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Assignment of Absolute Configuration of Natural Abundance Deuterium Signals Associated with (*R*)- and (*S*)-Enantioisotopomers in a Fatty Acid Aligned in a Chiral Liquid Crystal: Enantioselective Synthesis and NMR Analysis

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Abstract: Previous experimental natural abundance deuterium (NAD) NMR results have shown an odd/even-related alternation in the (^{2}H/^{1}H) ratio of the methylene groups of fatty acids (*ChemBioChem* **2001**, *2*, 425) and, by NAD NMR in CLC, a marked difference between enantiotopic deuterons for each methylenic site (*Anal. Chem.* **2004**, *76*, 2827). However, to date, the assignment of the absolute configuration for each deuterium has not been possible. To investigate further the origin of these effects, the assignment of NAD quadrupolar doublets observed in chiral oriented solvent is required. Here we describe the assignment of *R*- and *S*-isomers resulting from the isotopic substitution in positions 4 and 5 in the aliphatic chain of 1,1′-bis(thiophenyl)hexane **1** (BTPH) derived from natural linoleic acid of plant origin. This was achieved using an optimized synthetic strategy to obtain separately four regio- and stereoselectively deuterated enantiomers of BTPH. By reference to the deuterium spectra of these isotopically labeled reference compounds, we demonstrate that, on both 4 and 5 positions of BTPH, the isotopic enantiomers of *S* configuration are depleted relative to those of *R* configuration. This finding effectively explains the observed low (^{2}H/^{1}H) ratio in NAD of some ethylenic sites of unsaturated fatty acids.

Introduction

The diversity in the classes of fatty acids present in nature is due to the large number of modifications of their chain (introduction of a double bond or triple bond, conjugated double bond, hydroxylation, epoxidation, etc.). Such reactions involve small changes in the activity of enzymes of the same family for which the enzymatic mechanisms are not completely elucidated or are still unknown.[1,2] The well-established technique of quantitative ^{2}H NMR has proved powerful for analyzing both the natural deuterium distribution in bioproducts[3−5] and kinetic or physical isotope effects.[6,7] This approach applied to long-chain fatty acid biosynthesis has provided unique information about potential enzyme mechanisms.[8−12]

However, deuterium NMR spectra of long-chain fatty acids have too many overlapping signals to resolve fully all the hydrogen positions of interest: in particular, many of the methylenic and ethylenic positions resonate at coincident frequencies. To access a much larger number of unique deuterium signals, we have developed a series of chemical modifications of unsaturated methyl esters (methyl oleate and linoleate)[8,9] isolated quantitatively from oils. This sample preparation, followed by quantitative ^{2}H NMR analysis, has provided detailed data on the origin of hydrogens during elongation steps and the mechanisms of primordial chain reactions, such as desaturation,[8−10] hydroxylation,[11] and epoxidation.[12]

A notable feature of the (^{2}H/^{1}H) distribution observed in all fatty acids so far analyzed is a true relative impoverishment at one ethylenic site of each double bond.[8,9] In yeast, a strong normal primary ^{2}H-KIE has been shown to be associated with desaturation[13a,b] while no primary KIE has been measured from plant Δ^9 desaturase.[14] The impoverishment in deuterium at one

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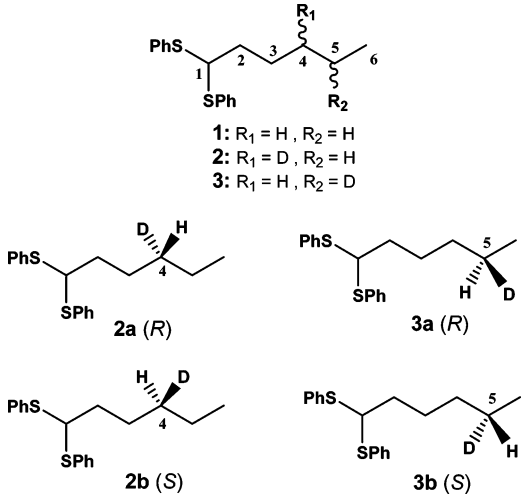

Figure 1. Structure, notation, and numbering of compounds investigated.

ethylenic site can be explained by two possible mechanisms: a secondary ^2H-KIE and/or a large difference in the ($^2H/^1H$) ratio at the pro-*R* and pro-*S* sites at the methylenic group in the substrate molecule. A direct measurement at the pro-*R* and pro-*S* sites at the methylenic group cannot, however, be obtained from conventional ^2H NMR spectra, because in achiral solvents these deuterium atoms are equivalent and hence always resonate at the same frequency.

We have recently demonstrated the potential of the natural abundance deuterium (NAD) 2D NMR technique, using a polypeptide chiral liquid crystal (CLC) as a weakly ordering solvent, to access individual deuterons in a methylenic site of a long-chain fatty acid.[15] Initially developed for analyzing monodeuterated enantiomers,[16] this original approach using commercially available polypeptides, such as poly-γ-benzyl-L-glutamate (PBLG) or poly-ϵ-carbobenzyloxy-L-lysine (PCBLL), has also proved to be a very powerful tool for distinguishing both between enantiomers of chiral molecules by virtue of their isotopic substitution $^2H/^1H$[17] and between enantiotopic directions in the case of prochiral molecules.[18] Strictly, for related parent molecules, the discrimination of the former is a consequence of the discrimination of the latter, as they both involve the same molecular recognition mechanisms.[18b]

During our previous investigation, the 1,1′-bis(thiophenyl)-hexane **1** (BTPH) derived from the natural linoleic acid of safflower (*Carthamus tinctorius*) was examined (Figure 1).[15] From a biosynthetic point of view, compound **1** is one of two products obtained by chemical modification of methyl linoleate;[8] hence it contains the natural deuterium substitution patterns of its parent molecule.[8,9]

