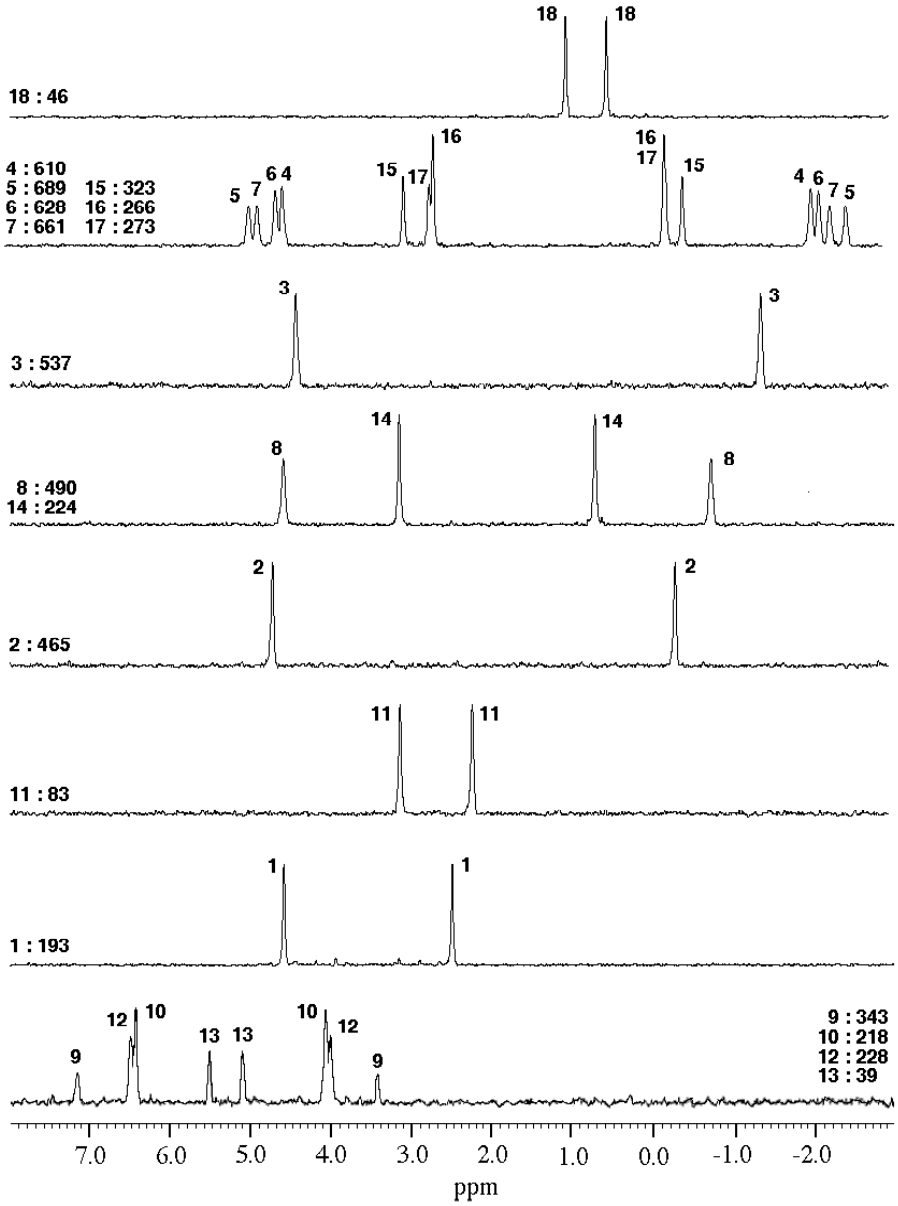

Figure SI-4. Series of ^{2}H-{^{1}H} 1D subspectra extracted from the tilted *Q*-COSY Fz map of methyl linoleate **2** dissolved in PBG/$CHCl_3$ at 300 K. The value of quadrupolar splittings are given in Hz.

