



**HAL**  
open science

# Nuclear Magnetic Resonance Spectroscopy-Applicable Elements-Hydrogen Isotopes: $^2\text{H}$ NMR

Philippe Lesot

► **To cite this version:**

Philippe Lesot. Nuclear Magnetic Resonance Spectroscopy-Applicable Elements-Hydrogen Isotopes:  $^2\text{H}$  NMR. Reference Module in Chemistry, Molecular Sciences and Chemical Engineering, 7, Elsevier, pp.152-167, 2018, 10.1016/B978-0-12-409547-2.14084-3 . hal-02334009

**HAL Id: hal-02334009**

**<https://hal.science/hal-02334009>**

Submitted on 18 Jul 2021

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

---

# Nuclear Magnetic Resonance Spectroscopy-Applicable Elements | Hydrogen Isotopes: $^2\text{H}$ NMR

Philippe Lesot\* 

[a] Institut de Chimie Moléculaire et des Matériaux d'Orsay (ICMMO), RMN en Milieu Orienté, UMR CNRS 8182, Bât. 410, Université de Paris Sud / Paris-Saclay, F-91405 Orsay cedex, France.

[b] Centre National de la Recherche Scientifique (CNRS), 3 rue Michel Ange, F-75016 Paris, France.

Correspondance : E-mail : [philippe.lesot@u-psud.fr](mailto:philippe.lesot@u-psud.fr)

ORCID ID:0000-0002-5811-7530

Researcher ID: M-3390-2017

Tel. : +33169154759 / Fax : +33169157017

## ABSTRACT :

As a second (stable) isotope of hydrogen with an abundance level of ca. 0.0155 % on average and spin  $I = 1$ , the deuterium nucleus ( $^2\text{H}$ ) has an important place from the NMR spectroscopy viewpoint with the advantage that it is present in all (bio)organic compounds. Among all quadrupolar nuclei ( $I > 1/2$ ), the deuteron plays a distinctive and relevant role because it possesses a very small nuclear electric quadrupole moment ( $Q_D$ ) that can be nicely spectrally exploited. Due to its intrinsic NMR properties and despite its low natural abundance,  $^2\text{H}$  nuclei can be used as a remarkable, natural probe or tracer for many analytical issues. Additionally, instrumental and methodological developments (magnetic fields, electronic, cryogenic probes, pulse sequences, processing) have significantly boosted the sensitivity and the potentialities of  $^2\text{H}$  NMR spectroscopy, in particular natural abundance deuterium NMR spectroscopy (NAD NMR). These new opportunities have opened up a diversified range of original applications, in liquid, liquid crystalline and solid states of matter. In this chapter, the main fundamentals of  $^2\text{H}$  NMR spectroscopy and an overview of the most significant applications in the three states of condensed matter (liquids, liquid crystals and solids) are presented. A special emphasis is placed on NAD NMR-based applications.

**Keywords :** Deuterium NMR, Natural abundance, Isotopomers, Liquids, Anisotropic media, SS-NMR, Chirality, Stereochemistry, Isotope fractionation

**Corresponding authors :** [philippe.lesot@universite-paris-saclay.fr](mailto:philippe.lesot@universite-paris-saclay.fr)

**ORCID numbers** : Philippe Lesot : [orcid.org/0000-0002-5811-7530](https://orcid.org/0000-0002-5811-7530)

## Introduction

Modern Nuclear Magnetic Resonance (NMR) spectroscopy is a unique and flexible technique for extracting valuable molecular information at atomic resolution (structural identification, dynamic behavior, isotopic analysis, ...) in the three states of condensed matter, namely in liquids (isotropic systems), in liquid crystals (anisotropic systems) and in solids (powders or single crystals). In practice, NMR can detect all common magnetically active nuclei (and some of their stable isotopes) naturally present in organic molecules ( $^1\text{H}$ ,  $^{13}\text{C}$ ,  $^{14,15}\text{N}$ ,  $^{17}\text{O}$ ,  $^{19}\text{F}$ ,  $^{31}\text{P}$ , ...) as well as more exotic nuclei such as  $^3\text{H}$ ,  $^{10,11}\text{B}$ ,  $^{51}\text{V}$ ,  $^{93}\text{Nb}$ ,  $^{181}\text{Ta}$ , ... Combined with significant instrumental, technical or methodological achievements during the three past decades, NMR spectroscopy has nowadays become a general and powerful method with a wide range of applications in modern organic chemistry and related domains (biology, biochemistry, ...). Within the broadened family of chemists, NMR spectroscopy is currently an essential analytical tool for most molecular investigations.

In the world of stable isotopes, the  $^2\text{H}$  nucleus, the second isotope of hydrogen has a special place from an NMR viewpoint for six interconnected reasons, at least: First, numerous and efficient synthetic approaches exist to incorporate deuterons in molecules: i) the use of acid-, base-, and metal-catalyzed hydrogen-isotope-exchange reaction yielding poly- and perdeuterated compounds; ii) specific chemical reactions leading to regio- or stereospecifically monodeuterated analytes (aromatic dehalogenation, borodeuteride, catalytic hydrogenation, methyl iodide methylation and reduction); iii) biosynthetic routes (enzymatic) for biomolecules (proteins). Second, deuterium atoms are naturally present at a level of ( $1.55 \times 10^{-2}\%$  compared to proton atoms) in any organic, bioorganic/organometallic compounds, hybrid materials or even proton conductors. As a consequence, information associated with (monodeuterated) isotopomers is essentially the same as that of corresponding deuterium-enriched analytes. Third, although the frequency dispersion of  $^2\text{H}$  spectra (in Hz) is 6.515 times smaller compared to  $^1\text{H}$  spectra (due to its low  $^2\text{H}$  gyromagnetic,  $\gamma(^2\text{H}) = 0.153 \times \gamma(^1\text{H})$ ), its range of chemical shifts ( $\delta$ ) is identical to that of  $^1\text{H}$  (electron clouds of deuterons are similar to proton ones), and hence we have  $\delta^{\text{iso}}(^2\text{H}) \approx \delta^{\text{iso}}(^1\text{H})$  in ppm. Fourth, for the same reasons, the magnitude of homonuclear ( $^2\text{H}$ - $^2\text{H}$ ) and heteronuclear ( $^2\text{H}$ - $^n\text{X}$ ) couplings (scalar or dipolar) are divided by a factor of 42.2 and 6.5, respectively, compared to coupled interatomic pairs involving  $^1\text{H}$ . Fifth, to simplify the spectral analysis of  $^2\text{H}$  spectra, proton nuclei can be decoupled. Thus in liquids and weakly orienting systems, classical CPD sequences such as Waltz-16 can be used, while for NMR in thermotropic liquid crystals or in solids, more powerful  $^1\text{H}$  decoupling is required. Beside, owing to the extremely low abundance ( $2.4 \times 10^{-6}\%$ ) of dideuterated isotopomers, the Natural

Abundance Deuterium (NAD NMR) spectrum of a molecule simply corresponds to the sum of independent  $^2\text{H}$  signals associated with each monodeuterated isotopomer of the mixture. Therefore, they are devoid of fine structures produced by  $^2\text{H}$ - $^2\text{H}$  scalar couplings ( $^n\text{J}_{2\text{H}-2\text{H}}$ ) in liquids, and both scalar and residual dipolar couplings (RDC), noted  $^n\text{D}_{2\text{H}-2\text{H}}$ , in orientationally ordered systems. Note finally that for weakly aligned, isotopically labeled analytes, the small magnitude of  $^2\text{H}$ - $^2\text{H}$  scalar couplings as well as ( $^2\text{H}$ - $^2\text{H}$ )-RDCs generally produces only a broadening of linewidths (compared to the NAD signal of the same site) and  $^2\text{H}$ - $^2\text{H}$  fine structures are rarely observed in  $^2\text{H}$  spectra.

Last but not least, the presence of a neutron inside the deuterium nucleus yields a spin  $I = 1$ , and so this nucleus is subject to a nuclear electric quadrupolar moment,  $Q_D$ . Interestingly, the small  $Q_D$  value ( $0.286 \text{ fm}^2$ ) leads to high-resolution  $^2\text{H}$  spectra (small linewidths) in liquids and LCs. This also limits the width of solid-state  $^2\text{H}$  1D spectra, thus facilitating its experimental detection. The dominance of the quadrupolar relaxation mechanism means that  $^1\text{H}$  irradiation (by decoupling) produces no or very negligible nOe. As a consequence, NAD- $\{^1\text{H}\}$  NMR spectra can be used for quantitative measurements (see below) when spectra are recorded under ‘quantitative’ conditions, namely  $T_{RD} > 5 \times T_1$  (the longest  $T_1$ ). Beside, in aligned solvents, the quadrupolar interaction,  $H_Q$ , affords valuable information on the orientational order (not allowed in liquids) of guest and host molecules, their structures, their dynamics or their isotopic profile. Exploiting this potential, many NMR developments based on this interaction have been designed and exploited.

Among stable NMR active isotopes, the deuterium at natural abundance level exhibits one of the lowest receptivities compared to that of protons ( $1.11 \times 10^{-6}$ ), both due to its low natural isotopic abundance, one of the smallest of the Periodic Classification of Elements (155 ppm according to V-SMOW standard value) and its low  $g$  that contributes to the decrease of sensitivity of the NMR detection of this isotope. Despite this drawback, NAD  $^2\text{H}$  NMR remains highly attractive for the molecular-level characterization of the three states of the matter, in particular with the new generations of high-field NMR instruments, the use of optimized probes and the development of specific methodologies.

In this Chapter, theoretical and practical aspects of  $^2\text{H}$  NMR spectroscopy will be examined and the most original analytical applications developed in liquids, oriented systems and solids will be overviewed. Special attention will be paid to NAD 2D-NMR in chiral liquid crystals (CLCs) that has been proposed as an efficient and versatile approach in various domains.

### **Instrumental and methodological advances in $^2\text{H}$ NMR**

NMR spectroscopy, arguably the most powerful tool in chemistry, is *a priori* known to be a relatively

insensitive method compared to other tools such as mass spectrometry. However continuous and exciting instrumental, hardware and/or methodological NMR developments have significantly boosted its sensitivity and in particular the experimental possibilities of NAD NMR. Major achievements in this domain have been: i) the detection with very high magnetic fields ( $\geq 14$  T) since the NMR sensitivity is proportional to  $B_0^{3/2}$ ; ii) the use of microcoil probes or cryogenically cooled  $^2\text{H}$  probes using gaseous helium at very low temperature (15 - 30 K), improving the RF efficiency and reducing the thermal noise generated by the coils of the probe as well as the electronic circuit of the preamplifier (70 - 80 K), leading to overall gains in sensitivity by a factor of 4-5 compared to a standard “hot” probe; iii) quality of hardware RF electronic circuitries; iv) cross polarization in solids; v) a new optimized NMR pulse sequence (CPMG); vi) new techniques in data acquisition as the NUS protocol combined with Cov or CST processing, for instance. Other methodological approaches such as Para-hydrogen Induced Polarization (PHIP) or Dynamic Nuclear Polarization (DNP) have been proposed and are currently in progress to boost the NMR sensitivity. However, the combination of high magnetic fields and selective  $^2\text{H}$  cryogenic probes remains currently the most competitive solution for liquids and partially oriented systems (in 2017), considering the specific prerequisites for the PHIP and DNP tools are not always compatible when applied to  $^2\text{H}$  nuclei. Note however the combination of  $^2\text{H}$  SS-NMR and DNP was explored successfully in 2016 [Rossini *et al.*, 2015].

### Isotropic $^2\text{H}$ NMR and applications

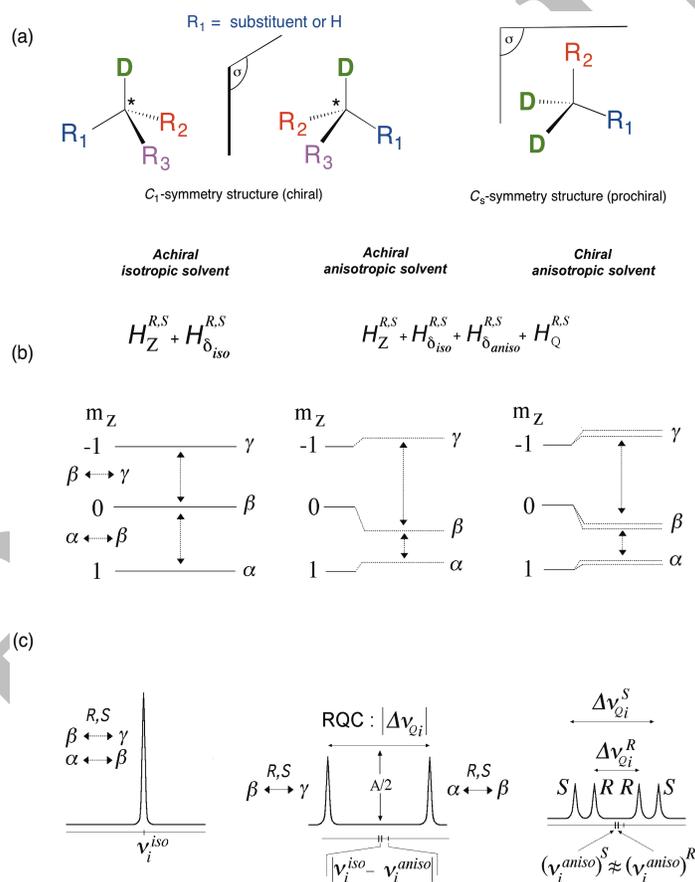
For a simple description of the total spin Hamiltonian,  $H_{\text{tot}}$ , between spin  $I = 1$  nuclei and  $B_0$ , only the case of  $^2\text{H}$  NMR applied to a monodeuterated molecule (or isotopomer for NAD NMR) recorded with  $^1\text{H}$  proton decoupling ( $^2\text{H}$ - $\{^1\text{H}\}$  NMR) will be considered, thus excluding the presence of  $^2\text{H}$ - $^2\text{H}$  and  $^2\text{H}$ - $^1\text{H}$  couplings. In isotropic solutions, an isolated  $^2\text{H}$  nucleus is only subject to  $H_{\text{Zeeman}}$ ,  $H_Z$ , (leading to three energy levels,  $a$ ,  $b$ ,  $g$ ) and the Hamiltonian corresponding to the isotropic chemical shift,  $H_\delta^{\text{iso}}$ , namely:

$$H_Z + H_\delta^{\text{iso}} = -\frac{\gamma_{^2\text{H}} B_0}{2\pi} \sum_i [1 - \sigma_i^{\text{iso}}] \mathbf{I}_{zi} \quad \text{with} \quad \sigma_i^{\text{iso}} = \frac{1}{3}(\sigma_{\text{iaa}} + \sigma_{\text{ibb}} + \sigma_{\text{icc}}) \quad [1]$$

where  $\sigma_i^{\text{iso}}$  is the isotropic part of the electronic shielding second-rank (3 X 3) tensor. These Hamiltonians result in two non-degenerated transitions ( $\alpha \leftrightarrow \beta$  and  $\beta \leftrightarrow \gamma$ ) between the three energy levels (see **Figure 1b**) and a  $^2\text{H}$  singlet (one resonance) at  $d^{\text{iso}}(^2\text{H})$  is expected. By extension, NAD- $\{^1\text{H}\}$  1D-NMR spectra consist of a sum of independent singlets associated with all chemically non-equivalent  $^2\text{H}$  sites (or monodeuterated isotopomers) of the analyte. Isotropic NAD- $\{^1\text{H}\}$  1D-NMR spectra show formally a resemblance to  $^{13}\text{C}$ - $\{^1\text{H}\}$  1D-NMR spectra where only the chemical shift data

of nuclei can be extracted. Compared to  $^1\text{H}$  NMR spectra, the reduction of frequency dispersion of  $^2\text{H}$  peaks (see above), combined with the stochastic fluctuations of the  $^2\text{H}$  quadrupolar interaction that accelerates the nucleus relaxation and increases the FWHM of peaks, tends to decrease the resolution of  $^2\text{H}$  1D spectra compared to that of  $^1\text{H}$  spectra. However the absence of fine structures due to  $^2\text{H}$ - $^2\text{H}$  couplings in NAD spectra limits this effect while facilitating the determination of  $\delta^{\text{iso}}(^2\text{H})$ 's.

From an application point of view,  $^2\text{H}$ - $\{^1\text{H}\}$  1D NMR spectroscopy of deuterium labeled molecules is revealed to be a helpful tool to mainly establish the presence/absence of expected product(s) as well as secondary or side-product(s). It can be also useful for following the increase/decrease in the concentration of any deuterated species (reactant/product) as a function of time (by integration of  $^2\text{H}$  resonances) as well as the detection of final secondary or side-products or also reaction intermediates that could be undetectable using other spectroscopic techniques (see below).



**Figure 1.** (a) Examples of generic deuterated chiral and prochiral structures. (b) Representation of energy level diagrams of a single deuteron under the effect of  $H_{\text{tot}}^{\text{iso}/\text{aniso}}(^2\text{H})$ . (c) Schematic  $^2\text{H}$ - $\{^1\text{H}\}$  spectral patterns expected for a pair of monodeuterated enantiomers in achiral isotropic solutions, in achiral LCs and in CLCs. The same patterns are obtained for a prochiral molecule dideuterated on the prostereogenic center and dissolved in a (C)LC, assuming that the ( $^2\text{H}$ - $^2\text{H}$ )-RDC between geminal positions is null or smaller than the linewidths in LCs and CLCs (Figure adapted from *Prog. NMR Spectrosc. J.*, 2009, 55, 128).

From these data, it becomes possible to confirm or understand mechanisms, evaluate regio- or enantiospecificity, and finally determine the kind of chemical reactions involved.

Being able to measure  $^2\text{H}\text{-}\{^1\text{H}\}$  NMR spectra of compounds at natural abundance is the basis of important various analytical applications that have strengthened the interest of  $^2\text{H}$  as a relevant spy nucleus without the drawback of isotopic enrichment. In the frame of structural analysis, the first modern milestone was reported in 1973 where it was demonstrated how the  $^1\text{H}$  isotropic chemical shifts,  $\delta^{\text{iso}}(^1\text{H})$ , of small organic molecules can be easily determined from the corresponding  $^2\text{H}$  ones,  $\delta^{\text{iso}}(^2\text{H})$ , measured in NAD- $\{^1\text{H}\}$  1D NMR spectra by Fourier transform (FT) and operating at 13.8 MHz (2.2 T) ([*Briggs et al, 1973*]. Around this idea, the same strategy was employed for a reliable assignment of  $\delta^{\text{iso}}(^1\text{H})$  of a series of representative polysubstituted distorted six-membered saturated rings (cyclohexanes) showing complex  $^1\text{H}$  spectral structures and very weak chemical shift dispersion; the analysis of results evidencing the influence of the steric distortion effects induced by large substituents or ring junctions on the  $^2\text{H}$  and  $^1\text{H}$  chemical shifts [*Curtis et al, 1989*]. In each example, the absence of  $^2\text{H}\text{-}^2\text{H}$   $^nJ$ -couplings was the key for facilitating the exact determination of  $\delta^{\text{iso}}(^2\text{H})$  values. Nowadays, the advent of complex homo- and heteronuclear 2D NMR experiments or the recent “pure shift”  $^1\text{H}$  NMR experiments eliminating  $^1\text{H}$  fine structures can provide efficient alternative for analyzing complex  $^1\text{H}$  spectra, but the  $^2\text{H}$  approach remains conceptually very elegant [*Zangger et al, 2015*].

In another analytical context, it was shown from the 1970's that many (bio)organic molecules contain an overall  $^2\text{H}$  percentage that can be different (variation up to 60 %) from the V-SMOW standard value (155 ppm). There may also be variations in the internal distribution of deuterons (site-specific) within given compound, which result from their different and complex chemical "histories". Hence, the determination of this isotopic profile is therefore an unrivalled method to determine and understand the (bio)chemical origin of molecules, in particular through the determination kinetic isotopic effects (KIE) [*Leopold et al, 1998*]. In the 1980's, the Site-specific Natural  $^2\text{H}$  Isotope Fractionation NMR (SNIF-NMR<sup>®</sup>) protocol was introduced to measure the ( $^2\text{H}/^1\text{H}$ ) isotope ratio for each spectrally resolved  $^2\text{H}$  signal of a molecule [*Martin et al, 1981*]. This method consists in the acquisition of NAD 1D-NMR spectra of isotropic solutions under quantitative conditions ( $T_{\text{RD}} > 5 \times T_1$ ). For the quantification purpose, the NAD spectrum must contain the signal of an internal chemical reference, such as TMU for which the ( $^2\text{H}/^1\text{H}$ )<sub>i</sub> ratio is known or also a calibrated electronical signal (ERETIC method) [*Billault et al, 2002*]. For a given molecular hydrogen site (i), the site-specific ratio, ( $^2\text{H}/^1\text{H}$ )<sub>i</sub>, expressed in parts *per* million (ppm) is defined as:

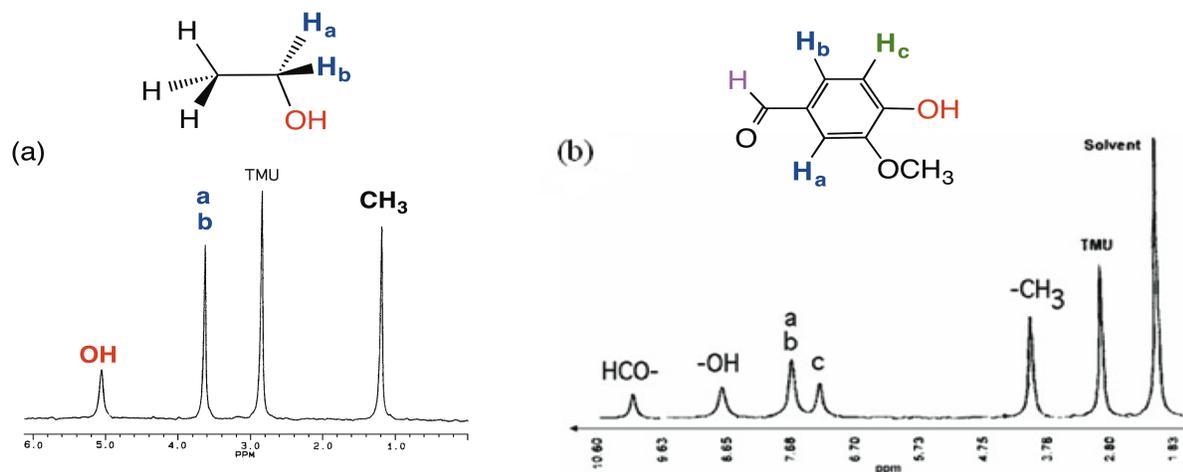
$$\left(\frac{{}^2\text{H}}{{}^1\text{H}}\right)_i = \frac{N_{2\text{H}_i}}{P_i \cdot N_{1\text{H}_i}} \times 10^6 \quad [2]$$

where  $N_{2\text{H}_i}$  is the number of mono-deuterated isotopomers with a deuterium nucleus at the site  $i$ ,  $N_{1\text{H}_i}$  is the number of fully protonated isotopomers, and  $P_i$  is the stoichiometric number of equivalent hydrogens at position  $i$ . Using the standard SNIF-NMR<sup>®</sup> protocol and TMU as calibrated isotopic reference, **Eq. 2** can be rewritten as:

$$\left(\frac{{}^2\text{H}}{{}^1\text{H}}\right)_i = \left(\frac{{}^2\text{H}}{{}^1\text{H}}\right)_{\text{TMU}} \times \left(\frac{S_i^{\text{anal}}}{S_{\text{TMU}}}\right) \times \left(\frac{P_{\text{TMU}}}{P_i^{\text{anal}}}\right) \times \left(\frac{m_{\text{TMU}}}{m_{\text{anal}}} \times \frac{M_{\text{anal}}}{M_{\text{TMU}}}\right). \quad [3]$$

In this equation,  $P_i^{\text{anal}}$  and  $P_{\text{TMU}}$  are the stoichiometric numbers of equivalent hydrogens in the site  $i$  of the analyte and in TMU,  $M_{\text{anal}}$ ,  $m_{\text{anal}}$  and  $M_{\text{TMU}}$ ,  $m_{\text{TMU}}$  are the molecular weights and the masses used, respectively, and  $S_i^{\text{anal}}$  and  $S_{\text{TMU}}$  are the areas of the NAD signals of analyte at site  $i$  and of TMU.

The analysis of intramolecular  ${}^2\text{H}$  isotope profile of natural compounds was intensively used to investigate primary and secondary  ${}^2\text{H}$  KIEs. These effects provide key data on the  ${}^2\text{H}$  enrichment or depletion effects by sites, and thereby on the understanding of enzymatic mechanisms producing them [Baillif *et al*, 2009] and/or the determination their geographical and/or botanical origin [Fronza *et al* 1998; Baillif *et al*, 2009]. Numerous derived applications of this method have been described for food control or identification of counterfeit products (adulteration). Among two illustrative and economically important examples using the SNIF-NMR<sup>®</sup> protocol are: i) the analysis of the NAD NMR spectrum of distilled ethanol (from wine) in order to determine the origin of sugar (natural or added beet sugar) and to control the chaptalisation process (amount of added of exogen sugar) by winegrowers (see **Figure 2a**) [Guillou *et al*, 1991]; (ii) and the analysis of the NAD spectrum of vanillin (one of the most used aroma in foods) to determine its origin, either natural or synthesized (see **Figure 2b**) [Remaud *et al*, 1997]. Although the method has been successfully applied against counterfeiting in both cases, the method does not permit the determination of the ( ${}^2\text{H}/{}^1\text{H}$ ) isotopic ratios associated with the enantiotopic hydrogen position ( $\text{CH}_2$  group) of ethanol (prochiral molecule) or three diastereotopic hydrogen positions of the aromatic group (achiral molecule) in vanillin. To circumvent this inherent drawback of NAD NMR in liquids, the SNIF-NMR<sup>®</sup> protocol has been extended by replacing the achiral isotropic solvent by anisotropic media made of polypeptide CLCs as enantiodiscriminating solvents (see below).



**Figure 2.** Two examples of NAD SNIF-NMR® 1D spectra obtained for (a) ethanol (61.4 MHz) and of (b) vanillin (77.8 MHz), both recorded using TMU as an isotopically calibrated internal reference. Note that the presence of a single resonance for the two enantio-isotopomers associated with the prostereogenic methylene group ( $H_a/H_b$ ) of ethanol, and the peak overlap for the two diastereo-isotopomers associated with aromatic sites ( $H_a/H_b$ ) of vanillin (Figure a: Courtesy of Guillou *et al*, *Analisis*, 1991, 19, 29, reproduced with permission from EDP Sciences; Figure b: Courtesy of Remaud *et al*, *J. Agric. Food Chem.*, 1997, 45, 859, reproduced with permission from ACS).