The NAD *Q*-COSY 2D spectrum of **1** recorded in the PBLG/$CHCl_3$ solvent on a 9.4 T NMR spectrometer has clearly shown a good spectral discrimination of all monodeuterated enantio-isotopomers on the basis of differences in quadrupolar splittings, $\Delta\nu_Q$.[15,16,19] In addition, this NMR tool allowed the separation of the ^2H signals associated with the even and odd sites (notated 4 and 5 in the aliphatic chain), which exhibit the same chemical shift in isotropic NAD NMR spectra. Thus, NAD NMR in CLC of compound **1** was demonstrated as a feasible method to evaluate quantitatively the natural ^2H distribution on *pro-R* and *pro-S* C−H(^2H) directions at a methylenic site within a fatty acid chain.[15]

Although this technique is able to overcome the limitations associated with classical isotropic methods, the absolute assignment of (*R*)- and (*S*)-isotopic enantiomers from the *Q*-COSY 2D maps is not possible when only anisotropic observables (dipolar couplings, quadrupolar splittings) extracted from spectra recorded in chiral oriented media are used.[20] This is due to the fact that enantiomers are mirror images; hence, for a coherent set of anisotropic data, the use of the *S*- and *R*-geometries in the calculation of order parameters does not affect the final results, thus preventing any attribution of a data set to the corresponding enantiomer when a racemic mixture is used.[20,21]

As a consequence, only chemical strategies enable the absolute configuration associated with an NMR signal to be determined. In our case, we have opted to synthesize selectively each monodeuterated enantiomer with the desired absolute configuration to assign the *R*- and *S*-stereodescriptors for quadrupolar doublets observed at either the odd or the even methylene groups of the aliphatic chain of **1**.[15]

In the present article, we first describe the synthesis of monodeuterated racemic compounds **2** and **3** and of the chiral compounds **2a**, **2b**, **3a**, and **3b** required for the assignment of ^2H signals in the CLC NMR spectrum. Key to the strategy adopted is the use of an elegant lipase-catalyzed resolution as a simple, efficient, and general method to introduce stereoselectively a deuterium atom into an aliphatic chain. Second, the deuterium assignments obtained with compounds **2a**, **2b**, **3a**, and **3b** are compared with previous results obtained from **1** using NAD NMR in CLC and discussed in relation to the observed natural distribution of ^2H within fatty acids.

Synthesis and NMR Results

Synthesis of Compounds. The synthesis of **2a/2b** and **3a/3b**, described in Scheme 1, is based on the preparation and lipase-catalyzed resolution of the racemic alcohols **8** and **9**. Detailed schemes are reported in the Supporting Information (see Scheme SI1). Both alcohols were synthesized in four steps using the same approach. The bromo- precursors possess a terminal double bond to introduce the phenyldithioacetal function via an aldehyde. The chain length of both starting materials, a bromoderivative and an aldehyde, defines the carbon number of the final compound as well as the final position of the deuterium atom in the chain, giving rise to **8** and **9**.

From these key intermediates **8** and **9**, the deuterium atom was introduced in two steps in 60% yield by first a tosylation (TsCl, pyridine) followed by a nucleophilic substitution in the presence of $LiAl^2H_4$ leading to racemic **2** and **3**.[22]

The enzymatic resolution of the racemic secondary alcohols **8** and **9** was carried out by stereoselective lipase-catalyzed

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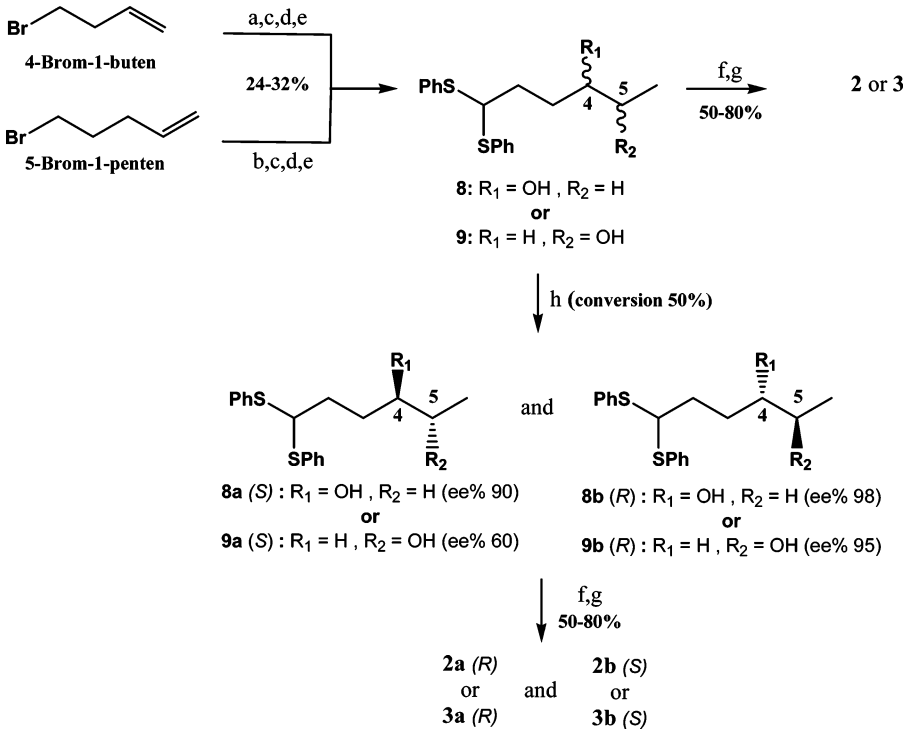
Scheme 1. Synthesis of Racemic Compounds **2** and **3** and Chiral Compounds **2a**,**2b** and **3a**,**3b**[a]



[a] Reagents and conditions: (a) Mg, Et_2O, propionaldehyde; (b) Mg, Et_2O, acetaldehyde; (c) OsO_4, *N*-methylmorpholine oxide, $CHCl_3/H_2O$; (d) $NaIO_4$, MeOH; (e) PhSH, $AlCl_3$, CH_2Cl_2; (f) TsCl, pyr.; (g) $LiAl^2H_4$, Et_2O; (h) 1-Lipase, vinyl acetate, hexane, 2-NaOH, MeOH.