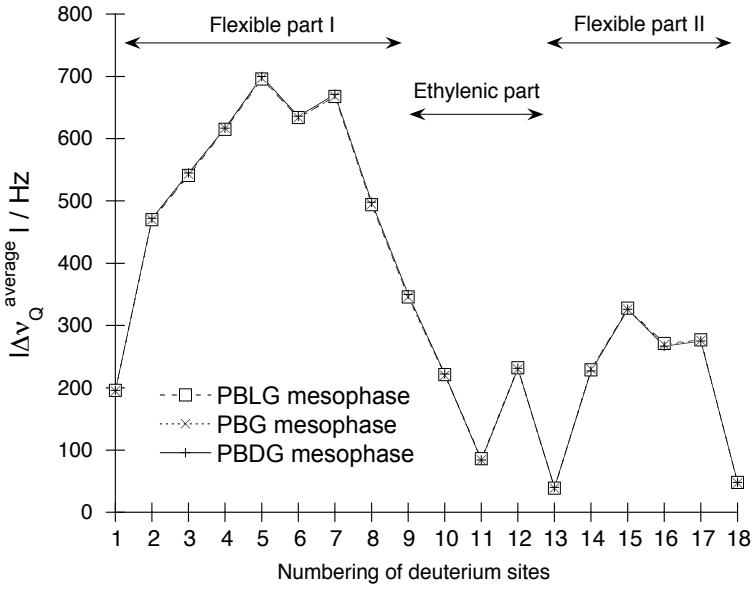

Figure SI-5. Comparison of the $\Delta\nu_Q$'s (absolute value) of methyl linoleate **2** dissolved in PBLG, PBG and PBDG mesophases *versus* the deuterium sites (1 to 18). In PBLG and PBDG solvents, the average value of $\Delta\nu_Q$'s for the methylene sites that are spectrally discriminated are reported on graph. ^{2}H data recorded PBG and PBDG have been normalized relative to PBLG data in order to be correctly compared. The values in PBG and PBDG were corrected relative to values measured in PBLG (see ref. 16).

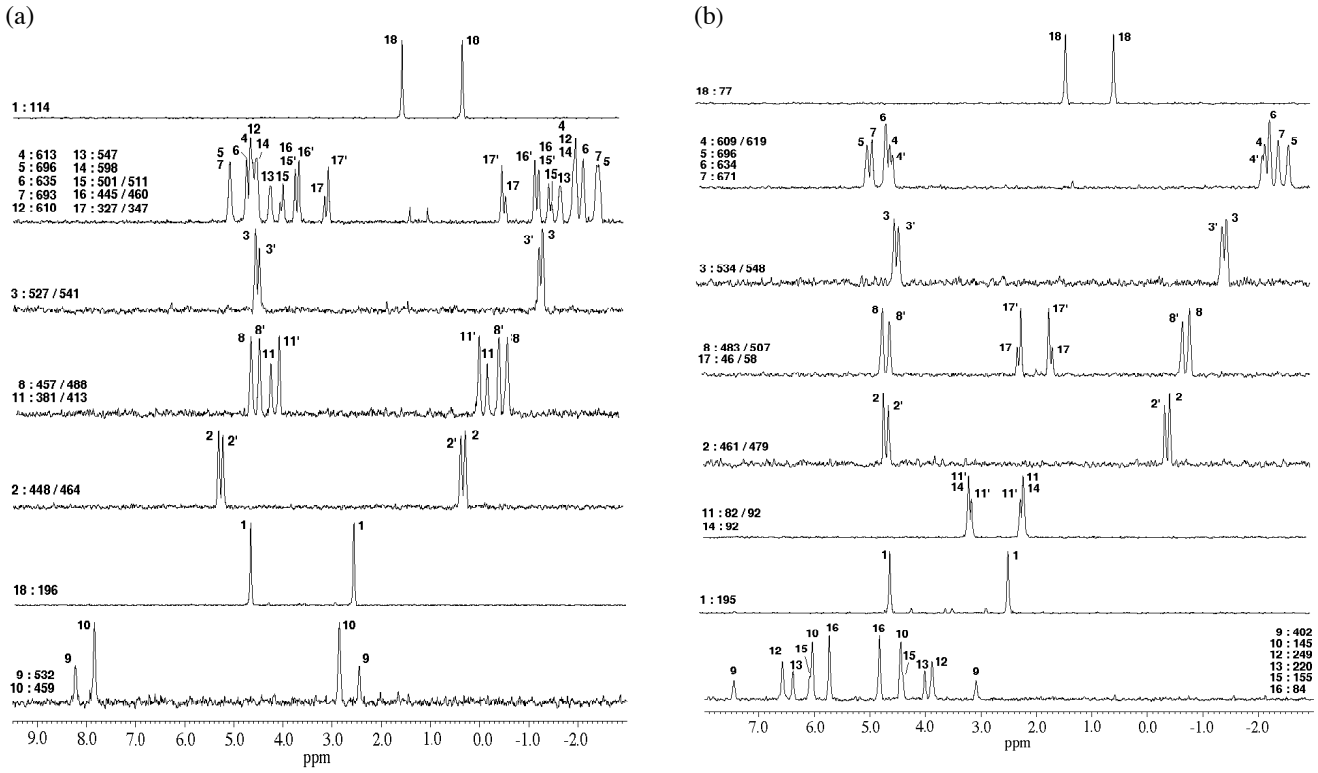

Figure SI-6. Series of ^{2}H-{^{1}H} 1D sub-spectra extracted from the tilted *Q*-COSY Fz map of (a) methyl oleate **1** and (b) methyl linolenate **3** both dissolved in the PBLG/$CHCl_3$ mesophase and recorded at 300 K. Zooms of 2D maps are reported below.