### Analytical wealth of anisotropic $^2\text{H}$ NMR

Liquid crystals (LCs) are typical anisotropic systems where the molecular organization is between crystalline solids and isotropic liquids. In this intermediate state of the matter, all molecules (LCs and solutes) are partially orientationally ordered on average and their orientational behavior can be defined by the “Saupe” matrix,  $\{S\}$ , a traceless and symmetric, second-rank ( $3 \times 3$ ) tensor. In this case, the Hamiltonian  $H_{\text{tot}}^{\text{aniso}}(^2\text{H})$ , describing their anisotropic  $^2\text{H}$  spectra is the sum of four terms:  $H_Z$ ,  $H_\delta^{\text{iso}}$  but also the Hamiltonian associated with the anisotropic chemical shift,  $H_\delta^{\text{aniso}}$ , and the quadrupolar Hamiltonian,  $H_Q$ . The sum of three terms (in Hz) is [Emsley and Lindon, 1975]:

$$H_Z + H_\delta^{\text{iso}} + H_\delta^{\text{aniso}} = -\frac{\gamma^2 B_0}{2\pi} \sum_i [1 - \sigma_i^{\text{iso}} - \Delta\sigma_i^{\text{aniso}}] \mathbf{I}_{zi} \quad \text{with} \quad \Delta\sigma_i^{\text{aniso}} = \frac{2}{3} \sum_{\alpha,\beta=a,b,c} \sigma_{i\alpha\beta} S_{\alpha\beta} \quad [4]$$

In Eq. 4,  $\Delta\sigma_i^{\text{aniso}}$  (denoted also RCSA) is the anisotropic part of the electronic shielding tensor, whose various elements are denoted  $S_{i\alpha\beta}$  and  $S_{\alpha\beta}$ , are elements of  $\{S\}$ . Note that the  $^2\text{H}$ -RCSA modifies the values of  $\delta_i^{\text{iso}}$ , however this contribution remains small for  $^2\text{H}$  nuclei, and hence  $\delta^{\text{iso}}(^2\text{H}) \approx \delta^{\text{aniso}}(^2\text{H})$ .

As  $H_Q$  is much smaller than  $H_Z$ , the Hamiltonian is restricted to its first-order term and can be then written (in Hz) as:

$$H_Q = \frac{1}{h} \sum_i \frac{eQ_D V_{izz}}{4} [3I_{zi}^2 - I_i^2] \quad [5]$$

where  $e$  is the electron charge and  $V_{izz}$  is the component of the EFG tensor along the  $B_0$  axis (z-axis) measured at the deuteron  $i$ . In an uniaxial LC,  $V_{izz}$  is related to the elements of the EFG tensor in the molecular frame ( $eq_{i\alpha\beta}$ ) and  $S_{\alpha\beta}$ 's.

The presence of the quadrupolar interaction modifies the Zeeman energy levels, thus leading to two possible transitions with distinct frequencies (see **Figure 1b**). Thus in LCs, the signal of a single  $^2\text{H}$  nucleus becomes a symmetric doublet centered on  $\delta^{\text{aniso}}(^2\text{H})$ , while  $\Delta\nu_{Q_i}$  denoted also as the residual quadrupolar coupling (RQC) is the separation (in Hz) between its two components (see **Eq. 6**). In the case of  $\text{C-}^2\text{H}$  directions aligned in a homogenous uniaxial LC, the EFG is usually assumed to be axially symmetric along the  $\text{C-}^2\text{H}$  vector (asymmetry parameter  $\eta = 0$ ), and hence  $\Delta\nu_{Q_i}$  can be simply expressed (in Hz) as a product of two terms: [*Emsley and Lindon, 1975; Sarfati et al., 2000*]

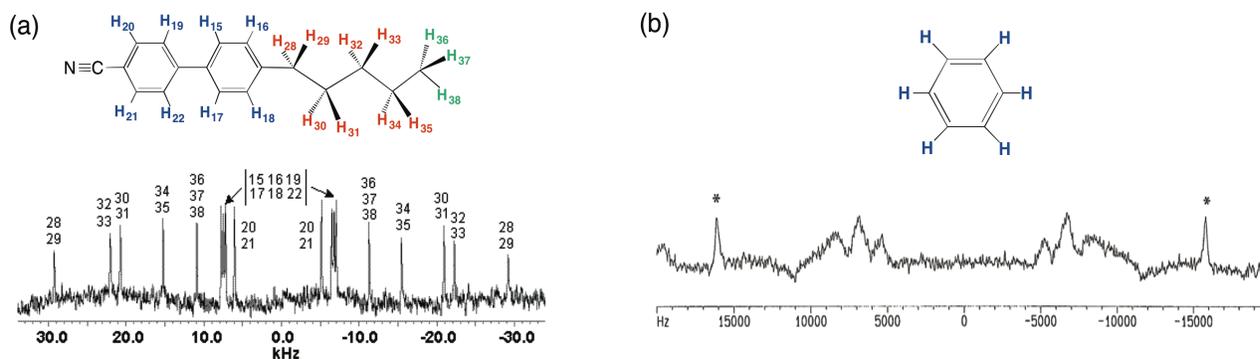
$$\Delta\nu_{Q_i} = \frac{3}{2} C_{Q_i} \times S_{\text{C-}^2\text{H}_i} = \frac{3}{2} \left( \frac{eQ_D V_{\text{C-}^2\text{H}_i}}{h} \right) \times \left( \frac{\langle 3\cos^2(\theta_{\text{C-}^2\text{H}_i}) - 1 \rangle}{2} \right). \quad [6]$$

In **Eq. 6**, brackets  $\langle \dots \rangle$  denote an ensemble average,  $S_{\text{C-}^2\text{H}_i}$  is the order parameter of the  $\text{C-}^2\text{H}_i$  bond and  $\theta_{\text{C-}^2\text{H}_i}$  is the angle between the  $\text{C-}^2\text{H}_i$  and  $B_0$  axis. Note that the value of  $C_Q$  varies approximately from 170 to 310 kHz, depending on the hybridization state of the bonded carbon atom as well as the electronic properties of the closest substituents. Note that the  $^2\text{H}_i$ -RQC value or splitting between components ( $\Delta\nu_Q$ ) of quadrupolar doublets, QDs, can be positive, negative or even null when  $\theta_{\text{C-}^2\text{H}_i}$  is equal to the magic angle ( $54.7^\circ$ ).

Due to the absence of  $^2\text{H-}^2\text{H}$  scalar and residual dipolar couplings (see above), NAD NMR spectra correspond to a sum of independent QDs, (instead of single lines as in liquids), each of them corresponding to a given inequivalent  $^2\text{H}$  position in the molecule. Compared to isotropic NMR, the presence of three new NMR interactions,  $^2\text{H}$ -RCSA,  $(^2\text{H-X})$ -RDC and  $^2\text{H}$ -RQC, extracted from oriented spectra is an important source of data for molecular analysis, thus illustrating the wealth of this anisotropic NMR.

The synthesis of new liquid crystals is generally followed by the investigation of microscopic properties of those mesogenic molecules, in particular by NMR spectroscopy. However in case of strongly orientationally ordered systems, the extraction of useful anisotropic data is often tricky for middle-size molecules owing to the large number and the magnitude of ( $^1\text{H}$ - $^1\text{H}$ )-RDCs, which result in a forest/or and the presence of unresolved broad signals. This issue can be circumvented by the selective or global deuteration of compounds and analysis by  $^2\text{H}$  NMR. Such an approach was extensively used for analyzing perdeuterated uniaxial liquid crystals such as 4-pentyl-4'-cyanobiphenyl (**5CB**) or 4-hexyloxy-4'-cyanobiphenyl (**6-OCB**) in their nematic phase [Dong *et al*, 2004]. However the isotopic labelling of large molecules is often arduous (multistep reaction pathway), time-consuming and hence expensive. When possible, the acquisition of NAD 1D-spectra in their nematic phase can be proposed as in the case of **5CB** reported in 1996 using a 7.1 T solid-state NMR spectrometer equipped with a 10-mm solenoid probe in order to increase the size of the sample and so the spin density [Prosser *et al*, 1996]. Typically the magnitude of  $^2\text{H}$ -RQCs (up to tens of kHz) is much larger than that of the classical range of  $^2\text{H}$  chemical shifts (1-2 kHz at most, depending on  $B_0$  strength) and the NAD 1D spectra of mesogens appear to be highly symmetrical, with linewidths for larger splittings reaching several hundred Hz. To limit decreasing of the SNR by excessive line broadening, the sensitivity of such spectra can be enhanced by the application of Carr-Purcell-Meiboom-Gill (CPMG) pulse trains inserted in  $^2\text{H}$  experiments such as quadrupolar echo or inversion-recovery [Prosser *et al*, 1996] or even by  $^1\text{H} \rightarrow ^2\text{H}$  CP transfer, but for this latter approach, the  $T_1(^1\text{H})$  relaxation times of mesogens are longer than those of  $^2\text{H}$ , and this limits the gain.

For thermotropics, the use of  $^2\text{H}$  cryogenic probes has been explored but this approach remains technically limited by the need of high-power  $^1\text{H}$  decoupling to suppress the strongest ( $^2\text{H}$ - $^1\text{H}$ )-RDCs, not compatible with the necessity to protect the cooled probehead with RF-field strengths smaller than 10-15 kHz [Emsley *et al*, 2008]. Another option consists of the use of MAS probes that easily support application of very high-power  $^1\text{H}$  decoupling during the  $^2\text{H}$  NMR experiments but without sample spinning. Applied to **5CB** (see **Figure 3a**) this approach was able to decrease the  $^2\text{H}$  linewidths below 100 Hz, thus significantly improving the SNR of the spectrum and the accuracy for the measurement of RQCs [Emsley *et al*, 2008]. By recording good quality NAD NMR spectra of mesogenic molecules in their nematic phase, it becomes possible to investigate the effect of selective deuteration of such systems on the molecular ordering by comparison between specifically the deuterated and undeuterated materials [Tabayashi *et al*, 1999].



**Figure 3.** (a) NAD- $\{^1\text{H}\}$  1D-NMR spectra of **5CB** in its nematic phase recorded at 107.5 MHz and 293 K with a 4-mm double resonance MAS probe under static conditions (Courtesy of Emsley *et al*, Figure from *Liq. Cryst.* **2008**, **35**, **443**, reproduced with permission from Taylor and Francis. (b) NAD- $\{^1\text{H}\}$  1D-NMR spectra of benzene recorded in the nematic thermotropic ZLI 1114 at 303K. using a 10-mm diameter sample tube and single RF-pulse sequence (64,000 scans). The  $^2\text{H}$ -QD marked by an asterisk is the NAD signal of benzene ( $C_6$  symmetry molecule with six equivalent hydrogen positions). The QDs with broad components originate from the mesogenic molecule (ZLI) (Courtesy of Khetrpal *et al*, Figure from *J. Magn. Reson.* **135** (1998) **265**, reproduced with permission from Elsevier).

The complexity of the  $^1\text{H}$  guest molecules in thermotropics has seriously discouraged chemists from using this approach for extracting molecular data of their compounds. In 1998 an elegant solution for simplifying the analysis of strongly oriented molecules by combining spectral data extracted from both the  $^1\text{H}$  and NAD spectra recorded with the same degree of alignment, namely at the same temperature, was described. Under this condition and knowing the geometry of compounds, the magnitude of  $^1\text{H}$ - $^1\text{H}$  residual dipolar couplings can be recalculated approximately from the  $^2\text{H}$ -RQCs values, assuming the  $C_Q$  values. This approach was first successfully tested in case of benzene, an highly symmetrical molecule ( $C_6$  symmetry), dissolved in the uniaxial thermotropic nematic ZLI-1114 (see Figure 3b) [Khetrpal *et al*, 1998]. Actually, this strategy is formally identical to that using isotropic NAD-NMR data as a starting point for analyzing the  $\delta(^1\text{H})$  in overcrowded  $^1\text{H}$  spectra recorded in isotropic solvents (see above).

## $^2\text{H}$ NMR and chiral anisotropic matrices

The spectral discrimination of enantiomers of chiral molecules by effective and robust NMR methods is an important challenge for asymmetric synthesis, and hence various analytical approaches have been proposed during the three last decades [Lesot *et al* 2003; Wenzel *et al*, 2007]. From the 1990's, new kinds of organosoluble or water-compatible lyotropic liquid crystals (LLCs) were reported and intensively exploited in various domains, with three advantages: i) providing weak aligning properties for solutes leading to simpler spectra compared to spectra recorded in thermotropics; ii) the propensity

to dissolve rather large amounts of material for some of them; iii) the possibility to discriminate the NMR signals of enantiomers or enantiotopic elements in prochiral analytes when the anisotropic environment is chiral. These three specificities afforded vigorous stimuli to the development and application of anisotropic  $^2\text{H}$  and NAD 1D/2D NMR in the field of stereochemical analysis, (D/H) isotopic measurement and structural analysis.