Table 1. ee % Obtained after Lipase-Catalyzed (PS-Amano) Resolution of Alcohols **8** and **9**

ee %	**8**	**9**
ee % residual alcohol (**8a** or **9a**)	90	60
(isolated yield)	(43%)	(47%)
ee % alcohol after acetate hydrolysis (**8b** or **9b**)	98	95
(isolated yield)	(43%)[a]	(40%)[a]

[a] Isolated yield of acetylated product.

acetylation. Enantio-enriched compounds **2a**, **2b** or **3a**, **3b** were obtained respectively from racemic alcohols **8** and **9** after enzymatic resolution and reduction (Scheme 1). The ee % of the alcohols **8a**, **8b**, **9a**, and **9b** was measured by chiral HPLC (Table 1) and confirmed by $^2H-\{^1H\}$ NMR in CLC on **2a**, **2b**, **3a**, and **3b** (see below).

The assignment of absolute configuration to the alcohols **8a** (*S*), **8b** (*R*), **9a** (*S*) and **9b** (*R*) was performed by ^1H NMR after classic derivatization by Mosher's acid chloride (see Scheme SI2).[23] The racemic alcohol **8** was esterified by (*S*)-MTPA-Cl to give the Mosher's esters mixture **14a/14b**, and the racemic alcohol **9** was esterified by (*R*)-MTPA-Cl to give the Mosher's esters mixture **15a/15b** (Figure 2). The proton NMR spectra of diastereoisomeric compounds **14a/14b** and **15a/15b** in each mixture were compared. The difference in chemical shifts for $-CH(SPh)_2$ and $-CH_2CH_3$ signals between both diastereoisomers were measured, and the $\Delta\delta$ values are given in Table 2.

The absolute configuration at C4 for **14a** or **14b**, and at C5 for **15a** or **15b**, was assigned using the model of the preferential conformation (Figure 2).[23] This predicts that, on the basis of the diamagnetic effect of the phenyl ring, protons in the shielding cone of this ring exhibit a ^1H chemical shift, which is shifted upfield relative to those not in this cone. Thus, on the basis of ^1H NMR chemical shift analysis, the arrangement of the terminal methyl of the alcohol can be as shown in Figure 2.

For example, the methyl proton in **14b** that is *cis*-configured relative to the phenyl ring of the Mosher's acid moiety displays a weaker chemical shift than that in **14a**. Also, the $CH(SPh)_2$ signal of **14a** shows a smaller chemical shift than the same signal in **14b**. Thus, alcohols **8a** and **8b** are, respectively, *S*- and *R*-configured. A similar procedure applied to compound **15** indicates that alcohols **9a** and **9b** are *S*- and *R*-configured, respectively.

Residual alcohols **8a** and **9a** obtained by lipase-catalyzed resolution were submitted to derivatization with Mosher's chloride, providing the corresponding diastereoisomer. Their associated ^1H NMR spectra were compared with the reference spectra obtained in racemic series, thus leading to an attribution of their absolute configurations. Thus, alcohols **8a** and **9a**, *S*-configured, are not substrates of the lipase PS "Amano" since this enzyme preferentially reacts with the *R*-isomers **8b** or **9b** to give the acetylated products. These results are in full agreement with the known selectivity for the resolution of a secondary alcohol using lipase PS "Amano".[24]

Deuterated compounds **2a**(*R*), **2b**(*S*), **3a**(*R*), and **3b**(*S*) were obtained, respectively, from alcohols **8a**(*S*), **8b**(*R*), **9a**(*S*), and **9b**(*R*) in two steps following a similar scheme to that used for the preparation of racemic **2** and **3** (Scheme 1). As the deuteration was performed by nucleophilic substitution, a strict

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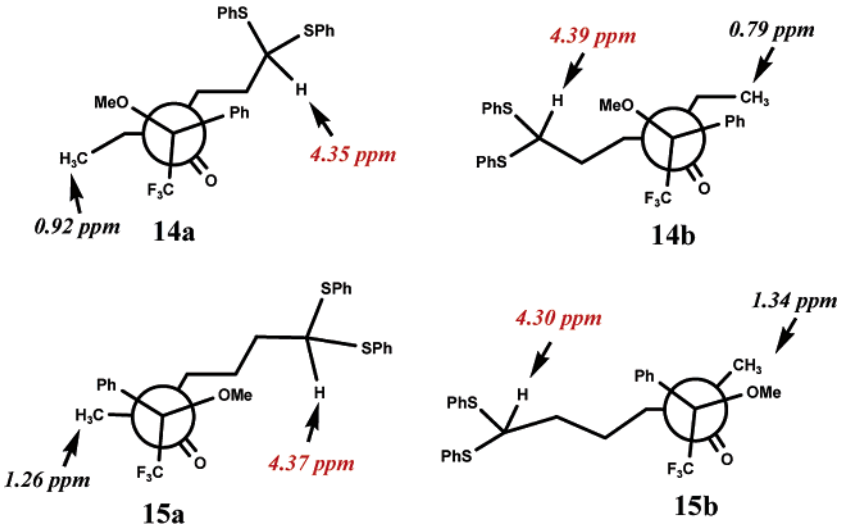

Figure 2. Models explaining the variation in proton chemical shifts of the methyl and methylene groups.