(a) (b)

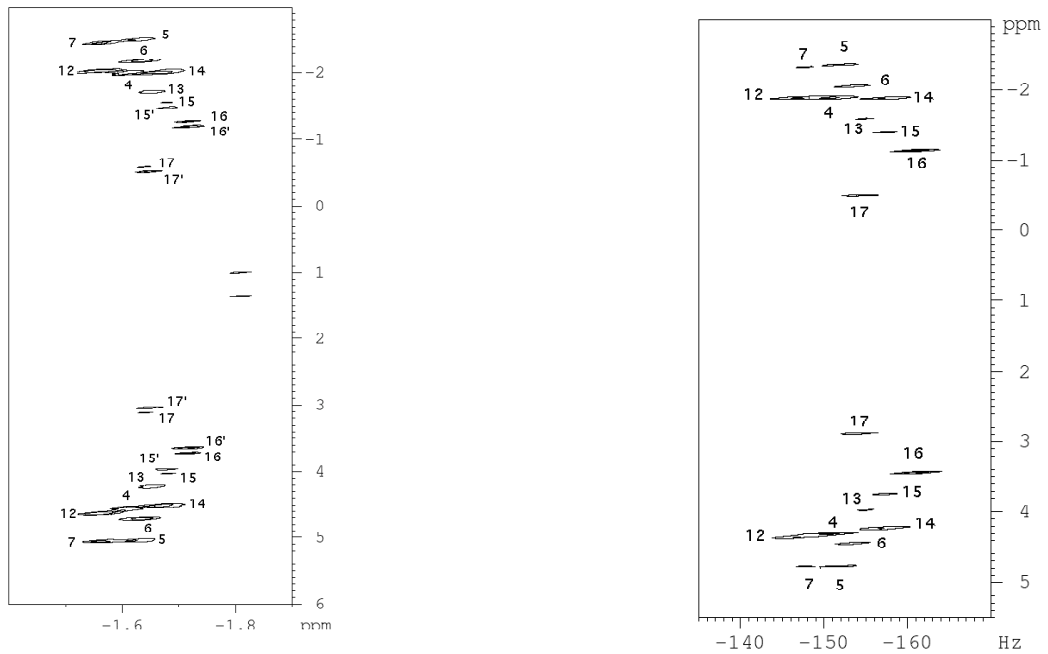

Figure SI-7. Zoom on the aliphatic region of the tilted *Q*-COSY Fz map of methyl oleate **1** dissolved in (a) PBLG/$CHCl_3$ and (b) PBG/$CHCl_3$ at 300 K.

(a) (b)

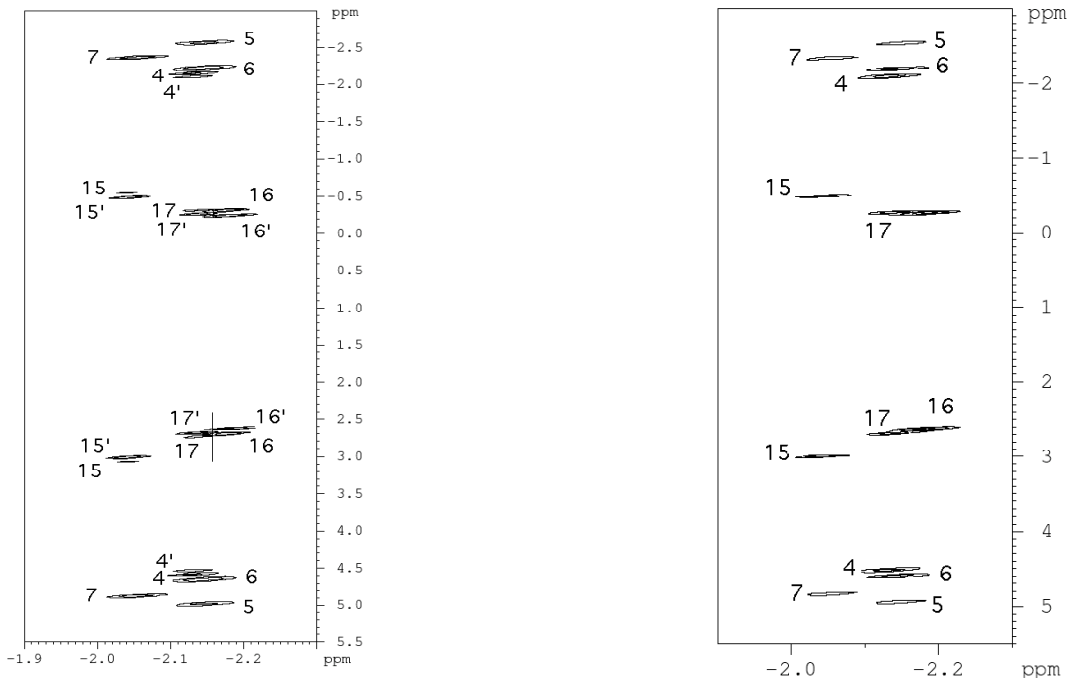

Figure SI-8. Zoom (on the aliphatic region) on the tilted *Q*-COSY Fz map of methyl linoleate **2** dissolved in (a) PBLG/$CHCl_3$ and (b) PBG/$CHCl_3$ at 300 K.