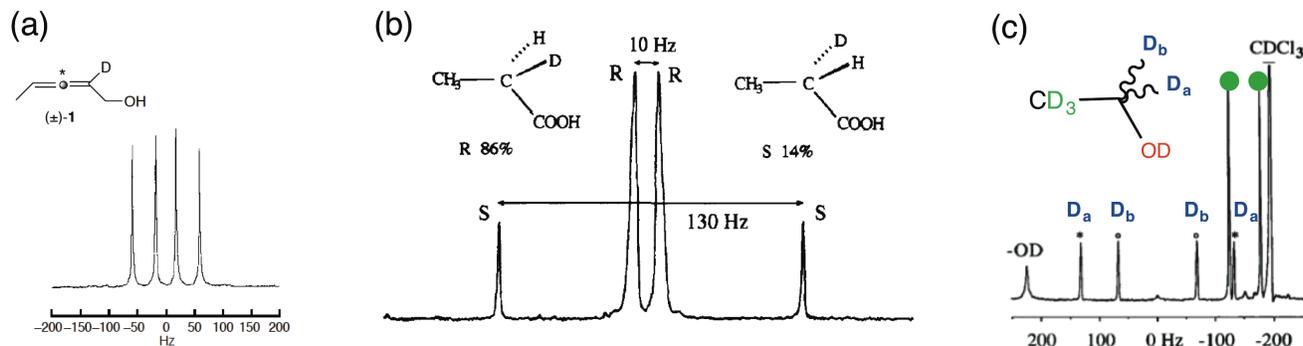
Exploiting the properties of the quadrupolar interaction,  $^2\text{H}$  NMR spectroscopy in helical weakening chiral LCs was revealed to be highly relevant to discriminate between deuterated enantiomers of chiral molecules (e.g.  $\text{R-C}^*\text{D-R}'$ ,  $-\text{R}''$ ), including the case of chiral molecules by virtue of the isotopic substitution (e.g.  $\text{R-C}^*\text{DH}$ ,  $-\text{R}''$ ) but also enantiotopic elements (nuclei, internuclear directions ...) in prochiral molecules (e.g.  $\text{R-CD}_2\text{-R}'$ ). The key idea is to orient two mirror-image C-D vectors between enantiomers ( $\mathbf{S}^S \neq \mathbf{S}^R$ ) or between enantiotopic C-D bonds ( $\mathbf{S}^{\text{pro-S}} \neq \mathbf{S}^{\text{pro-R}}$ ) differently on average relative to  $B_0$  by replacing (achiral) isotropic solvents with CLCs (see Figure 1). Thus  $^2\text{H}$  NMR spectra show two spectral signatures, one for each enantiomer, or one for each enantiotopic element. In both cases, a doubling of  $^2\text{H}$  signals compared to an achiral environment is generally observed, together with the presence of two QDs with different splittings for each deuterated site, namely [Meddour et al, 1994; Merlet et al, 1998; Sarfati et al, 2000; Lesot et al, 2015a]:

$$\Delta\nu_{\text{Qi}}^R \neq \Delta\nu_{\text{Qi}}^S \quad \text{or} \quad \Delta\nu_{\text{Qi}}^{\text{pro-R}} \neq \Delta\nu_{\text{Qi}}^{\text{pro-S}} \quad [7]$$

Three illustrative examples of  $^2\text{H}$ - $\{^1\text{H}\}$  1D-NMR spectra of mono- or perdeuterated analytes dissolved in organic solutions of polypeptide (PBLG) are reported in **Figure 4**: i) the case of a chiral solute with or without (H/D) isotopic substitution (where two QDs are observed, one for each enantiomer); ii) the case of a prochiral compound where the enantiotopic C-D directions of the  $\text{CD}_2$  group are spectrally discriminated yielding also two QDs [Meddour et al, 1994; Smadja et al, 1997]. In practice, the observation of enantiomers through  $^2\text{H}$  NMR spectroscopy in polypeptide CLCs can be obtained by incorporation of deuterium labels on the asymmetric carbon or more distant positions within the molecule, including the particular case of exchangeable sites such as hydroxyl group [Palomino et al, 2012]. The analysis of mixtures of deuterated *threo-erythro* isomers bearing remote chiral centers has been also elegantly solved by  $^2\text{H}$ - $\{^1\text{H}\}$  NMR in PBLG-based CLC [Canlet et al, 2000].

Recording the  $^2\text{H}$ - $\{^1\text{H}\}$  spectrum under quantitative conditions ( $T_R > 5T_1$ ) as in liquids is an interesting way to accurately determine the enantiomeric excess ( $ee = |A^R - A^S| / (A^R + A^S)$ ) of a

mixture (enantiopure, scalemic or racemic) by integrating the peak surfaces or deconvolving the peak shapes associated with pairs of enantiomers.



**Figure 4.** Examples of  $^2\text{H}\{-^1\text{H}\}$  1D spectra of mono- and perdeuterated solutes dissolved in organic solutions of PBLG and showing spectral enantiodiscriminations on the basis of  $^2\text{H}$ -RQC differences: (a) a chiral molecule of axial chirality, (b) a chiral molecule by virtue of the ( $^2\text{H}/^1\text{H}$ ) isotopic substitution with  $ee = 72\%$ ; (c) a prochiral molecule ( $C_s$  symmetry) with enantiotopic directions. Only one component of the QD of  $\text{CDCl}_3$  is shown here. (Figure (a): Courtesy of Smadja *et al*, *Chem. Commun.* 1997, 2031, reproduced with permission from RSC; Figures (b): Courtesy of Meddour *et al*, *J. Am. Chem. Soc.*, 1994, 116, 9652, reproduced with permission from ACS; Figures (c): Figure adapted from *J. Am. Chem. Soc.*, 1998, 120, 963).

Other orienting chiral systems possessing enantiodiscrimination properties exploitable by  $^2\text{H}$  NMR spectroscopy are possible, such as mixtures of polypeptides [Lesot *et al*, 2008], polyacetylene-based LLCs [Meyer *et al*, 2012], tunable alignment chiral systems (stretched or compressed gels), [Kobzar *et al*, 2005, Naumann *et al*, 2007; Naumann *et al*, 2009] or even the use of non-polymeric alignment media based on benzenetricarboxamides which self-assemble into rod-like supramolecules [Leyendecker *et al*, 2017]. For hydrosoluble analytes,  $^2\text{H}$  spectral enantiodiscriminations can be detected using water-compatible orienting chiral systems such as polysaccharide systems [Solgadié *et al*, 1994] or DNA-fragment media [Lesot *et al*, 2011b].

Interestingly, as the magnitude of a  $^2\text{H}$ -RQC is temperature dependent through molecular ordering, the  $^2\text{H}$  NMR spectra of deuterated analytes dissolved in a CLC can be used as a powerful tool for investigating the intramolecular conformational dynamic processes of small prochiral / chiral molecules. Thus, deuterated model compounds such as *cis*-decalin or derivatives of methyl-5-(1'-naphthyl)benzene (atropoisomers) were successfully investigated [Sarfati *et al*, 2000; Lesot *et al*, 2006; Lafon *et al*, 2007] but also complex macromolecules such as 18-membered hexathia-metacyclophanes [Lesot *et al*, 2011a]. From the analysis of anisotropic  $^2\text{H}$  spectra, it becomes possible to extract thermodynamic parameters (e.g. energy barriers). Exploiting the enantiodiscrimination properties of orienting chiral systems, it has been demonstrated that it is possible to follow *in situ* and in real-time

kinetics of reactions by  $^2\text{H}$  NMR in DNA-based CLCs (water compatible). The proof of concept has been demonstrated with Alanine racemase, an enzyme leading to the racemization of enantiopure alanine. Thus the analysis of variation of peak areas of (*L* and *D*)-alanine- $\text{d}_3$  on a series of  $^2\text{H}$ - $\{^1\text{H}\}$  1D-NMR spectra *versus* time allows the derivation of some kinetic parameters of the reaction as the turnover numbers  $k_{\text{catL}}$  and  $k_{\text{catD}}$  of the enzyme in a reversible Michaelis-Menten model [*Chan-Huot et al, 2013*]. The excellent agreement between data obtained by anisotropic  $^2\text{H}$  NMR spectroscopy and classical methods showed the robustness of this new approach, thus providing a change of paradigm in the analysis of enzymatic systems. A further, advanced methodological development of this strategy is the possible use of ultra-fast  $^2\text{H}$  2D experiments in anisotropic solvents (ADUF) for recording  $^2\text{H}$  2D QUOSY experiments (see below) within a sub-second duration, such a protocol being crucial for following very rapid kinetics with the additional advantage of obtaining 2D maps simplifying the analysis of complex  $^2\text{H}$  spectra [*Lesot et al, 2016*].

To overcome the isotopic enrichment step of analytes, incompatible for a routine analytical tool, the method was extended to  $^2\text{H}$  NMR spectroscopy at the natural abundance level. This tool has the advantage of detecting all monodeuterated diastereo- and/or enantio-isotopomers of mixture in a single NMR experiment, but with the drawback of making the spectral analysis for large molecules more difficult because of the complex distribution of components of  $^2\text{H}$  DQs that can generate peak overlap or ambiguity in peak order) [*Lesot et al, 1998*]. In this case, recording 2D/3D NMR experiments belonging to the class of QUOSY (QUadrupole Ordered Spectroscopy) in order to group the two components of each QD and their assignment on the basis of the  $\delta^{\text{aniso}}(^2\text{H})$  facilitates the analysis of complex NAD 1D spectra [*Merlet et al, 1999; Lesot et al, 2003; Lafon et al, 2007*]. Interestingly, this kind of experiment allows the decomposition of NAD 2D maps into a sum of NAD 1D sub-spectra associated to each pair of diastereo- or enantio-isotopomers (see **Figure 5c**). One recent methodological development of such 2D experiments for reducing experimental time of NAD 2D-NMR spectroscopy was the acquisition of  $^2\text{H}$  data using non-uniform sampling (NUS) combined with an adapted signal reconstruction algorithm (Cov or CST), instead of classical  $^2\text{H}$  data combined with FT [*Lesot et al., 2015b*].

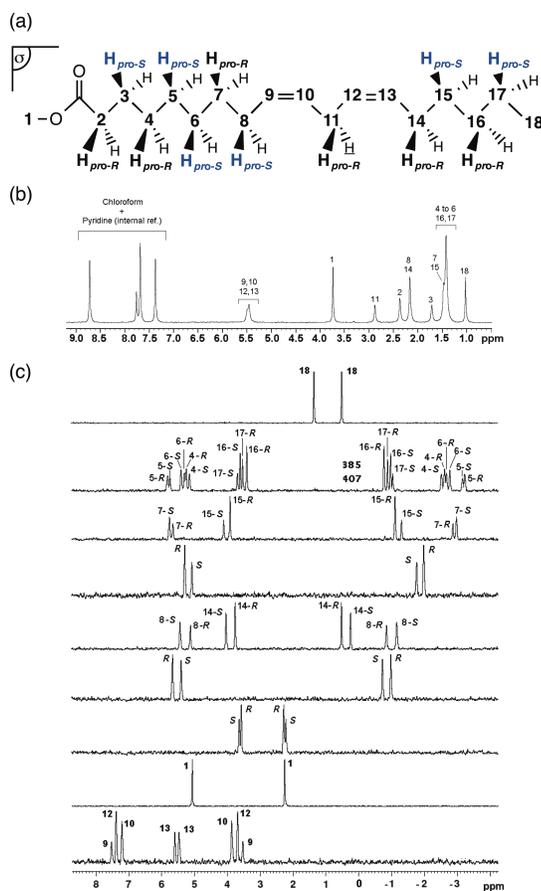
Among significant applications in chemistry and related area, the usefulness of NAD 2D-NMR spectroscopy in polypeptide CLC has been demonstrated for: i) the stereochemical control of chiral precursors in the synthesis of enantiopure natural products [*Parenty et al, 2002*]; ii) the analysis of rigid and flexible chiral hydrocarbons [*Lesot et al, 2003*]; iii) the study of enantiotopic discriminations in prochiral molecules [*Lesot et al, 2015a*]; iii) the determination of the natural deuterium distribution by

quantitative  $^2\text{H}$ - $\{^1\text{H}\}$  NMR spectroscopy for characterizing the biosynthesis pathways of natural products such as fatty acid methyl esters [Baillif *et al*, 2009] or terpenic molecules as miliacin used as a geo-biomarker (see below) [Berdagué *et al*, 2015].

Although the SNIF-NMR® approach is a powerful analytical tool, this approach is limited by the small  $^2\text{H}$  chemical shift dispersion that can lead to fortuitous but cumbersome peak overlaps (e.g the case of vanillin) and, more importantly, by the impossibility of measuring the natural ( $^2\text{H}/^1\text{H}$ ) ratios for enantiotopic hydrogen sites (in methylene groups) of prochiral molecules as in the case of ethanol, a very simple  $C_s$  symmetry structure, as the solvent is achiral (see **Figure 2**). In both cases, a single resonance is observed for two hydrogen or more sites, thus reducing the isotopic information accessible. Applied to more flexible complex prochiral compounds, NAD 2D-NMR spectroscopy in CLC was successfully extended to analyze unsaturated FAMES, such as methyl linoleate (ML) [Serhan *et al*, 2010], conjugated FAMES [Lesot *et al*, 2011a], and saturated FAMES [Serhan *et al*, 2012], such as methyl stearate, prior finally to be applied to homogeneous triglycerides (glyceryl tributyrates) [Lesot *et al*, 2012b]. **Figure 5c** displays the series of NAD 1D sub-spectra (extracted from a  $Q$ -COSY Fz map) of **ML** dissolved in the PBLG/pyridine mesophase [Serhan *et al*, 2010] and recorded on a 14.1 T spectrometer equipped with a selective  $^2\text{H}$  cryogenic probe [Kovacs *et al*, 2005]. In this example, only 30% of the deuterium signals (8/26 of inequivalent  $^2\text{H}$  sites) are observable by isotropic NAD NMR (**Figure 5b**), whereas in the anisotropic NAD 2D spectrum recorded in the PBLG/pyridine CLC, all monodeuterated isotopomers and enantio-isotopomers of the mixture (corresponding to all inequivalent hydrogen sites) are now spectrally discriminated (**Figure 5c**) and in turn, their isotopic ratios ( $^2\text{H}/^1\text{H}$ ) can be evaluated by peak deconvolutions. Applied to prochiral compounds such as **ML** or ethanol, it becomes possible to determine the site-specific enantio-isotopomeric excesses, *eie* (%), for each  $\text{CH}_2$  group using Eq. 8 [Serhan *et al*, 2012]:

$$eie(\%) = \frac{\left| \left( \left( \frac{^2\text{H}}{^1\text{H}} \right)_i^R - \left( \frac{^2\text{H}}{^1\text{H}} \right)_i^S \right) \right|}{\left( \frac{^2\text{H}}{^1\text{H}} \right)_i^R + \left( \frac{^2\text{H}}{^1\text{H}} \right)_i^S} \times 100. \quad [8]$$

The determination of all isotopic information in achiral, prochiral and chiral natural molecules as well as their *eie* (or their diastereo-isotopomeric excesses (*die*) for diastereotopic sites of chiral molecules) is essential for the understanding of the different incorporation mechanisms of hydrogens and their origins (water, glucose, metabolite, ...) during the complex enzymatic processes leading to their synthesis.



