Electronic Supplementary Material (ESI) for Analyst.

Potential and Performance of Anisotropic ^{19}F NMR for the Enantiomeric Analysis of Fluorinated Chiral Active Pharmaceutical Ingredients

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Table of content

I. Chemicals and chiral lyotropic liquid crystals

Chemicals. (*S*)-Efavirenz was purchased at TCI with a purity of 98%. *Rac*-Efavirenz was purchased at Toronto Research Chemical (TRC) with a purity of 97%. (*R*)-Flurbiprofen was purchased at Acros Organics with a purity of 97%. *Rac*-Flurbiprofen was purchased at Sigma-Aldrich (Merck) with a purity of 98%. The polymer PBLG (DP = 849) was purchased at Sigma-Aldrich (Merck).

NMR sample preparation. Nearby 100 mg of PBLG were weighted directly within a 5 mm NMR tube. Then, a solution of $CHCl_3$ containing the chiral solute to be studied (racemic or scalemic series) was added. The mass of chloroform (about 600 mg) was here adjusted so that the total wt% of PBLG (*i.e.,* m_{PBLG} / m_{tot}) is maintained around 14%. The NMR tubes is then flame sealed to avoid $CHCl_3$ evaporation during NMR acquisitions and several low-speed centrifugation cycles of the tube at short time repetition (*e.g.,* 500 rpm during 20 s) are carried out to limit solute orientation inhomogeneities (mainly due to matter gradients). The NMR tube is inverted between each centrifugation.

II. NMR sample composition, *ee*(%) and uncertainty

The composition of the PBLG-based weakly oriented samples used in this work is detailed in **Table SI-1** and **SI-2** below. The expected enantiomeric excess (ee_{Theo}) is derived as (here for an excess of *S*-isomer):

$$ee_{Theo}(S) = \frac{m(S) - m(R)}{m(S) + m(R)} = \frac{m(S)}{m(S) + m(rac)} \qquad \textbf{(SI-1)}$$

In this equation, the masses take into account the purity of chemicals. In case of *R*-isomer in excess, the ee_{Theo} is determined by interchanging *R* and *S* descriptors.

The enantiopur and racemic drugs were weighted with a AB204 METTLER TOLEDO balance claiming a standard deviation of 0.1 mg. Hence, weighing included an uncertainty u of $\pm$ 0.2 mg for a 95% confidence interval. These uncertainties are propagated as below to provide the total uncertainty ee_{Theo} (*S*) $\pm$ U:

$$U = ee_{Theo}(S)\sqrt{\left(\frac{u}{m(S)}\right)^2 + \frac{2u^2}{(m(S) + m(rac))^2}} \qquad \textbf{(SI-2)}$$

In case of (*R*)-isomer in excess in the scalemic mixture, u is determined by interchanging *R* and *S* stereodescriptors.

Table SI-1: Mesophase composition of Efavirenz samples

***ee*(*S*)** (%)	m_{PBLG} **(DP = 849)** (mg)	$m_{analyte}$ **(*S*)** (mg)	$m_{analyte}$ ***(rac.)*** (mg)	$m_{analyte}$ **(tot.)** (mg)	$m_{solv.}$[b] (mg)	$m_{poly.}/m_{tot}$ (%)
100	113.1	30.3	-	30.3	602.6	15.1
100	101.4	20.1	-	20.1	599.4	14.1
100	102.2	10.9	-	10.9	599.3	14.4
100	102.2	5.8	-	5.8	607.0	14.3
0	99.6	0	20.5	20.5	601.0	13.8
71.5	101.1	22.4	9.0	31.4	600.8	13.8
90.5	103.4	27.5	2.9	30.4	605.7	14.0

Table SI-2: Mesophase composition of Flurbiprofen samples

ee(*R*) (%)	m_{PBLG} **(DP = 849)** (mg)	$m_{analyte}$ **(*R*)** (mg)	$m_{analyte}$ ***rac*** (mg)	$m_{analyte}$ **tot** (mg)	$m_{solv.}$[b] (mg)	$m_{poly.}/m_{tot}$ (%)
0	99.8	-	30.0	30.0	600.1	13.7
0	99.3	-	20.2	20.2	605.8	13.7
0	100.9	-	14.8	14.8	607.3	14.0
0	99.9	-	10.2	10.2	597.2	14.1
0	99.1	-	4.8	4.8	603.2	14.0
9.6	100.5	2.9	27.0	29.9	608.4	13.6
58.2	101.2	18.0	12.8	30.8	599.2	13.8
88.7	102.6	27.0	3.4	30.4	615.2	13.7