(a) (b)

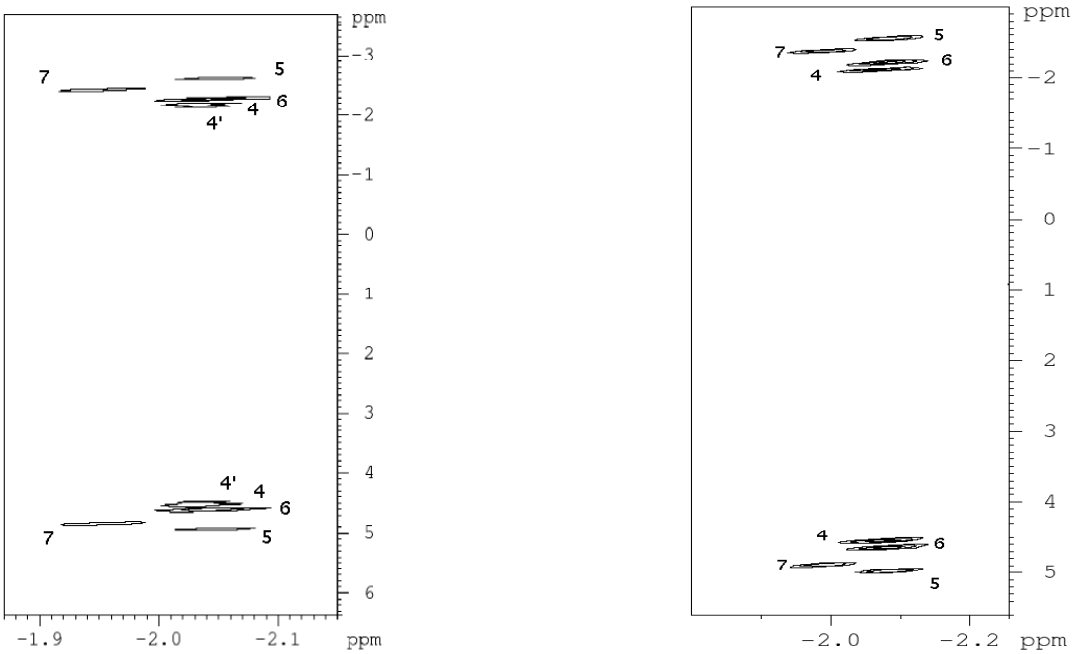

Figure SI-9. Zoom (on the aliphatic region) on the tilted *Q*-COSY Fz map of methyl linolenate **3** dissolved in (a) PBLG/$CHCl_3$ and (b) PBG/$CHCl_3$ at 300 K.

(a) (b)