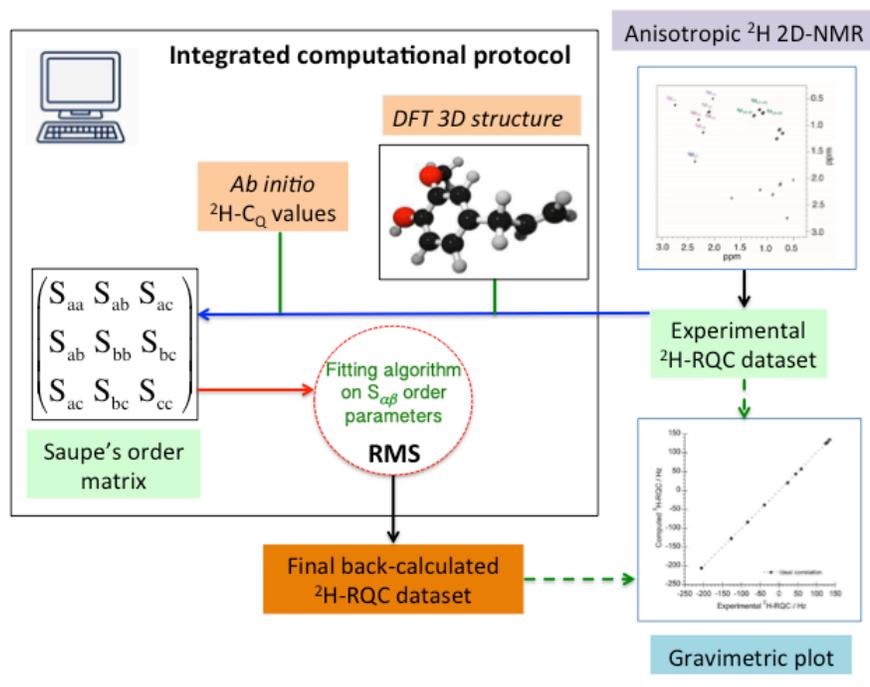
**Figure 5.** (a) Structure of methyl linoleate (ML). (b) Isotropic SNIF-NMR<sup>®</sup> spectrum (92.1 MHz) of **ML** (pyridine used as internal reference for quantification). (c) Series of NAD 1D-NMR sub-spectra extracted from the tilted *Q*-COSY Fz map recorded in PBLG/Py. For each CH<sub>2</sub> site, the absolute configuration of the natural, major enantio-isotopomer (CIP rules applied to **ML**) is given (**Figure adapted from Chem. Commun. 2010, 46, 6599**).

From a structural viewpoint, NAD NMR spectra in oriented phases can also provide key information about the molecular geometry since the relevant <sup>2</sup>H-RQC<sub>i</sub> values are mathematically related to the Saupe molecular order matrix of a solute, *S*<sub>ab</sub>, but also on the orientation of the C-D bonds within an axis system fixed to the molecule. The structure determination from NAD NMR spectra is based on Eq. 9 [Sarfati et al, 2000; Aroulanda et al, 2001]:

$$\frac{2}{3} \frac{\Delta v_{Qi}}{C_Q} = \sum_{\alpha, \beta = a, b, c} \cos \theta_{C-D, i}^{\alpha} \cos \theta_{C-D, i}^{\beta} S_{\alpha \beta} \quad [9]$$

Thus by comparing the experimental <sup>2</sup>H-RQC data,  $\Delta v_{Qi}^{Exp}$ , to theoretical data (magnitude and sign),  $\Delta v_{Qi}^{Calc}$ , back-calculated from the Saupe matrix (applying iterative computational calculation), for a given (minimized energy geometrical structure obtained from molecular modeling), it is possible to

assess the degree of compatibility between the anisotropic data and the molecular structure. In practice, a very good agreement (evaluated by gravimetric plots), between  $\Delta\nu_Q^{exp}$ , and,  $\Delta\nu_Q^{calc}$ , derived from  $S_{\alpha\beta}$  parameter calculations means that the anisotropic data and the molecular structure are fully compatible (see **Figure 6**). Otherwise the proposed dataset of  $^2\text{H}$ -RQCs is inconsistent with the structure, and hence a new assignment or a new geometry must be tested. This strategy was successfully applied first to revisit the  $^1\text{H}$  assignment of apolar bridged ring systems, as quadricyclane, a  $C_{2v}$  symmetry structure, exhibiting enantiotopic elements [Aroulanda *et al*, 2009]. In 2017, an hyphenated computational protocol (Programme MSpin-RQC) was proposed for analysing natural abundance  $^2\text{H}$ -RQC data of norbornen (a  $C_s$  symmetry prochiral bridged molecule) and a chiral molecule (fenchone) dissolved in polypeptide CLCs. The interest of this computational tools is the integration of all steps of the analysis process (from the *ab-initio* determination (DFT calculations) of the quadrupolar coupling,  $C_Q$ , (see **Eq. 6**), the miminized energy structure, and iterative calculation to match calculated and experimental  $^2\text{H}$ -RQC data [Navarro-Vazquez *et al.*, 2017]. The general principle of this integrated computational program is schematically depicted in **Figure 6**.

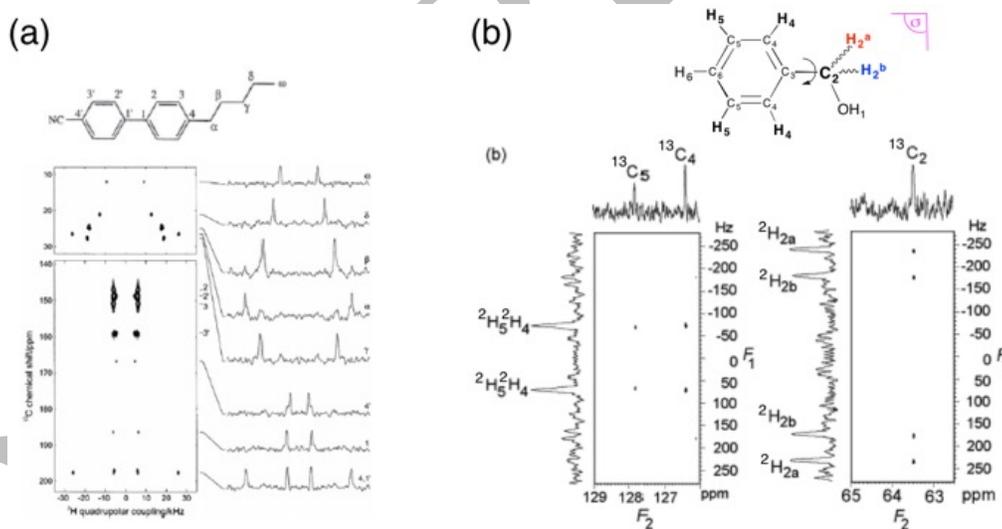


**Figure 6.** Simplified flow chart showing the principle of molecular structural analysis through  $^2\text{H}$ -RQCs extracted from anisotropic  $^2\text{H}$  (or NAD) 2D NMR experiments, and using an integrated computational program (MSpin-RQC program). The gravimetric plot ( $^2\text{H}\text{-RQC}^{calc}$  versus  $^2\text{H}\text{-RQC}^{exp}$ ) simply visualizes the quality of fit of experimental data.

## Detection of $^2\text{H}$ - $^{13}\text{C}$ isotopomers: the ultimate frontier?

Recording  $^2\text{H}$  1D/2D NMR spectra with spectrometers operating at higher fields increases the  $^2\text{H}$  spectral width (in Hz) and can facilitate the assignment of  $^2\text{H}$  signals of deuterated analytes. Another more challenging alternative consists in the correlation of the (isotropic or anisotropic) spectral information between  $^2\text{H}$  and  $^{13}\text{C}$  nuclei by heteronuclear 2D experiments. Various approaches have been explored such as a modified/adapted HETCOR scheme [Auger *et al*, 1998] or HMQC scheme [Sandström *et al*, 2000] or more recently the DAPT approach for which it is not need to satisfy matching conditions unlike the Hartmann–Hahn cross-polarization [Jayanthi *et al*, 2010]. In this case, the extremely large range of  $^{13}\text{C}$  chemical shifts (up to 250 ppm, instead of 15 ppm for  $^2\text{H}$ ) is a benefit that can facilitate the assignment of  $^2\text{H}$  signals from that of  $^{13}\text{C}$  signals, while no  $^{13}\text{C}$  isotopic enrichment is required. These approaches were experimentally demonstrated in perdeuterated mesogenic molecules (as 5CB) in their nematic phase using direct or inverse correlation schemes (see Figure 7a) prior to be extended to the case of isotopically enriched solutes dissolved in weakly orienting systems such as organic solutions of polypeptides [Lafon *et al*, 2004].

effect of  $^2\text{H}$  nuclei on  $d(^{13}\text{C})$ ,  $^1D(^{13}\text{C}) = d(^2\text{H}) - d(^1\text{H})$ , without any need for  $^2\text{H}$  isotopic enrichment.



**Figure 7.** (a) Example of a  $^2\text{H}$ - $^{13}\text{C}$  HMQC 2D spectrum of 5-CB- $d_{19}$  (neat) in its nematic phase and under static conditions. The total acquisition was 24 hours (Courtesy of Sandström *et al*, *J. Phys. Chem.*, 2003, 104, 1490, reproduced with permission from ACS). (b) Two expansions (aromatic and aliphatic region) of the Refocused-NASDAC 2D spectrum of benzyl alcohol recorded in PBLG polypeptide recorded at 21.1 T and 18 hours of experimental time (Figure adapted from *Anal. Chem.*, 2012, 84, 4569).

Various NMR strategies to correlate between distant deuterons through a  $^{13}\text{C}$  relay scheme have also been proposed when aligned solvents are considered [Lesot *et al*, 2014]. Obviously, the ideal approach consists in recording the two nuclides at their respective abundance level. Considering the

natural abundances of  $^2\text{H}$  and  $^{13}\text{C}$  nuclei ( $1.5 \times 10^{-2}$  and 1.1 % relative to  $^1\text{H}$ , respectively), the relative abundance of isotopomers simultaneously containing both atoms coupled together is reduced to  $1.7 \times 10^{-4}\%$ , namely one molecule in 580,000. Despite a very low isotopic abundance, it was demonstrated in 2012 that the detection of  $^2\text{H}$ - $^{13}\text{C}$  isotopomers in isotropic liquids and chiral oriented solvents is experimentally possible by taking advantage of a very high-field NMR spectrometer (21.1 T) with a 5-mm inverse (TCI) cryogenic probe and the 2D NASDAC or Refocused-NASDAC heteronuclear 2D sequence optimized to eliminate the signals of  $^2\text{H}$ - $^{12}\text{C}/^1\text{H}$ - $^{13}\text{C}$  isotopomers [Lesot *et al*, 2012] (see **Figure 7b**). Interestingly, in the chiral LCs, the sensitivity of the experiment was enough to detect for the first time the pair of  $^{13}\text{C}$ - $^2\text{H}$  enantio-isotopomers associated to enantiotopic directions of the  $\text{CH}_2$  group of benzylalcohol. From an isotopic effect viewpoint, the difference in  $\delta(^{13}\text{C})$  measured between the  $F_2$  projection of the NASDAC 2D map and the  $^{13}\text{C}$ - $\{^1\text{H}\}$  1D spectrum and in isotropic or anisotropic solvent allows a simple evaluation of the isotope effect of  $^2\text{H}$  nuclei on  $\delta(^{13}\text{C})$  without any need for  $^2\text{H}$  isotopic enrichment.

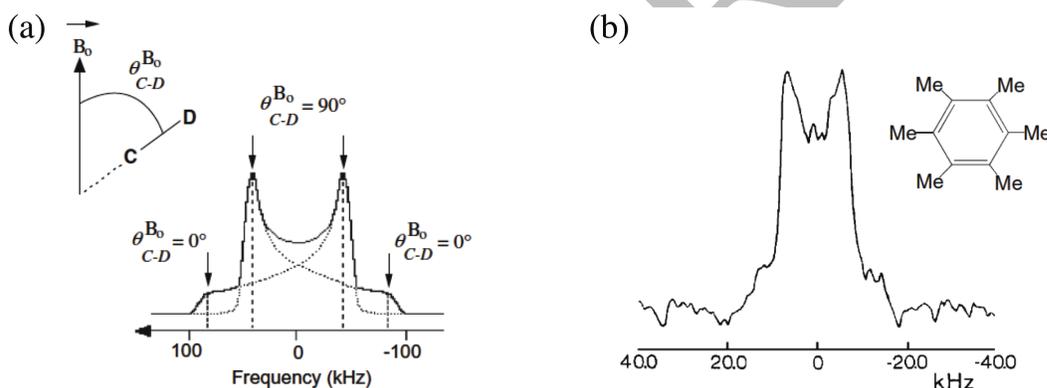
### Exploiting the potential of $^2\text{H}$ solid-state NMR spectra

As seen above, the  $^2\text{H}$ -RQC values depend on the orientation of the C-D bond with respect to the  $B_0$  static magnetic field. In a powder, the molecules take a variety of orientations and the  $^2\text{H}$  SS-NMR spectrum under static condition (no rotation of the sample) is a superposition of QDs with different  $^2\text{H}$ -RQC values. As the asymmetry parameter of the EFG tensor is usually zero, the  $^2\text{H}$  powder pattern is symmetrical where the full width of this pattern is equal to  $3C_Q/2$  as shown in **Figure 8a**. The "horns" and the "feet" of the spectral envelope (a Pake doublet) correspond to the situation when the principal component of the EFG tensor of deuterons is perpendicular to  $B_0$  ( $\theta_{\text{C-D}} = 90^\circ$ ) and parallel to  $B_0$  ( $\theta_{\text{C-D}} = 0^\circ$ ); the difference of intensity between the horns and feet results from the difference of probability of finding the principal axis parallel or perpendicular to  $B_0$ , this latter being statistically more relevant. A typical example of an experimental NAD powder pattern is shown in **Figure 8b** in the case of isotopically normal hexamethylbenzene ( $C_6$  symmetry molecule with 18 equivalent monodeuterated isotopomers).