Table 2. ^1H NMR Chemical Shift and Absolute Configuration of C4 for **14a/14b** and of C5 for **15a/15b**

	δ(ppm)			δ(ppm)		
	14a	**14b**	Δδ	**15a**	**15b**	Δδ
$CH(SPh)_2$	4.35	4.39	**−0.04**	4.37	4.30	**+0.07**
CH_2CH_3	0.92	0.79	**+0.13**	1.26	1.34	**−0.08**
configuration	(+)4-*S*	(−)4-*R*		(+)5-*S*	(−)5-*R*	
alcohol *n*°	**8a**	**8b**		**9a**	**9b**	

inversion of configuration was expected.[25] This was seen to have occurred.

Study by ^2H NMR in Chiral Liquid Crystal. Before examining the ^2H spectra of deuterated isotopomers **2a**, **2b**, **3a**, and **3b** recorded in oriented solvents, the NAD NMR signals associated with deuterons 4,4′ and 5,5′ of **1** dissolved in the achiral phase PBG/$CHCl_3$ and chiral phase PBLG/$CHCl_3$ must be described (see Figure 3).

Due to quadrupolar interaction that is no longer averaged to zero as in an isotropic medium, the NMR signal of a deuterium atom (spin $I = 1$) in oriented phases is characterized by a quadrupolar doublet, whose splitting between both components is noted $\Delta\nu_Q$. In the achiral medium, where no enantiodiscrimination is possible,[26] only two doublets centered on the same chemical shift are observed for the methylene groups 4 and 5 (Figure 3a). The large difference in peak intensity enables both doublets to be assigned, based on the well-characterized differences in the $^2H/^1H$ ratio at even- and odd-numbered positions in the fatty acid aliphatic chain. In contrast, in the chiral oriented matrix where enantioselective interactions exist, a doubling of quadrupolar doublets appears due to the discrimination of isotopic enantiomers (Figure 3b), and for both methylene groups (4 and 5), the peak intensity (and the peak area) is different. However, as emphasized in the Introduction, it is not possible to assign the absolute configuration for each quadrupolar doublet. We can now compare the various deuterated isotopomers (**2a**, **2b**, **3a**, and **3b**) associated with methylenic groups 4 and 5 with the NAD NMR spectrum of **1**. Due to the fact that order parameters are dependent on various external parameters, such as temperature or the sample composition, the comparison of the two sets of data (isotopically normal and enriched compound) is only valid if the spectra are recorded under the same experimental conditions. In our previous study, we prepared the NMR sample with 14.3% w/w of polymer and 28.6% w/w of solute (see Table SI1 in Supporting Information). To overcome the intrinsically low sensitivity of NAD NMR spectroscopy, we used a large amount of isotopically normal solute (200 mg).[15] To keep the same w/w relative proportions for samples with deuterated solutes, we have therefore prepared a mixture of **1** isotopically normal (∼190 mg) and deuterated compounds **2** or **3** (∼10 mg). As the orientational behaviors for the two kinds of solute (see above) are identical, we can assume a priori that we are comparing "identical" samples. It could be argued that the compensation of deuterated solute by a larger amount of chloroform to keep the % w/w of polymer identical should also provide "identical" samples. In fact, this option is not valid, since we have observed experimentally a significant increase in quadrupolar splittings (around 30%) of deuterated BPTH (**2** and **3**) compared with values measured on the NAD NMR spectrum of **1** (see Tables SI2 and SI3 in the Supporting Information). This suggests that molecules of BTPH generate a larger orientational disorder inside the PBLG phase compared to chloroform, thus reducing the magnitude of the order parameters for the solute (as well as for the cosolvent). From this, we clearly show that the effects of solute and cosolvent on the liquid crystalline properties of the phase are not identical. These differences can be attributed to differences in dielectric constant, polarity, and molecular size of the BTPH compared with chloroform, as well as the fact that BTPH does not dissolve the PBLG fibers.

Figure 4 shows the six $^2H-\{^1H\}$ 1D NMR spectra associated with BTPH selectively deuterated on the methylene group 4 or 5 in racemic and enantio-enriched series. Spectral data of each spectrum as well as data obtained for isotopically normal compound **1** are described in the Supporting Information (Tables SI2 and SI3).

The assignment of each quadrupolar doublet in methylene groups 4 and 5 of (±)-**2** and (±)-**3** is made by a direct comparison of the spectra with those recorded with enantio-

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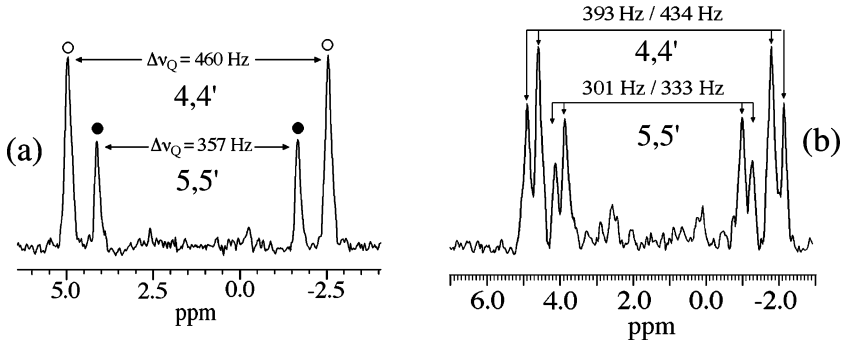

Figure 3. Quadrupolar doublets associated with deuterons 4,4′ and 5,5′ (sum of columns) extracted from the NAD *Q*-COSY 2D map of **1** dissolved in (a) the PBG/$CHCl_3$ achiral phase and (b) the PBLG/$CHCl_3$ chiral phase (see ref 15 for experimental details). The quadrupolar splitting, $\Delta\nu_Q$, between both components is equal to $(^3/_2)e^2 \times Q_D \times q_{C-D} \times S_{C-D}$ where Q_D is the deuterium quadrupole moment, q_{C-D} is the electric field gradient (EFG) along the C−^{2}H bond, and S_{C-D} is the order parameter of the C−^{2}H bond.