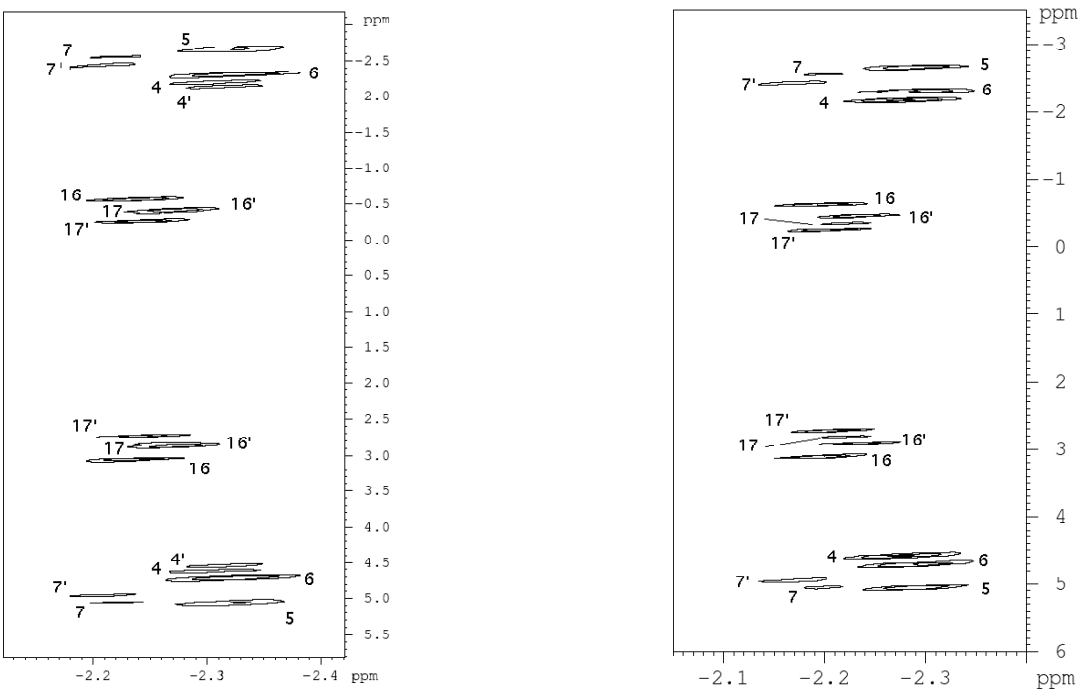

Figure SI-10. Zoom (on the aliphatic region) on the tilted *Q*-COSY Fz map of methyl vernoleate **4** dissolved in (a) PBLG/$CHCl_3$ and (b) PBG/$CHCl_3$ at 300 K.

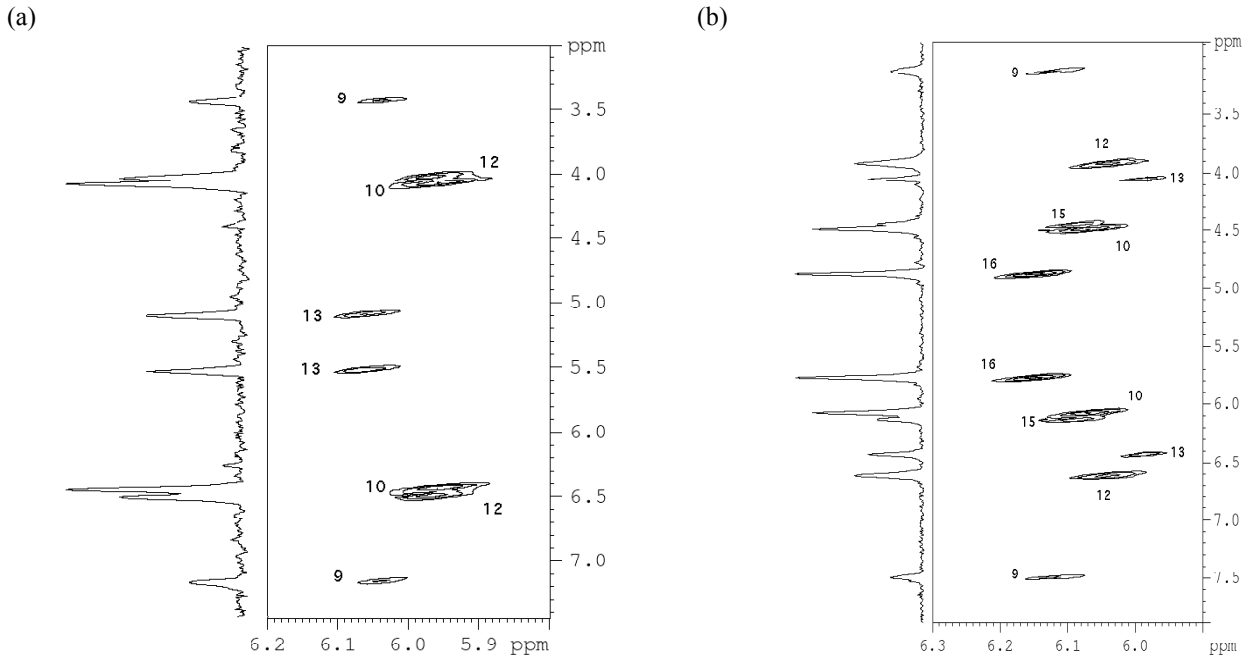

Figure SI-11. Zoom (on the ethylenic region) on the tilted *Q*-COSY Fz map of (a) methyl linoleate **2** and (b) methyl linolenate **3**, both dissolved in PBG/$CHCl_3$ mesophase at 300 K.