Providing analytically exploitable high-resolution, proton decoupled  $^2\text{H}$  SS-NMR spectra is possible by the combination of three necessary tools in solid NMR: i) the Magic Angle Spinning (MAS) technique where the sample rotates at high speed (10-80 kHz) at an angle of  $54.7^\circ$  relative to the  $B_0$  axis of the magnet, thus averaging the first-order quadrupolar interaction; ii) high power proton decoupling

(HPPD) techniques which provide an efficient decoupling of proton signals; iii) cross polarization (CP) from  $^1\text{H}$  to the X nuclei.

In practice, the MAS frequency of the rotor is generally much smaller than the width (in Hz) of the  $^2\text{H}$  spectrum, and then the center band of the  $^2\text{H}$  (or NAD) MAS NMR spectrum is accompanied by many sidebands, the envelope of which corresponds to the powder pattern obtained under static conditions. As an example of applications, the combination of MAS and HPPD techniques has allowed the acquisition of high-resolution NAD SS-NMR spectra of plastic crystals. In these very specific crystals, the  $^2\text{H}$  quadrupolar interaction is averaged out by the molecular motions. As an example, **Figure 9a** reports the NAD SS-NMR spectra of 2,2-dimethyl-1,3-propanediol (**DMPD**) and 2-hydroxymethyl-2-methyl-1,3-propanediol (**HMPD**) recorded under MAS and HPPD conditions, at a temperature where they are in the plastic-crystalline state. Interestingly, in both samples, the combination of these two techniques allows the resolution of all  $^2\text{H}$  chemical shifts of molecules [*Aliev et al, 1994*].



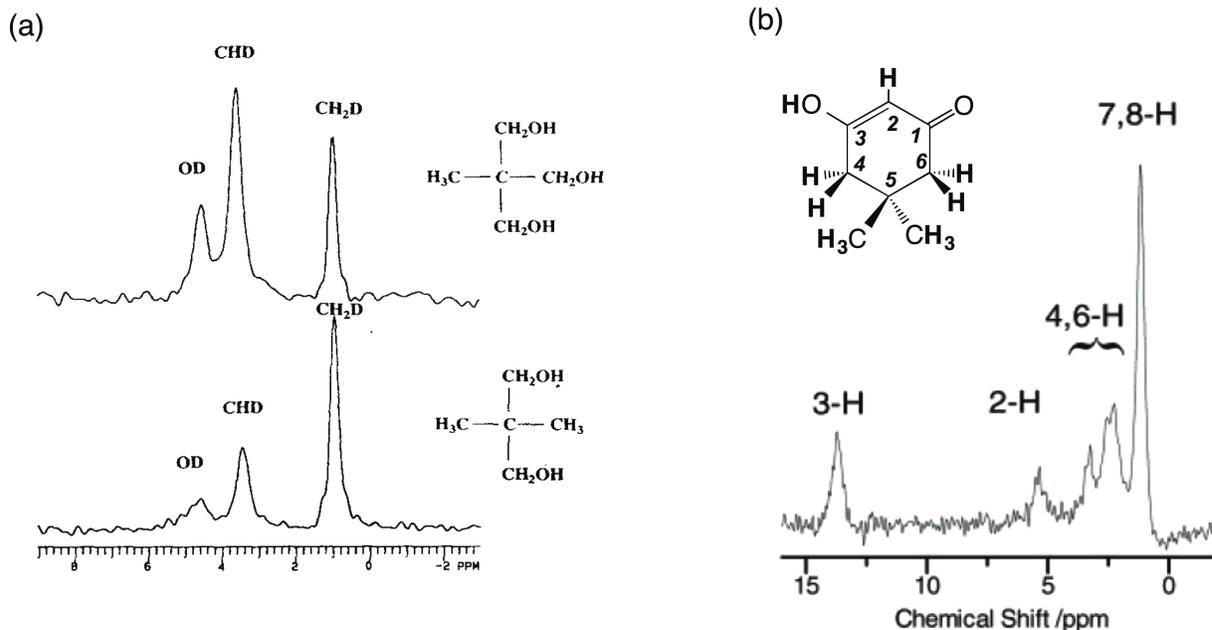
**Figure 8.** (a) Theoretical quadrupolar Pake's doublet observed in  $^2\text{H}$  static SS-NMR spectra of a monodeuterated amorphous or polycrystalline sample assuming all possible orientations of the C–D bond relative to  $B_0$  and an axially symmetric quadrupole coupling. The superposition of two dash lines provides the Pake pattern (continuous line) with two ‘‘horns’’ at  $q_{\text{C-D}} = 90^\circ$ . The fit of such spectra allows the evaluation of  $C_Q$  and  $\eta$ . (b) Example of experimental NAD powder pattern recorded for hexamethylbenzene (**Courtesy of Jansen-Glaw et al, J. Chem. Phys. 2012, 90, 6858, reproduced with permission from the American Institute of Physics**).

To overcome the low sensitivity of NAD SS-NMR,  $^1\text{H}$  to  $^2\text{H}$  cross-polarization (CP) can be also applied in the case of rigid solids. Combined with the addition of Spinning Side Bands (SSB) to the center band of the NAD spectrum, the  $^1\text{H} \rightarrow ^2\text{H}$  CP-MAS scheme can significantly improve the SNR of NAD SS-NMR spectra as demonstrated in the case of dimedone (**DMD**) where the experimental resolution reached in the NAD 1D spectrum allowed assignment of all resonances on the basis of their  $^2\text{H}$  chemical

shifts (see **Figure 9b**), [Mizuno *et al*, 2006]. For partially deuterated DMD, the  $^1\text{H}\rightarrow^2\text{H}$  CP enhancement factor,  $S_{\text{CP}}$ , obtained was closed to the theoretical maximum given by **Eq. 10** [Muller *et al*, 1980; Muller *et al*, 1981]:

$$\frac{S_{\text{CP}}}{S_0} = \frac{\gamma(^1\text{H})}{\gamma(^2\text{H})} \times \left(1 + \frac{8N(^2\text{H})}{3N(^1\text{H})}\right)^{-1} \quad [10]$$

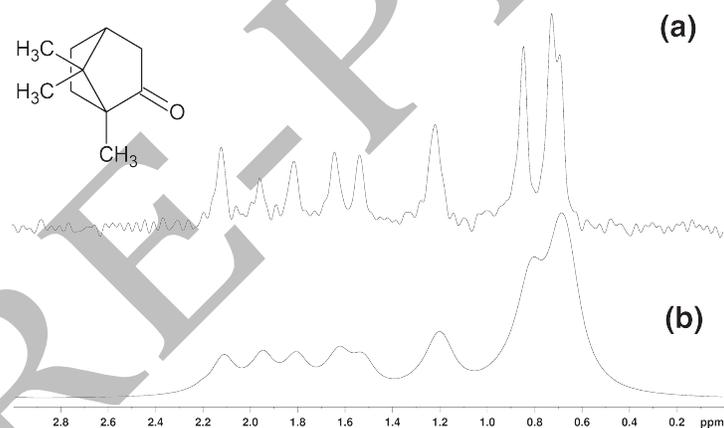
where  $S_0$  is the unenhanced thermal equilibrium polarisation,  $g(^1\text{H})$  and  $g(^2\text{H})$  are the  $^1\text{H}$  and  $^2\text{H}$  gyromagnetic ratio and  $N(^1\text{H})$  and  $N(^2\text{H})$  are the number of  $^1\text{H}$  and  $^2\text{H}$  spin in the molecule. Actually the almost ideal CP enhancement for  $^2\text{H}$  is possible when MAS (that modulates the quadrupolar coupling) realizes the matching of the Hartmann–Hahn condition for a  $^1\text{H}\text{-}^2\text{H}$  pair during a rotor cycle [Muller *et al*, 1980; Muller *et al*, 1981]. For very fast MAS, the efficiency of  $^1\text{H}\rightarrow^2\text{H}$  CP can be improved by generating an amplitude/frequency modulation for  $^2\text{H}$  irradiation during the CP period [Murakami *et al*, 2009].



**Figure 9.** (a) 76.8 MHz NAD SS-NMR spectra of **DMPD** (bottom) and **HMPD** (top) recorded under MAS conditions at high Temperature ( $> 370$  K) and using a single pulse sequence with HPPD during the acquisition (Courtesy of Aliev *et al*, *Chem. Phys. Lett.*, 1994, 226, 193, reproduced with permission from Elsevier). (b) NAD  $^1\text{H}\rightarrow^2\text{H}$  CP-MAS 1D spectrum of solid DMD at a MAS frequency of 5 kHz with  $^1\text{H}$  HPPD during the acquisition. The  $-14^{\text{th}}$  to the  $+14^{\text{th}}$  spinning sidebands as well as the centre band are added together here. The strength of the  $^1\text{H}$  TPPM decoupling was 61 kHz (Courtesy of Mizuno *et al*, *J. Am. Chem. Soc.*, 2006, 128, 9683, reproduced with permission from Elsevier).

In order to increase the dispersion of resonances in the  $^2\text{H}$  SS-NMR spectra, but also to overcome the low sensitivity of NAD MAS-NMR measurements, the use of very high magnetic fields is an undeniable advantage. For instance, it was shown that NAD SS-NMR of plastic crystals under MAS conditions can be acquired within a few hours at 20 T (130.5 MHz for  $^2\text{H}$  observation) (see **Figure 10a**). Additionally, the comparison of  $^1\text{H}$  and NAD MAS SS-NMR spectra shows that the latter exhibits significantly sharper resonances. This difference originates from the fact that the  $^2\text{H}$  first-order quadrupolar interaction is inhomogeneous, whereas the  $^1\text{H}$ - $^1\text{H}$  dipolar couplings are homogeneous. As seen in **Figure 10b**, the NAD 1D-NMR spectrum of camphor allows the resolution of the resonances for almost all  $^2\text{H}$  environments, including the two geminal methyl groups.

In another application, NAD SS-NMR spectroscopy was also used to investigate the dynamics of crosslinked elastomer networks. These polymeric materials generally possess molecular motions that are not fully isotropic, especially near cross-link and entanglement points. As a consequence, NAD SS-NMR spectroscopic measurements of the residual quadrupolar interactions allowed the monitoring of the variation in the molecular dynamics between the various regions of the polymer (i.e. near or far from the cross-link).



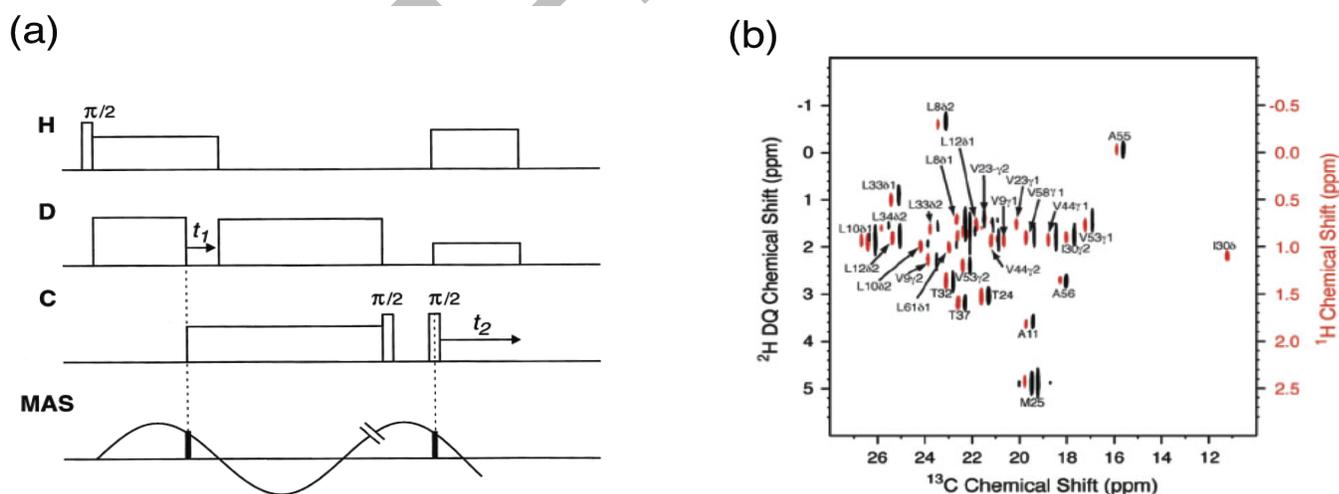
**Figure 10.** Comparison of the 130.5 MHz NAD MAS 1D-NMR spectrum (a) of (*S*)-camphor in the plastic-crystalline state recorded with only 2344 scans and a recycle delay of 3 s with the corresponding 850 MHz  $^1\text{H}$  MAS 1D NMR spectrum (b) of the same sample. Note the advantage of  $^2\text{H}$  NMR over the  $^1\text{H}$  NMR in terms of peak resolution (Courtesy of Aliev et al, *J. Phys. Chem. A.*, 2011, 115, 5568, reproduced with permission from ACS).

A recent important methodological achievement in SS-NAD NMR spectroscopy was experimentally demonstrated in 2015 by combining the high-field (9.4T) DNP approach and the MAS technique at about 100 K [Rossini et al, 2015]. This combination enables the acquisition in two hours of high quality natural abundance  $^1\text{H}\rightarrow^2\text{H}$  CP-MAS SS-NMR spectra of organic solids impregnated

with tetrachloroethane solutions of TEKPOL biradical. In this highly promising DNP-NMR experiment, the polarization of unpaired electrons is first transferred to  $^1\text{H}$  by microwave irradiation, then the DNP-enhanced  $^1\text{H}$  polarization is distributed throughout the organic solids via  $^1\text{H}$ - $^1\text{H}$  spin diffusion and is finally transferred to  $^2\text{H}$  nuclei by CP. Interestingly, the spectral resolution for these NAD SS-NMR spectra recorded with this strategy is comparable to that of  $^1\text{H}$  NMR spectra obtained with « state of the art » of the homonuclear decoupling techniques.