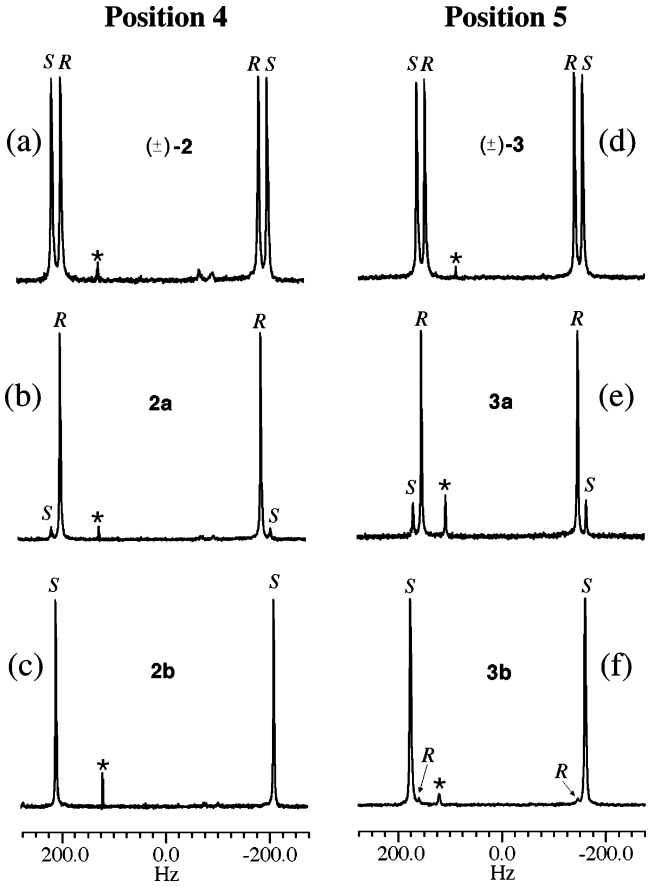

Figure 4. 61.4 MHz ^{2}H−{^{1}H} 1D NMR spectrum of BTPH deuterated on the methylene group 4 (left column) or 5 (right column) recorded at 300 K in the PBLG/$CHCl_3$ phase. (a and d) Racemic sample corresponding to **2** and **3**, respectively; (b and e) *R*-enriched sample corresponding to **2a** and **3a**, respectively; (c and f) *S*-enriched sample made up of **2b** and **3b**, respectively. The 1D spectra of racemic and enantio-enriched mixtures were recorded by adding 2700 and 4000 scans, respectively. For all spectra, no filtering window was applied. The spectra are plotted with the same frequency scale. The signal labeled with an asterisk corresponds to the shielded component of the quadrupolar doublet of chloroform.

enriched compounds, **2a**, **2b** and **3a**, **3b**. For both methylene positions, the comparison indicates that the inner and outer quadrupolar doublets correspond to the *R*- and *S*-enantiomers, respectively (Figure 4). As a consequence, the depletion observed in the CLC NMR spectra of **1** corresponds to the *S*-enantio-isotopomers. In other words, the *pro-S* site in both 4 and 5 prostereogenic methylene groups possesses the smaller isotopic (^{2}H/^{1}H) ratio.

Discussion

In previous work, we have demonstrated that NAD 2D NMR spectroscopy in chiral polypeptide liquid crystals (PBLG) can be exploited to analyze the isotopic (^{2}H/^{1}H) ratio at *pro-R* and *pro-S* sites in a methylene position in an aliphatic chain in a natural fatty acid, via the derivative 1,1′-bis(thiophenyl)hexane.[15] The initial experimental NMR results identified the same odd/even variation in the ^{2}H/^{1}H ratio as that observed in the achiral medium[8,9] but, additionally, distinguished between the enantiotopic deuterons for each stereogenic site. We have now demonstrated that it is possible to assign the absolute configuration for each deuterium signal to each enantiotopic C−H(^{2}H) direction for all accessible stereogenic sites. To reach this goal, we have developed an optimized synthetic strategy to obtain separately four regio- and stereoselective deuterated enantiomers in positions 4 and 5 in the aliphatic chain of BTPH and used these to assign unambiguously the NAD NMR signals in the PBLG phase.

Fatty acid biosynthesis is dominated by a succession of enantioselective reactions in the FAS complex.[27] In all organisms, the origin of hydrogens in the aliphatic chain of fatty acids is different between odd and even methylene sites and also between pro-*R* and pro-*S* sites.[28] At even methylene sites, hydrogens are provided by acetate and water, while both hydrogens at odd methylene sites are introduced by two reduction steps, which involve a keto and an enoyl reductase operating with the cofactor NAD(P)H. Moreover, each organism displays a different stereochemistry for the introduction of hydrogens from acetate or water and for the reduction steps; thus the natural distribution of deuterium at pro-*R* and pro-*S* should change in fatty acids isolated from different organisms (mammals, plants, bacteria, algae, fungi). In contrast to mammalian and bacterial sources, the stereochemical sequence of steps performed by the FAS is still not well described in plants, algae, and fungi.[29]

After elongation, important modifications of the fatty acid chain occur, such as desaturation, hydroxylation, or epoxidation.

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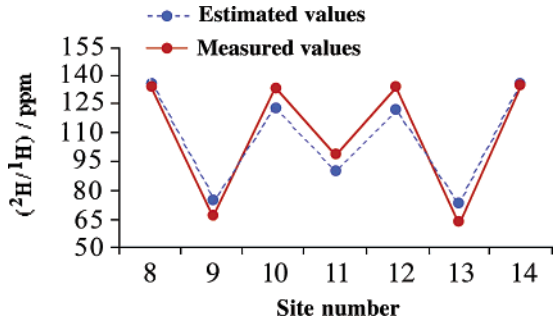

Figure 5. Comparison of the relative values of the ($^2H/^1H$) ratios in BTPH with the pro-*R* and pro-*S* positions of the original methyl linoleate.