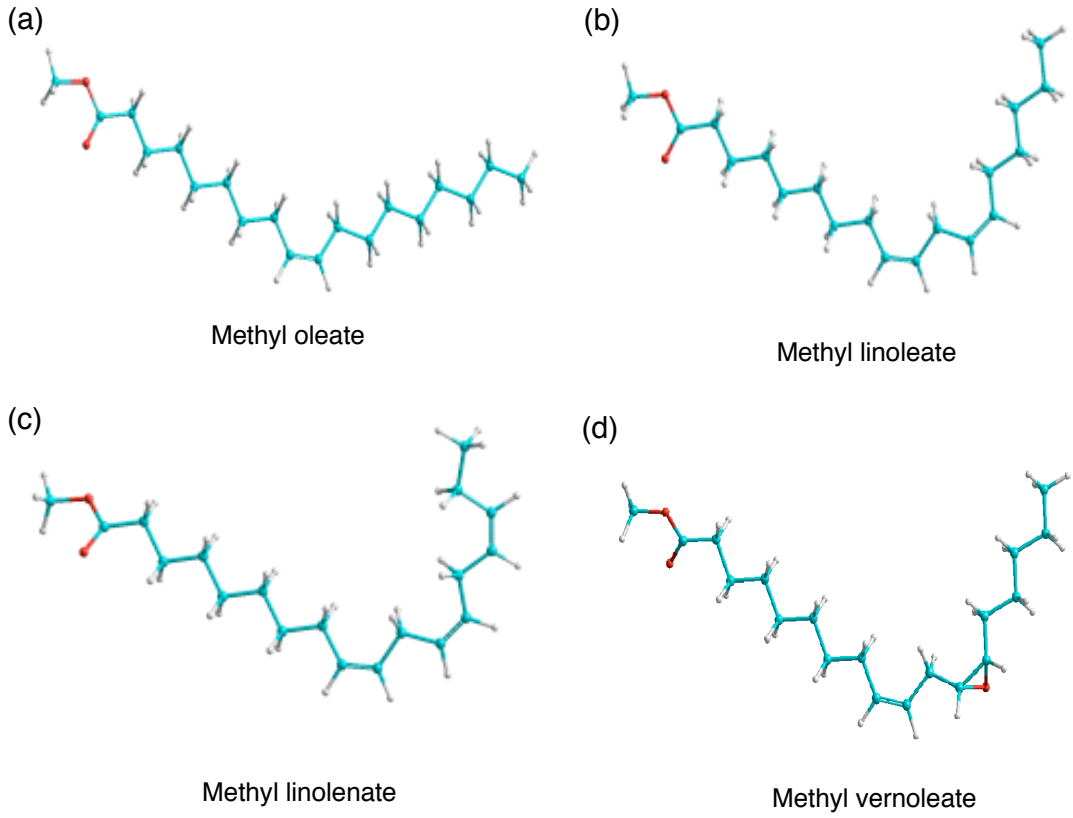

Figure SI-12. Lowest-energy conformer for (a) the methyl oleate **1**, (b) the methyl linoleate **2**, (c) the methyl linolenate **3** and (d) the methyl vernoleate **4** determined by molecular modelling using Hyperchem 5.0 software. The calculation uses the semi-emprical model (AM1) and the Polack-Ribiere algorithm.

Table SI-1. Quadrupolar splittings (in absolute value and in Hz) of nonequivalent deuterium sites in compounds **1** to **4** (PBLG and PBG)

^{2}H site	Group	Methyl oleate 1 PBLG	Methyl oleate 1 PBG	Methyl linoleate 2 PBLG	Methyl linoleate 2 PBG	Methyl linolenate 3 PBLG	Methyl linolenate 3 PBG	Methyl vernoleate 4 PBLG	Methyl vernoleate 4 PBG
-	$CHCl_3$	451	427	446	441	434	432	433	432
1	CH_3O-	196	182 (193)[b]	196	193 (195)	195	195 (196)	195	195 (195)
2	-CH_2-	448 / 464 {456}[a]	432 (455)	462 / 478 {470}	465 (470)	461 / 479 {470}	470 (472)	459 / 477 {468}	468 (469)
3	-CH_2-	527 / 541 {534}	506 (534)	534 / 548 {541}	537 (543)	534 / 548 {541}	546 (547)	537 / 553 {545}	543 (544)
4	-CH_2-	613	574 (606)	610 / 620 {615}	610 (617)	609 / 619 {614}	614 (615)	617 / 629 623	624 (625)
5	-CH_2-	696	657 (690)	696	689 (697)	696	693 (695)	709	711 (711)
6	-CH_2-	635	598 (632)	634	628 (634)	634	624 (625)	646	647 (648)
7	-CH_2-	693	653 (690)	668	661 (668)	671	670 (672)	681 / 701 {690}	682 / 702[c] (682 / 702)
8	-CH_2-	457 / 488 {472}	447 (472)	484 / 506 {495}	490 (495)	483 / 507 {495}	495 (496)	514 / 538 {526}	500 / 554 (501 / 555)
9	-HC=CH-	532	504 (531)	346	343 (346)	402	403 (404)	335	335 (336)
10	-HC=CH-	459	430 (454)	221	218 (220)	145	146 (147)	233	228 (229)
11	-CH_2-	381 / 413 {397}	373 (393)	86	83 (84)	82 / 92 {87}	86 (86)	23 / 197 (110)	19 / 193 (19 / 194)
12	-CH_2-	610	572 (604)	- -	- -	- -	- -	- -	- -
12	-COC-	- -	- -	- -	- -	- -	- -	434	413 (414)
12	-HC=CH-	- -	- -	232	228 (230)	249	249 (250)	- -	- -
13	-CH_2-	547	513 (542)	- -	- -	- -	- -	- -	- -
13	-COC-	- -	- -	- -	- -	- -	- -	301	326 (326)
13	-HC=CH-	-	- -	39	39 (39)	220	219 (220)	- -	- -
14	-CH_2-	598	563 (595)	218 / 240 {229}	224 (230)	92	93 (93)	115 / 387 {251}	119 / 405 (119 / 406)
15	-CH_2-	501 / 511 {506}	473 (499)	322 / 334 {328}	323 (326)	- -	- -	289 / 491 {390}	289 / 498 (289 / 499)
15	-HC=CH-	-	-	-	-	155	155 (155)	-	-
16	-CH_2-	445 / 460 {452}	422 (447)	264 / 278 {271}	266 (269)	- -	- -	302 / 334 {318}	310 / 345 (310 / 345)
16	-HC=CH-	- -	- -	- -	- -	84	83 (84)	- -	- -
17	-CH_2-	327 / 347 {334}	311 (328)	272 / 282 {277}	273 (276)	46 / 58 (52)	52 (52)	275 / 297 {286}	274 / 292 (274 / 293)
18	-CH_3	114	107 (113)	48	46 (47)	77	77 (77)	51	52 (52)