As in liquids and liquid-crystalline phases, correlation between quadrupolar interactions of deuterons (labelled compounds) and  $^{13}\text{C}$  chemical-shielding interactions in spatial proximity is possible by 2D MAS SS-NMR spectroscopy. The heteronuclear correlations between  $^{13}\text{C}$  and  $^2\text{H}$  nuclei are established by CP under slow MAS conditions over the whole frequency ranges of both nuclei virtually uniformly. As seen in **Figure 11a**, the sample rotation and the start of the  $t_1$  and  $t_2$  evolution periods are synchronized in order for them to occur at exactly the same position of the rotor [*Robyr et al, 2000*].

Applied to the simple amino acid glycine,  $^{13}\text{C}$  labelled at the carboxylic group, and  $^2\text{H}$  labelled at the methylene group, the analysis of correlation 2D NMR spectra provided key molecular structural information, such as torsional angles around the  $\text{CO}-\text{C}_\alpha$  bond evaluated by measuring the relative orientations between the  $^{13}\text{C}$ -RCSA tensor and the two  $^2\text{H}$ -quadrupolar tensors associated to the enantiotopic deuterium sites. The technique was also used to characterize the conformation of synthetic and bio-polymers.



**Figure 11.** (a) Schematic diagram of MAS  $^2\text{H}$ - $^{13}\text{C}$  correlation 2D experiment under slow magic-angle sample spinning proposed by Robyr et Meier (*Courtesy of Robyr et al, Chem. Phys. Lett., 2000, 327, 319, reproduced with permission from ACS*). (b) 14.1 T  $^{13}\text{C}$ -detected  $^1\text{H}$ ,  $^{13}\text{C}$  (red) and the  $^2\text{H}$ -DQ,  $^{13}\text{C}$  (black) correlation map of the methyl region of  $^2\text{H}$ -,  $^{13}\text{C}$ -,  $^{15}\text{N}$ -labeled sample of the SH3 domain of chicken R-spectrin. The MAS frequency of 20 kHz is used. Note the correspondence between  $^2\text{H}$ -DQ and the  $^1\text{H}$ -SQ chemical shift modulo a factor 1/2 (*Courtesy of Agarwal et al, J. Am. Chem. Soc. 2009, 131, 2, reproduced with permission from ACS*).

In “biological” NMR applications, the combined use of the MAS technique and solid-state  $^2\text{H}$ - $^{13}\text{C}$  correlation 2D spectra for analyzing isotopically enriched peptides and perdeuterated proteins (as for the chicken R-spectrin) has been evaluated [Agarwal et al., 2009]. Excitation of the  $^2\text{H}$  double quantum coherence (DQC) was allowed to evolve during  $t_1$  followed by reconversion to  $^2\text{H}$  single-quantum coherence (SQC) before CP with Hartman-Hahn matching yielded very high-resolution spectra in the  $^2\text{H}$  dimension as can be seen in **Figure 11b**. In this specific experiment, the gain in resolution compared to  $^2\text{H}$  SQC originates from: i) the doubling of  $^2\text{H}$  DQCs chemical shifts while linewidths are not affected; ii) the insensitivity of  $^2\text{H}$  DQCs to magic angle offset effects, and the absence of line broadening due to molecular dynamics as in case of  $^2\text{H}$  SQCs.

### **Brief outlook and perspectives**

Considering the NMR spectral properties of deuterons, these nuclei are remarkable nuclear spies to probe molecular properties and are the heart of numerous analytical applications without the need for isotopic enrichment of solutes. In this chapter, we have overviewed several original aspects of  $^2\text{H}$  NMR including NAD NMR spectroscopy in the three states of condensed matter. The interest of NAD NMR spectroscopy compared to NMR spectroscopy of other quadrupolar nuclei is that deuterons are naturally present in almost all molecules and do not present serious drawbacks associated with quadrupolar nuclei with large  $Q_D$  values.

During the past two decades, special attention has been paid to the development of NAD multidimensional NMR spectroscopy in combination with the use of CLC solvents, thus opening up a wide analytical field in three important distinct domains: i) the measurement of enantiomeric purity in chiral analysis; ii) stereochemical study (configurational study) as a tool for structure elucidation; iii) the investigation of isotope fractionation in natural compounds dedicated to the elucidation of biochemical mechanistic pathways. Lastly, developments of NAD SS-NMR spectroscopy combined with DNP and CP are also promising and should quickly open new analytical opportunities.

Eventually, a challenging complementary research line could be the development of  $^2\text{H}$  NMR spectroscopy on isotopically labelled or even normal analytes (pure molecules) detected on the recently developed 60-80 MHz benchtop NMR spectrometers, whose quality and technical and methodological possibilities have been significantly improved since the 2010's [Auguste et al, 2017 Blumich et al, 2016].

## References

- Agarwal**, V. Faelber, K., Schmieder, P., Reif, B. (2009). High-Resolution Double-Quantum Deuterium Magic Angle Spinning Solid-State NMR Spectroscopy of Perdeuterated Proteins, *J. Am. Chem. Soc.* 131, 2-3. (FIGURE 11b)
- Aliev**, A.E., Harris, K.D.M., Apperley, D.C. (1994). Natural Abundance High-resolution Solid State  $^2\text{H}$  NMR Spectroscopy. *Chem. Phys. Lett.*, 226 193. (FIGURE 9a)
- Aliev**, A. E. Mann, S. E., Luga, D., Hughes, C. E., Harris, K. D. M. (2011). Natural-Abundance Solid-State  $^2\text{H}$  NMR Spectroscopy at High Magnetic Field. *J. Phys. Chem.*, 115, 5568–5578. (FIGURE 10a/b)
- Aroulanda**, C., Lesot, P., Merlet, D., Courtieu J. (2003). Structural Ambiguities in Bridged Ring Systems Resolved using Natural Abundance Deuterium NMR in Chiral Liquid Crystals. *J. Phys. Chem. A.*, 107, 10911-10918.
- Auger**, C., Lesage A., Caldarelli, S., Hodgkinson, P., Emsley, L. (1998). Assignment and Measurement of Deuterium Quadrupolar Couplings in Liquid Crystals by Deuterium-Carbon NMR Correlation Spectroscopy. *J. Phys. Chem. B*, 120, 3718-3723.
- Auguste**, E., Lucas, E., Poignez, L., Ramamurthy, D., Vaneckhoutte, H., Lesot, P. (2017). RMN de Paillasse à Transformée de Fourier: Le Renouveau de la RMN à Bas Champ. *Spectra Analyse*, 315, 32-62.
- Baillif**, V., Robins, R. J., Le Feunten, S., Lesot, P., Billault I. (2009). Investigation of Fatty Acid Elongation and Desaturation Steps in *Fusarium lateritium* by Quantitative Two-dimensional Deuterium NMR Spectroscopy in Chiral Oriented Media. *J. Biol. Chem.*, 284, 10783-10792.
- Berdagué**, P., Lesot, P., Jacob, J., Terwilliger, V.-J., Le Milbeau C. (2016). Contribution of NAD 2D-NMR in Liquid Crystals to the Determination of Hydrogen isotope Profile of Methyl Groups in Miliacin. *Geo. Cosmo. Acta*, 173, 337-351.
- Billault**, I., Robins, R. J., Akoka, S. (2002). Determination of Deuterium Isotope Ratio by Quantitative  $^2\text{H}$  NMR spectroscopy: the ERETIC Method as a Generic Reference Signal Analytical Chemistry. *Anal. Chem.*, 74, 5902 - 5906.
- Blumich**, B. (2016). Introduction to Compact NMR: A Review of Methods, *TRAC-Trends in Anal. Chem.*, 83, 2-11.
- Briggs**, J. M., Farnell, L. F., Randall, E. W. (1973). Proton-Noise-Decoupled Deuterium Resonance at Natural Abundance by Fourier Transform. *Chem. Commun.*, 70-71.
- Canlet**, C., Merlet, D., Lesot, P. et al., J. (2000). Deuterium NMR Stereochemical Analysis of *Threo-Erythro* Isomers Bearing Remote Chiral Centres in Racemic and Non-Racemic Liquid Crystalline Solvents. *Tetrahedron: Asymmetry*, 11, 1911-1918.
- Chan-Huot**, M., Lesot, P., Pelupessy et al. (2013). ‘On-the-fly’ Kinetics of Enzymatic Racemization using Deuterium NMR in DNA-based, Oriented Chiral Solvents. *Anal. Chem.*, 85, 4694-4697.
- Curtis**, J., Grant, D. M., Pugmire, R.J., (1989), Distortion in Six-Membered Saturated Rings by Natural Abundance Deuterium NMR, *J. Am. Chem. Soc.*, 111, 7711-7716.
- Dong**, R. Y. (2004). Advances in NMR Studies of Liquid Crystals. *Ann. Rep. on NMR Spect.*, 53, 67-155.
- Emsley** J. W., Lindon J.C. (1975). Chapter 2, *NMR Spectroscopy Using Liquid Crystal Solvents*, Pergamon Press, Oxford.
- Emsley**, J. W., Lesot, P., De Luca, G., Lesage, A., Merlet, D., Pileio, G. (2008). A Comparison of Proton-detected  $^{13}\text{C}$  Local Field Experiments with Deuterium NMR at Natural Abundance for Studying Liquid Crystals. *Liq. Crystals*, 35, 443-464. (FIGURE 3a)

- Fronza, G.**, Fuganti, C., Guillou, C., Reniero, F., Joulain, D. (1998). Natural Abundance  $^2\text{H}$  NMR Study of the Origin of Raspebery Ketone. *J. Agric. Food. Chem.*, 46, 248-254.
- Guillou, C.** (1991). Isotopic Analysis of Wines, *Analysis*, 1, 29-33. (FIGURE 2a)
- Jansen-Glaw, B.**, Rössler, E., Taupitz M., Vieth, H. M. (1989). Hexamethylbenzene As a Sensitive Nuclear Magnetic Resonance Probe for Studying Organic Crystals and Glasses. *J. Chem. Phys.* 90, 6858-6866. (FIGURE 8b)
- Jayanthi, S.**, Ramanathan, K.V. (2010). Deuterium–Carbon Correlation in Static Oriented Systems using DAPT: A Windowless Multiple Pulse Sequence. *Chem. Phys. Lett.*, 487, 122-128.
- Khetrapal, C.L.**, Ramanathan, K. V., Suryaprakash, N., Vivekanandan, S. (1998). Natural Abundance  $^2\text{H}$  NMR Spectra of Molecules Oriented in Liquid Crystals. *J. Magn. Reson.* 135, 265-266. (FIGURE 3b)
- Kobzar, K.**, Kessler, H., Luy, B. (2005). Stretched Gelatin Gels as Chiral Alignment Media for the Discrimination of Enantiomers by NMR Spectroscopy. *Angew. Chem. Int. Ed.*, 44, 3145-3147.
- Kovacs, H.**, Moskau, D., Spraul, M. (2005). Cryogenically cooled probes - a leap in NMR technology. *Prog. Nucl. Magn. Reson. Spectrosc.* 46, 131-155.
- Lafon, O.**, Berdagué, P., Lesot, P. (2004). Use of Two-dimensional Correlation between  $^2\text{H}$  Quadrupolar Splittings and  $^{13}\text{C}$  CSA's for Assignment of NMR Spectra in Chiral Nematics, *Phys. Chem. Chem. Phys.*, 6, 1080-1084.
- Lafon, O.**, Lesot, P. (2005). Deuterium Three-dimensional NMR Experiments for Analysing Weakly Aligned, Enriched Molecules, *Chem. Phys. Lett.*, 404, 90-94.
- Lafon, O.**, Lesot P., Fan C.A., Kagan H.B. (2007). Analysis of intramolecular dynamic processes in enantiomeric diaryl atropisomers and related derivatives through  $^2\text{H}$  NMR in polypeptide liquid crystals. *Chem. Eur. J.*, 13, 3772-3786.
- Leyendecker, M.**, Nils-Christopher Meyer, N.-C., and Christina M. Thiele, C.M. (2017) Development of New Supramolecular Lyotropic Liquid Crystals and their Application as Alignment Media for Organic Compounds. *Angew. Chem. Int. Ed.*, 56, 11471-11474.
- Leopold, M. F.**, Epstein, W. W., Grant, D. M. (1998). Natural Abundance Deuterium NMR as a Novel Probe of Monoterpene Biosynthesis: Limonene. *J. Am. Chem. Soc.*, 111, 616-617.
- Lesot, P.**, Merlet, D., Loewenstein, A., Courtieu, J. (1998). Enantiomeric Visualisation using Proton-Decoupled Natural Abundance Deuterium NMR in Poly(g-benzyl-L-glutamate) Liquid Crystalline Solutions. *Tetrahedron: Asymmetry*, 9, 1871-1881.
- Lesot, P.**, Sarfati, M., Courtieu, J. (2003). Natural Abundance Deuterium NMR Spectroscopy in Polypeptide Liquid Crystals as a New and Incisive Means for Enantiodifferentiation of Chiral Hydrocarbons. *Chem. Eur. J.*, 9, 1724-1745. (FIGURE 1)
- Lesot, P.**, Lafon, O., Kagan, H.B., Fan, P. (2006). Study of Molecular Rotational Isomerism using Deuterium NMR in Chiral Oriented Solvents. *Chem. Commun.*, 389-391.
- Lesot, P.**, Lafon, O., Aroulanda, C., Dong, R. (2008).  $^2\text{H}$  NMR Studies of Two-homopolypeptide Lyotropic Mesophases: Toward the Quantification of Solute-Fiber Interactions *Chem. Eur. J.*, 14, 4082-4092.
- Lesot, P.**, Aroulanda, C., Berdagué, P., Zimmerman, Z., Luz Z. (2011). Conformation and Dynamics of 18-membered Hexathia-metacyclophanes: The two Steps Chirality Averaging as Studied by Deuterium NMR in Chiral Liquid Crystalline Solutions. *J. Phys. Chem. B*, 115, 11793-11804.
- Lesot, P.**, Serhan, Z., Billault, I. (2011). Recent Advances in the Analysis of the Site-specific Isotopic Fractionation of Metabolites such as Fatty Acids using Anisotropic Natural Abundance  $^2\text{H}$  NMR Spectroscopy: Application on Conjugated Linolenic Methyl Esters. *Anal. Bioanal. Chem.*, 399, 1187-1200.