Once again, these reactions are stereoselective. In the case of desaturation, it has been reported that both pro-*R* hydrogens are eliminated, giving rise to a *cis*-configured double bond.[30] Previously, we have measured the ($^2H/^1H$) ratio at even and odd sites of unsaturated fatty acids isolated from plants. In all cases, odd ethylenic sites were strongly depleted while even sites were not. Initially, we interpreted this observation as the result of a strong secondary KIE on the odd site during the desaturation step, considering the existence of a large difference in the deuterium distributions between pro-*R* and pro-*S* sites in the substrate molecule improbable, as both hydrogens are introduced by two reduction steps with the same pool of cofactor (NAD(P)H).

However, an initial analysis by CLC NMR indicated that this second explanation is justified, one hydrogen of the methylene group being richer in ^2H than the other (see Table 3 in ref 15). In the present study, we have extended this analysis and shown that it is the pro-*R* hydrogens that are enriched in ^2H and the pro-*S* that are, relatively, depleted. Hence, as the stereochemistry of desaturation exclusively eliminates the pro-*R* hydrogens during double-bond formation,[2,31] it can be expected that the retained hydrogens will be impoverished relative to the mean of the methylenic groups. The relative values of the ($^2H/^1H$) ratios in BTPH can now be compared with the pro-*R* and pro-*S* positions of the original methyl linoleate (Figure 5). For this comparison, the ($^2H/^1H$) ratios of pro-*S* and pro-*R* of the 2, 3, 4, and 5 positions of BTPH are used, these being equivalent, respectively, to the 14, 15, 16, and 17 positions of methyl linoleate (see Figure 6).

From Figures 5 and 6, it can be seen that the predicted values of ^2H in sites 8 to 14 of methyl linoleate are in good agreement (within 10−15 ppm) with the experimentally determined values.[8] Notably, positions 9 and 13 are strongly impoverished relative to the 10 and 12 positions. Thus, the apparent relative impoverishment at the odd ethylenic positions is explained by the $\Delta(^2H/^1H)$ for the pro-*S* and pro-*R* enantiotopic sites in the methylenic groups from which they are derived.

Conclusion

In this work, we have shown for the first time that the ($^2H/^1H$) ratio of pro-*S* enantiotopic sites in methylenic groups (even and odd) within a fatty acid isolated from a plant (*Carthamus tinctorius*) is strongly depleted relative to the pro-*R*. This disparity is sufficient to explain the differences observed at natural abundance between the ($^2H/^1H$) ratios for the *n* and *n* + 1 ethylenic hydrogens at the positions of unsaturation.

The next question concerning fatty acid biosynthesis in plants is to establish which hydrogen (pro-*R* or pro-*S*) comes from water and which from acetate in the even methylene sites and which comes from the reduction steps by a keto and an enoyl reductase in the odd sites. As we demonstrate here, we have at our disposal a very specific analytical method with which to tackle this question. At present, the ($^2H/^1H$) ratios in fatty acids isolated from *Fusarium lateritium*, an oleaginous fungus, cultured in controlled media (enriched in ^2H in water or in glucose) are under investigation.

Experimental Section

General Methods. Lithium aluminum deuteride, $LiAl^2H_4$ (98% ^2H), (*R*)- and (*S*)-α-methoxy-α-trifluoromethyl-phenyl-acetyl chloride (98−99%) were purchased from Sigma-Aldrich; Lipases PS, AY, and GC20 were purchased from Amano. ^1H and ^{13}C NMR spectra were recorded with a Bruker DRX 500 or DPX 400 spectrometer. Chemical shifts are given in ppm from TMS. Flash chromatography was performed on silica gel Normasil (40−60 μm, Prolabo). Reactions were followed by gas chromatography on an HP-5 capillary column (30 m × 0.32 mm, film thickness 0.52 μm); carrier gas, He; flow, 1.2 mL min^{-1}; split, 1:40; FID temp, 280 °C; thermal gradient 100 °C initially for 1 min, ramped at 10 °C min^{-1} to 240 °C for 1 min, ramped at 10 °C min^{-1} to 280 for 1 min, then ramped at 10 °C min^{-1} to 290, and held at 290 for 10 min. All solvents were distilled before use. Reactions were performed at room temperature (20 °C). Enantiomeric excess (ee %) was determined by HPLC on an HP series 1100 apparatus; UV detector operating at 254 nm; Column: Chiracel AD-H (250 mm × 4.6 mm) elution with hexane/2-propanol (95/5) at 0.5 mL min^{-1} for product **8**; Chiracel OD-H (250 mm × 4.6 mm) elution with hexane/2-propanol (98/2) at 0.5 mL min^{-1} for product **9**.

1,1′-Dithiophenyl-4-^{2}H-hexane (2). To a solution of the racemic tosylate **10** (140 mg, 0.3 mmol) in anhydrous Et_2O (3 mL) was added $LiAl^2H_4$ (37.8 mg, 0.9 mmol), and the reaction mixture was stirred for 2 h at room temperature. The reaction was cooled and then quenched by the addition of several drops of water. The mixture was diluted with Et_2O and washed with H_2O. The aqueous layer was extracted with Et_2O, and the combined organic phases were dried ($MgSO_4$), filtered, and evaporated. The crude residue was purified by flash chromatography (petroleum ether) and gave **2** (66 mg, 72%) as a colorless oil. ^1H NMR (400 MHz, $CDCl_3$) δ 0.89 (t, *J* = 7 Hz, 3H), 1.29 (m, 3H), 1.62 (m, 2H), 1.87 (qd, *J* = 7 Hz, 2H), 4.82 (t, *J* = 6.7 Hz, 1H), 7.25−7.5 (m, 10H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.1, 22.5, 26.8, 31.03 (t, *J* = 19 Hz), 36.0, 57.7, 127.8, 129.0, 132.9, 134.6. GC−MS (EI): 303, 194, 123, 109, 123, 84, 56. EI-MS: calcd = 303.1220; found = 303.1299.