[a] The notation "(X)" corresponds to corrected data with : $\Delta\nu_Q(\text{corr}) = \Delta\nu_Q(\text{exp})\times[\Delta\nu_Q(CHCl_3 \text{ in PBLG}) / \Delta\nu_Q (CHCl_3 \text{ in PBG})]$ (ref. 20)

[b] The notation "{X}"corresponds to the average of $\Delta\nu_Q$'s (A and B), *i.e.*: $\Delta\nu_Q(\text{aver}) = [\,|\Delta\nu_Q(A)| + |\Delta\nu_Q(B)|\,]/2$.

[c] The notation "X / X" means that the methylene sites are diastereotopically discriminated.

Table SI-2. Quadrupolar splittings (in Hz) of all nonequivalent deuterium sites of **2** dissolved in PBLG, PBG and PBDG mesophases

^{2}H site	Group	PBLG	PBG		PBDG	
		$\Delta\nu_Q$(exp)	$\Delta\nu_Q$(exp)	$\Delta\nu_Q$(corr)[b]	$\Delta\nu_Q$(exp)	$\Delta\nu_Q$(corr)[d]
-	$CHCl_3$	446	441	446	465	465
1	CH_3O-	196	193	195	204	196
2	-CH_2-	{470}[a]	465	470	483 / 501 [c] {492}	{472}
3	-CH_2-	{541}	537	543	561 / 575 {568}	{545}
4	-CH_2-	{615}	610	617	638 / 648 {643}	{617}
5	-CH_2-	696	689	697	730	700
6	-CH_2-	634	628	634	663	636
7	-CH_2-	668	661	668	700	671
8	-CH_2-	{495}	490	495	506 / 532 {519}	{498}
9	-**H**C=CH-	346	343	346	365	350
10	-HC=C**H**-	221	218	220	231	222
11	-CH_2-	86	83	84	88	84
12	-**H**C=CH-	232	228	230	241	231
13	-HC=C**H**-	39	39	39	42	40
14	-CH_2-	{229}	224	230	226 / 248 {237}	{227}
15	-CH_2-	{328}	323	326	333 / 347 {340}	{326}
16	-CH_2-	{271}	266	269	276 / 280 {278}	{267}
17	-CH_2-	{277}	273	276	282 / 292 {287}	{275}
18	-CH_3	48	46	47	50	48

[a] The notation "{X}"corresponds to the average of $\Delta\nu_Q$'s (A and B), *i.e.* : $\Delta\nu_Q(\text{aver}) = [\,|\Delta\nu_Q(A)| + |\Delta\nu_Q(B)|\,]/2$. [b] Corrected data using: $\Delta\nu_Q(\text{corr}) = \Delta\nu_Q(\text{exp})\times[\Delta\nu_Q(CHCl_3 \text{ in PBLG}) / \Delta\nu_Q(CHCl_3 \text{ in PBG})]$ (ref. 16). [c] The notation "X / X" corresponds to the $\Delta\nu_Q$'s for each discriminated enantiotopic direction. [d] Corrected data using: $\Delta\nu_Q(\text{corr}) = \Delta\nu_Q(\text{exp})\times[\Delta\nu_Q(CHCl_3 \text{ in PBLG}) / \Delta\nu_Q(CHCl_3 \text{ in PBDG})]$ (ref. 16).