- Lesot, P., Reddy, V., Suryaprakash, N. (2011).** Exploring the Enantiodiscrimination Potentialities of DNA-based orienting Media using Deuterium NMR Spectroscopy. *Chem. Commun.*, 47, 11736-11738.
- Lesot, P., Lafon, O. (2012).** Experimental Detection of Achiral and Chiral Naturally Abundant  $^{13}\text{C}$ - $^2\text{H}$  Isotopomers by 2D-NMR in Liquids and Chiral Oriented Solvents. *Anal. Chem.*, 84, 4569-4573. (FIGURE 7b)
- Lesot, P., Serhan Z., Aroulanda, C. Billault I. (2012).** Analytical Contribution of NAD 2D-NMR Spectroscopy in Polypeptide Mesophases to the Investigation of Triglycerides. *Magn. Reson. Chem.*, 50, S2-S11.
- Lesot, P., Lafon, O., Berdagué P., (2014).** Correlation 2D-NMR Experiments Involving both  $^{13}\text{C}$  and  $^2\text{H}$  Isotopes in Oriented Media: Methodological Developments and Analytical Applications. *Magn. Reson. Chem.*, 52, 595-613.
- Lesot, P., Aroulanda, C., Zimmermann, H., Luz Z., (2015).** Enantiotopic Discrimination in the NMR Spectrum of Prochiral Solutes in Chiral Liquid Crystals. *Chem. Soc. Rev.*, 44, 230-275.
- Lesot, P., Kazimierczuk, K., Trébosc, J., Amoureux, J.-P., Lafon, O. (2015).** Fast Acquisition of Multidimensional NMR Spectra of Solids and Mesophases using Alternative Sampling Methods. *Magn. Reson. Chem.*, 53, 927-939.
- Lesot, P., Berdagué, P., Giraudeau, P., (2016).** Detection of Quadrupolar Nuclei by Ultrafast 2D-NMR: Exploring the Case of Deuterated Analytes Aligned in Chiral Oriented Solvents. *Chem. Commun.*, 52, 2122-2125.
- Martin, G. J., Martin, M.L. (1981).** Deuterium Labelling at the Natural Abundance level as Studied by High Field Quantitative  $^2\text{H}$  NMR. *Tetrahedron. let.* 22, 3525-3528.
- Meddour, A., Canet, I., Loewenstein A., Péchiné, J.-M., Courtieu, J. (1994).** Observation of Enantiomers, Chiral by Virtue of Isotopic Substitution, through Deuterium NMR in a Polypeptide Liquid Crystal. *J. Am. Chem. Soc.*, 116, 9652-9656. (FIGURE 4b)
- Merlet, D., Loewenstein, A., Smadja, W., Courtieu, J., Lesot, P. (1998).** Quantitative Description of the Facial Discrimination of Molecules Containing a Prochiral Group Oriented in a Chiral Liquid Crystalline Solution. *J. Am. Chem. Soc.*, 120, 963-969. (FIGURE 4c)
- Merlet, D., Ancian, B., Courtieu, J., Lesot, P. (1999).** Two-dimensional Deuterium NMR Spectroscopy of Chiral Molecules Oriented in a Polypeptide Liquid Crystal: Application for the Enantiomeric Analysis through Natural Abundance Deuterium NMR. *J. Am. Chem. Soc.*, 121, 5249-5258,
- Meyer, N.-C., Krupp, A., Schmidts, V., Thiele, C.-M., Reggelin, M. (2012).** Polyacetylenes as Enantiodifferentiating Alignment Media. *Angew. Chem. Int. Ed.*, 51, 8334-8338.
- Mizuno T., Nemoto, T., Tansho, M., Shimizu, T., Ishii, H., Takegoshi, K. (2006).**  $^2\text{H}$  Natural-abundance MAS NMR Spectroscopy: An Alternative Approach to Obtain  $^1\text{H}$  Chemical Shifts in Solids. *J. Am. Chem. Soc.*, 128, 9683-9686. (FIGURE 9b)
- Muller, L., Eckman, R., Pines, A. (1980).** Proton-enhanced Deuterium NMR in Rotating Solids. *Chem. Phys. Lett.* 76, 149-154.
- Muller, L. (1981).** Proton-Deuterium Polarization Transfer In Magic Angle Spinning Polycrystalline Solids in the Rotating Frame. *Chem. Phys.*, 61, 235-248.
- Murakami, M., Shimizu, T., Tansho, M., Takegoshi, K. (2009).** Improvement of  $^1\text{H}$ - $^2\text{H}$  Cross Polarization under Magic-angle Spinning by using Amplitude/Frequency Modulation. *Solid State Nucl. Magn. Reson.*, 36, 172-176.
- Navarro-Vazquez, A., Berdagué, P., Lesot, P. (2017).** Integrated Computational Protocol for Analyzing Quadrupolar Splittings from Natural Abundance Deuterium NMR Spectra in (Chiral) Oriented Media. *ChemPhysChem.*, 18, 1252-1266.

- Naumann, C.**, Bubb, W. A., Chapman, B. E., Kuchel, P.W., (2007). Tunable-Alignment Chiral System Based on Gelatin for NMR Spectroscopy, *J. Am. Chem. Soc.*, 129, 5340-5341.
- Naumann, C.**; Kuchel, P. W. (2009). NMR (Pro)chiral Discrimination Using Polysaccharide Gels. *Chem. Eur. J.* 15, 12189-12191.
- Palomino, M.**, Khudr H., Courtieu, J., Merlet, D., Meddour A. (2012). The Use of Exchangeable Nuclei to Observe Enantiomers Through Deuterium NMR in Chiral Liquid Crystalline Solvents. *Magn. Reson. Chem.* 50, S12-S16.
- Prosser, R. S.**, Heaton, N. J., Kothe, G. (1996). Application of the Quadrupolar Carr-purcell-Meiboom-Gill Pulse train for the sensitivity Enhancement in Deuteron NMR of Liquid Crystals. *J. Magn. Reson. B*, 112, 51-57.
- Remaud G. S.**, Martin Y.-L., Martin G. G, Martin G. J. (1997). Detection of Sophisticated Adulterations of Natural Vanilla Flavors and Extracts: Application of the SNIF-NMR Method to Vanillin and *p*-Hydroxybenzaldehyde *J. Agric. Food Chem.* 45, 859-866. (FIGURE 2b)
- Robyr, P.**, Meier, B. H. (2000). Deuterium-carbon NMR Correlation Spectroscopy: A Tool for Structural Characterization of Solids. *Chem. Phys. Lett.*, 327, 319-324. (FIGURE 11a)
- Rossini, A. J.**, Schlagnitweit, J., Lesage, A. Emsley L. (2015). High-resolution NMR of Hydrogen in Organic Solids by DNP Enhanced Natural Abundance Deuterium Spectroscopy. *J. Magn. Reson.*, 259, 192-198.
- Sandström, D.**, Zimmermann. H. (2000). Correlation of Deuterium Quadrupolar Couplings and Carbon-13 Chemical Shifts in Ordered Media by Multiple-Quantum NMR. *J. Phys. Chem. B*, 104, 1490-1493. (FIGURE 7a)
- Sarfati, M.**, Lesot, P., Merlet, D., Courtieu J., (2000). Theoretical and Experimental Aspects of Enantiomeric Differentiation using Natural Abundance Multinuclear NMR Spectroscopy in Polypeptide Liquid Crystals *Chem. Commun.*, 2069-2081.
- Sarfati, M.**, Aroulanda, C., J. Courtieu, J., Lesot. P. (2001). Enantiomeric Recognition of Chiral Invertomers through NMR in Chiral Oriented Solvents: A Study of the *cis*-Decalin. *Tetrahedron: Asymmetry*, 12, 737-744.
- Serhan, Z.**, Martel, L., Billault, I., Lesot, P. (2010). Complete Determination of Site Specific Bio-enantiomeric Excesses in Linoleic Acid using Natural Abundance Deuterium 2D NMR in Polypeptide Mesophase. *Chem. Commun.*, 46, 6599-6601. (FIGURE 5)
- Serhan, Z.**, Billault, I., Borgogno, A., Ferrarini, A., Lesot P. (2012). Analysis of NAD 2D-NMR Spectra of Saturated Fatty Acids in Polypeptide Aligning Media by Experimental and Modeling Approaches. *Chem. Eur. J.*, 18, 117-126.
- Smadja, W.**, Auffret, S., Berdagué, P., Merlet, D., Canlet, et al, (1997). Visualisation of Axial Chirality using  $^2\text{H}\{-^1\text{H}\}$  NMR in poly(*g*-benzyl-*L*-glutamate), a Chiral Liquid Crystal Solvent, *Chem. Commun.*, 2031-2032. (FIGURE 4a)
- Solgadi, A.**, Meddour, A., Courtieu, J. (2004). Enantiomeric Discrimination of Water Soluble Compounds using Deuterium NMR in a Glucocon/Buffered Water/*n*-Hexanol Chiral Lyotropic Liquid Crystal. *Tetrahedron: Asymmetry*, 15, 1315-1318
- Tabayashi, K.**, Akasaka, K. (1999). Natural Abundance  $^2\text{H}$  NMR for Liquid Crystal Studies: Deuterium Isotope Effect on Microscopic Order, *Liquid Crystals*, 26, 127-129.
- Wenzel T. J.** (2007). Discrimination of Chiral Compounds using NMR Spectroscopy, J. Wiley & Sons.
- Zangger, K.** (2015). Pure Shift NMR. *Prog. Nucl. Magn. Reson. Spectrosc.* 86-87, 1-20.

## Glossary :

ADUF	Anisotropic Deuterium Ultra Fast
$B_0$	static magnetic field of spectrometer
CLC	Chiral Liquid Crystal
Cov	Covariance transform
CP	Cross Polarization
CPD	Composite Pulse Decoupling
CPMG	Carr-Purcell-Meiboom-Gill sequence
$C_Q$	Quadrupolar coupling constant
CSA	Chemical Shift Anisotropy
CST	Compressed Sensing Theory
DAPT	Dipolar Assisted Polarization Transfer
DFT	Density Function Theory
DQC	Double Quantum Coherence
DIE	Diastereo-Isotopomeric Excess
DNA	DesoxyriboNucleic Acid
DNP	Dynamic Nuclear polarization
QD	Quadrupolar Doublet
$\Delta\nu_Q$	Quadrupolar splitting (in Hz)
FAME	Fatty Acid Methyl Ester
FWHM	Full Width at Half Maximum
FT	Fourier Transform
EE	Enantiomeric Excess
EFG	Electric Field Gradient
EIE	Enantio-Isotopomeric Excess
ERETIC	Electronic REference To access In vivo Concentrations
HETCOR	HETeronuclear CORrelation
HMQC	Heteronuclear MultiQuantum Coherence
HPPD	High-Power Proton Decoupling
KIE	Kinetic Isotope Effect
LC	Liquid Crystal
MAS	Magic Angle Spinning
NAD	Natural Abundance Deuterium
NMR	Nuclear Magnetic Resonance
(N)US	(Non) Uniform Sampling
PBLG	Poly-g-Benzyl-L-Glutamate
QD	Quadrupolar Doublet
$Q_D$	$^2\text{H}$ quadrupolar moment
QUOSY	QUadrupole Ordered SpectroscopY
Q-COSY	Quadrupole COrelation SpectroscopY
NASDAC	Natural Abundance Spectroscopy for Deuterium And Carbon
nOe	Nuclear Overhauser effect
RCSA	Residual Chemical Shift Anisotropy
RQC	Residual Quadrupolar Coupling
RDC	Residual Dipolar Coupling
RF	Radio Frequency
SSB	Spinning Side Band
SQC	Single Quantum Coherence
SS-NMR	Solid-State NMR

---

$S_{ab}$	Saupe's order matrix elements
SNR	Signal-to-Noise Ratio
SNIF	Site-specific Natural Isotopic Fractionation
$T_{RD}$	Recycle Delay
TMU	TetraMethyl Urea
V-SMOW	Vienna Standard Means Ocean Water

## Author Biography and Photography

Dr. Philippe Lesot, born in 1967, is affiliated to the French National Center for Scientific Research (CNRS). He has been Research Director since 2007 at the Institut de Chimie Moléculaire et des Matériaux d'Orsay (ICMMO, UMR 8182) located at the University of Paris Sud / Paris-Saclay in France (Orsay). He received his Ph.D degree (cum laudae) from the University of Paris Sud in 1995 under the supervision of Prof. J. Courtieu, and then pursued his research activities as a post-doctoral fellow at University of Southampton (UK) with



Prof. JW. Emsley (one of the pioneers of anisotropic NMR spectroscopy), supported by a grant from the Royal Society of London and the French Foreign Ministry (Lavoisier). After holding a temporary assistant professor position for one year, he joined the CNRS in 1997 as tenured researcher. He was a recipient of the 2001 Bronze Medal from CNRS for young scientists for his pioneering work on the NAD nD NMR in CLCs. By 2017, he has published over 125 articles including 100 peer-review papers and 8 book chapters, in collaboration with numerous French or foreign chemists (H. Kagan, I. Marek, R. Grée, ...) or internationally recognised NMR scientists (G. Bodenhausen, A. Loewenstein, Z. Luz, ...) and given more than 50 invited talks on the analytical and methodological developments of anisotropic NMR spectroscopy. His current research interests focus on the natural isotope fractionation of bioproducts by anisotropic NAD NMR spectroscopy and the fight against counterfeiting.