(*R*)-1,1′-Dithiophenyl-4-^{2}H-hexane (2a) and (*S*)-1,1′-Dithiophenyl-4-^{2}H-hexane (2b). These compounds were prepared from **10a**-(*S*) and **10b**-(*R*) following the same protocol as that for **2** from **10**. Deuterated compounds **2a**-(*R*) (130 mg), from **10a**-(*S*), and **2b**-(*S*) (120 mg), from **10b**-(*R*), were obtained in 66% and 64% yield, respectively. Their ^1H NMR and ^{13}C NMR spectra were in complete agreement with spectra obtained from the racemic mixture **2**.

1,1′-Dithiophenyl-5-^{2}H-hexane (3). To a solution of racemic tosylate **11** (118 mg, 0.25 mmol) in anhydrous Et_2O (3 mL) was added $LiAl^2H_4$ (21 mg, 0.5 mmol), and the reaction mixture was stirred for 3 h at room temperature. The reaction was cooled and then quenched by the addition of several drops of water. The mixture was diluted with Et_2O and washed with H_2O. The aqueous layer was extracted with Et_2O, and the combined organic phases were dried ($MgSO_4$), filtered, and evaporated. The crude residue was purified by flash chromatography (petroleum ether) and gave **2** (69 mg, 90%) as a colorless oil. ^1H NMR (400 MHz, $CDCl_3$): δ 0.88 (d, *J* = 6.6 Hz, 3H), 1.27 (m, 3H), 1.62

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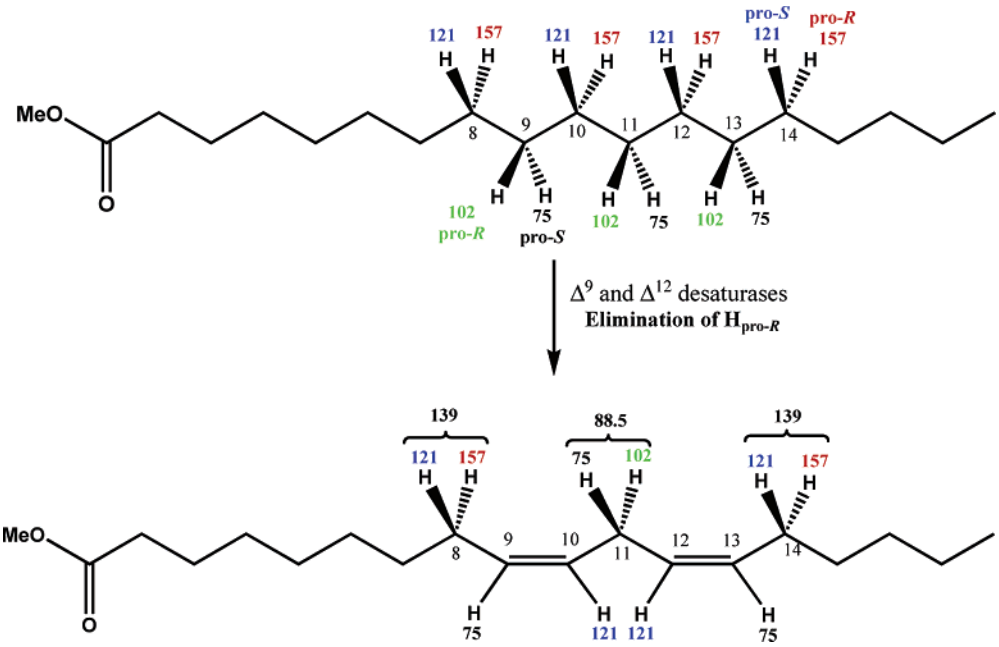

Figure 6. Estimated (^{2}H/^{1}H) ratios from the height of doublet peaks from CLC ^{2}H NMR spectra of **1** (ref 15). $(^2H/^1H)_{pro-S}$ at odd sites is the mean of (^{2}H/^{1}H) values found from sites 3*S* and 5*S*, $(^2H/^1H)_{pro-R}$ at odd sites is the mean of (^{2}H/^{1}H) values found from sites 3*R* and 5*R*, $(^2H/^1H)_{pro-S}$ at even sites is the mean (^{2}H/^{1}H) values found from sites 2*S* and 4*S*, and $(^2H/^1H)_{pro-R}$ at even sites is the mean (^{2}H/^{1}H) values found from sites 2*R* and 4R.

(qt, J = 7.5 Hz, 2H), 1.87 (qd, J = 7 Hz, 2H), 4.42 (t, J = 6.7 Hz, 1H), 7.25−7.5 (m, 10H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.2, 22.4 (t, J = 19.07 Hz), 27.1, 31.5, 36.2, 58.9, 128.0, 129.2, 133.0, 134.8. GC−MS (EI): 303, 194, 123, 109, 123, 84, 56. EI-MS: calcd = 303.1220; found = 303.1201.

(*R*)-1,1′-Dithiophenyl-5-^{2}H-hexane (3a) and (*S*)-1,1′-Dithiophenyl-4-^{2}H-hexane (3b). These compounds were prepared from **11a**-(*S*) and **11b**-(*R*) following the same protocol for **3** from **11**. Deuterated compounds **3a**-(*R*) (150 mg), from **11a**-(*S*), and **3b**-(*S*) (110 mg), from **11b**-(*R*), were obtained in 69% and 73% yield, respectively. Their ^{1}H NMR and ^{13}C NMR spectra were in complete agreement with spectra obtained from the racemic mixture **3**.