Table SI-3. $(^2H/^1H)_i$ ratios (in ppm) measured for methyl linoleate **2** dissolved in PBLG/$CHCl_3$ (with T_R = 1 sec) and using isotropic SNIF-NMR

	Anisotropic medium							**Isotropic medium**	
^2H site	$(^2H/^1H)_i$ Exp. 1	$(^2H/^1H)_i$ Exp. 2	$(^2H/^1H)_i$ Exp. 3	Mean[a] $(^2H/^1H)_i$	SD[b]	Mean by site[c] $(^2H/^1H)_i$	SD	$(^2H/^1H)_i$[e]	SD
2	147.5	158.7	143.5	149.9	7.9	141.3	-	141.3	2.5
2'	135.1	123.9	139.1	132.7	7.9				
3	140.3	148.1	140.1	142.8	4.6	126.5	-	126.5	4.4
3'	112.7	104.9	112.9	110.2	4.6				
4[i]	129.0	130.1	118.6	125.9	6.3	125.9	6.3	119.9[f]	1.7
5	101.9	104.4	113.6	106.6	6.2	106.6	6.2		
6	127.8	135.1	140.8	134.6	6.5	134.6	6.5		
7	113.5	105.0	112.8	110.4	4.7	110.4	4.7		
8	136.3	132.1	140.9	136.4	4.4	120.9	2.9	140.7[g]	2.0
8'	103.8	104.8	107.2	105.3	1.8				
9	73.7	64.6	68.3	68.9	4.6	68.9	4.6	99.8[h]	2.0
10	143.1	146.6	138.7	142.8	4.0	142.8	4.0		
11	98.6	98.6	98.6	98.6	-	98.6	-	98.6	2.1
12	108.1	107.2	113.9	109.7	3.6	109.7	3.6	99.8[h]	2.0
13	74.3	80.8	78.3	77.8	3.3	77.8	3.3		
14	146.9	142.7	141.7	143.7	2.8	160.5	2.9	140.7[g]	2.0
14'	175.8	183.2	173.0	177.3	5.3				
15	83.5	84.3	87.6	85.1	2.2	127.0	1.0	119.9[f]	1.7
15'	171.4	171.0	164.2	168.9	4.1				
16[i]	122.9	121.7	117.0	120.6	3.1	120.6	3.1		
17[i]	122.9	121.7	117.0	120.6	3.1	120.6	3.1		
18	119.0	119.0	119.0	119.0	-	119.0	-	119.0	1.9

[a] Mean values from Exp. 1 to Exp 3. [b] Standard Deviation. [c] Mean values by site [d] Mean of standard deviations (from column 6). [e] Values given are the mean of $(^2H/^1H)_i$ ratios determined from three NAD 1D experiments. [f] Deuterium sites 4-7 and 15-17 resonate at the same frequency. [g] Deuterium sites 8 and 14 resonate at the same frequency. [h] Deuterium sites 9, 10, 12 and 13 resonate at the same frequency. [i] Due to the small spectral enantiodiscrimination and large peak overlapping observed on NAD spectra, the measurement of $(^2H/^1H)_i$ ratios for each enantiomeric isotopomers cannot be performed accurately.

Table SI-4. Composition of liquid-crystalline NMR samples

Solute	*Polymer*	*Dp*[a] *of polypeptide*	*Mw*[b] *of polypeptide*	*Co-solvent*	*Polymer / mg*[c]	*Solute / mg*[c]	*Co-solvent / mg*[c]	*% of polymer in weight*	*Sample*
1	PBLG	782	171300	$CHCl_3$	100	100	650	11.8	4
1	PBG	782 / 914	171300 200200	$CHCl_3$	50 / 51	100	650	11.8	5
2	PBLG	782	171300	$CHCl_3$	100	100	649	11.8	1
2	PBG	782 / 914	171300 200200	$CHCl_3$	50 / 51	100	650	11.8	2
2	PBDG	914	200200	$CHCl_3$	100	100	649	11.8	3
3	PBLG	782	171300	$CHCl_3$	100	100	650	11.8	6
3	PBG	782 / 914	171300 200200	$CHCl_3$	50 / 51	100	651	11.8	7
4	PBLG	782	171300	$CHCl_3$	100	100	651	11.8	8
4	PBG	782 / 914	171300 200200	$CHCl_3$	50 / 51	100	650	11.8	9

[a] DP : Degree of polymerization of polypeptide. [b] Value in g/mol. [c] The accuracy of the weighing is ± 1 mg.