Enzymatic Resolution. (+)-(*S*)-1,1′-Dithiophenyl-4-hydroxy-hexane (8a) and (*R*)-1,1′-Dithiophenyl-4-acetyl-hexane (12). To a solution of the racemic alcohol **8** (576 mg, 1.81 mmol) in hexane (30 mL) were added the lipase PS Amano (1 g) and vinyl acetate (157 μL, 1.7 mmol). The mixture was stirred at room temperature. After 24 h, the conversion [%] of **8** to acetylated compound **12** reached about 50 (measured by GC). The mixture reaction was filtered, and the solvent was evaporated under a vacuum. The crude residue was purified by flash chromatography to give the residual alcohol **8a** (major configuration *S*) (275 mg, 0.86 mmol, 47%) and **12** (major configuration *R*) (283 mg, 0.786 mmol, 44%) as colorless oils. The ^{1}H NMR spectrum of **8a** agreed with the ^{1}H NMR spectrum of the racemic alcohol **8**. The ee % of **8a** measured by chiral HPLC was 90% to 98%. $[\alpha]_D^{20} = +3.2$ (5.4×10^{-3}, $CHCl_3$, ee % = 97%).

NMR data of **12**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.87 (t, J = 7.4 Hz, 3H), 1.54 (qt, J = 7.2 Hz, 2H), 1.83−1.89 (m, 4H), 1.97 (s, 3H), 4.42 (t, J = 6.1 Hz, 1H), 4.78 (m, 1H), 7.2−7.5 (m, 10H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 9.7, 21.3, 27.2, 30.9, 31.5, 58.3, 74.7, 128.03, 129.1, 133.0, 133.1, 134.1, 134.2, 171.0.

(−)-(*R*)-1,1′-Dithiophenyl-4-hydroxy-hexane (8b). To a solution of **12** (283 mg, 0.786 mmol) in MeOH (10 mL) was added NaOH (160 mg, 4 mmol). After the solution was stirred overnight at room temperature, solvent was evaporated and the crude residue was diluted with CH_2Cl_2 and then washed with water. The aqueous layer was extracted twice with CH_2Cl_2, and the combined organic phases were dried ($MgSO_4$), filtered, and evaporated. The crude residue was filtered on silica gel (CH_2Cl_2) to give the alcohol **8b**-(*R*) (244 mg, 98%). The ^{1}H NMR spectrum was in complete agreement with the ^{1}H NMR spectrum of the racemic alcohol **8**. The ee % of **8b** measured by chiral HPLC was 98%. $[\alpha]_D^{20} = -4.5$ (4.9×10^{-3}, $CHCl_3$, ee % = 98%).

(+)-(*S*)-1,1′-Dithiophenyl-5-hydroxy-hexane (9a) and (*R*)-1,1′-Dithiophenyl-5-acetyl-hexane (13). To a solution of the racemic alcohol **9** (330 mg, 1.0 mmol) in hexane (15 mL) were added the lipase PS Amano (0.6 g) and vinyl acetate (78 μL, 0.84 mmol). The mixture was stirred at room temperature. After 24 h, the conversion [%] of **9** to acetylated compound **13** reached about 50 (measured by GC). The reaction mixture was filtered, and the solvent was evaporated under a vacuum. The crude residue was purified by flash chromatography to give alcohol **9a** (major configuration *S*) (0.41 mmol, 47%) and **13** (major configuration *R*) (0.48 mmol, 40%) as colorless oils. The ^{1}H NMR spectrum of **9a** was in complete agreement with the ^{1}H NMR spectrum of the racemic alcohol **9**. The ee % of **9a** measured by chiral HPLC was 60% to 94.5%. $[\alpha]_D^{20} = +3.5$ (5.8×10^{-3}, $CHCl_3$, ee % = 94.5%).

NMR data of **13**: ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.21 (d, J = 6.2 Hz, 3H), 1.43−1.71 (m, 4H), 1.87 (m, 2H), 2.03 (s, 3H), 4.40 (t, J = 6.6 Hz, 1H), 4.88 (sp, J = 6.3 Hz, 1H), 7.25−7.5 (m, 10H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 20.1, 22.1, 23.7, 36.0, 36.5, 59.1, 71.4, 128.5, 129.7, 133.5, 134.9, 171.4.

(−)-(*R*)-1,1′-dithiophenyl-5-hydroxy-hexane (9b). To a solution of **13** (150 mg, 0.416 mmol) in MeOH (5 mL) was added NaOH (80 mg, 2.08 mmol), and the mixture was stirred at room temperature. After 3.5 h, the solvent was evaporated, and the crude residue was diluted in CH_2Cl_2. The organic phase was washed with water, and the aqueous phases were extracted with CH_2Cl_2. The combined organic phases were dried ($MgSO_4$), filtered, and evaporated to afford **9b**-(*R*) (108 mg, 82%). The ^{1}H NMR spectrum was in complete agreement with the ^{1}H NMR spectrum of the racemic alcohol **9**. The ee % of **9b** measured by chiral HPLC was 95% to 96%. $[\alpha]_D^{20} = -3.8$ (6.4×10^{-3}, $CHCl_3$, ee % = 96%).

NMR in Oriented Solvent. The various samples were composed of around 100 mg of PBLG (Sigma) with MW ~171 300 g/mol (DP = 782), ~200 mg of solute mixture, and ~700 mg of dry $CHCl_3$. The exact composition for each oriented NMR sample is given in Table SI1 of the Supporting Information. The mixture components were directly weighed into 5-mm NMR tubes. Then they were homogenized

by centrifugation (see ref 15) until an optically homogeneous birefringent phase was obtained. The $^2H-\{^1H\}$ 1D NMR experiments were performed at 9.4 T on a Bruker DRX 400 high-resolution spectrometer equipped with a selective ^2H probe operating at 61.4 MHz for deuterium and a standard variable temperature unit. Sample temperature was carefully controlled at 300 K. The protons were broadband decoupled using the WALTZ-16 composite pulse sequence. For other details on the method, see refs 15, 16, and 19.

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Supporting Information Available: Details of synthesis. Experimental data concerning ^2H NMR in the chiral liquid-crystalline phase. This material is available free of charge via the Internet at http://pubs.acs.org.

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