III. Assignment of ^{13}C-^{19}F satellites on ^{19}F-{^{1}H} spectra of Flurbiprofen (FLU)

Some weak lines surround the ^{19}F signal of **FLU**. In order to differentiate resonances coming from ^{13}C-^{19}F coupling (satellites) and potential fluorinated impurities (purity = 97%), assignment of satellite lines was achieved by comparing ^{19}F-{^{1}H}, ^{19}F-{^{1}H,^{13}C} and ^{13}C-{^{1}H} spectra recorded on an isotropic sample of **FLU**.

Identification of satellites is non-trivial on ^{19}F spectra as the doublets arising from ^{13}C-^{19}F coupling in ^{13}C-isotopomers (**Figure SI-1a**) is not centered on the ^{19}F chemical shift of the main ^{12}C-isotopomer. Indeed, the substitution of a ^{12}C by a ^{13}C at the vicinity of fluorine modifies the chemical environment sufficiently to vary the value of the ^{19}F δ (isotopic effect). This is clearly seen in **Figure SI-1b**, where the center of the two satellite lines from $|^{1}J_{CF}|$ = 248.6 Hz is shifted by around 0.1 ppm from the main line. In principle, this effect is also perceptible on two distant bonds (isotopomers in green and black in **Figure SI-1a**). This gives rise to two $^{2}J_{CF}$ doublets, the left-hand components of which are covered by the main ^{19}F signal, while the right-hand components are observed and marked by the green and black dots (**Figure SI-1b**). The values of the related $^{2}J_{CF}$ can be measured on a ^{13}C-{^{1}H} spectrum as shown in **Figure SI-2**. The assignment of satellites is then confirmed by a ^{19}F-{^{1}H, ^{13}C} spectrum, in which the two doublets of $|^{1}J_{CF}|$ = 248.6 and $|^{2}J_{CF}|$ = 23.8 Hz are decoupled as highlighted in **Figure SI-1c**. Note that under ^{13}C-decoupling the resulting singlet coming from the doublet of $|^{2}J_{CF}|$ = 13.7 Hz (green isotopomer) is overlapped by the main ^{19}F signal.

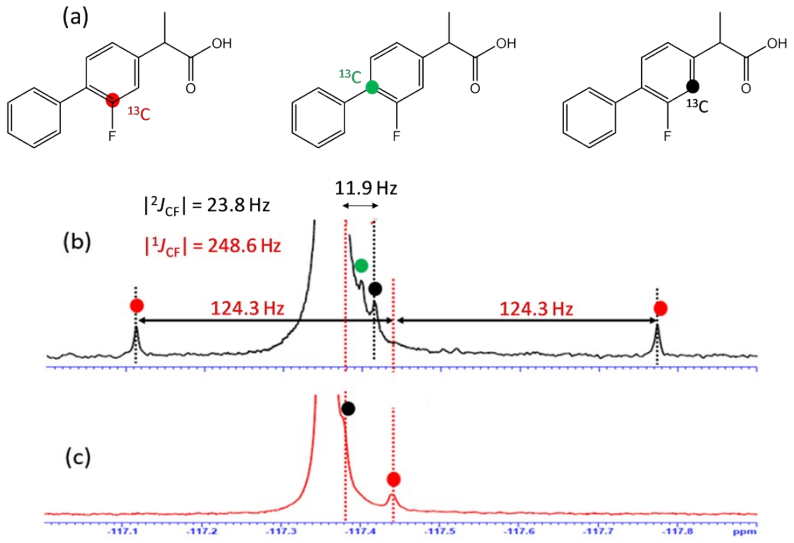

Figure SI-1. The three ^{13}C-isotopomers **(a)** leading to the ^{13}C-^{19}F satellites highlighted on the isotropic ^{19}F-{^{1}H} spectrum **(b)**. The assignment of satellite lines is confirmed by the ^{19}F-{^{1}H, ^{13}C} spectrum **(c)**. Spectra were recorded at 376.4 MHz with a triple resonance QXO NMR probe.

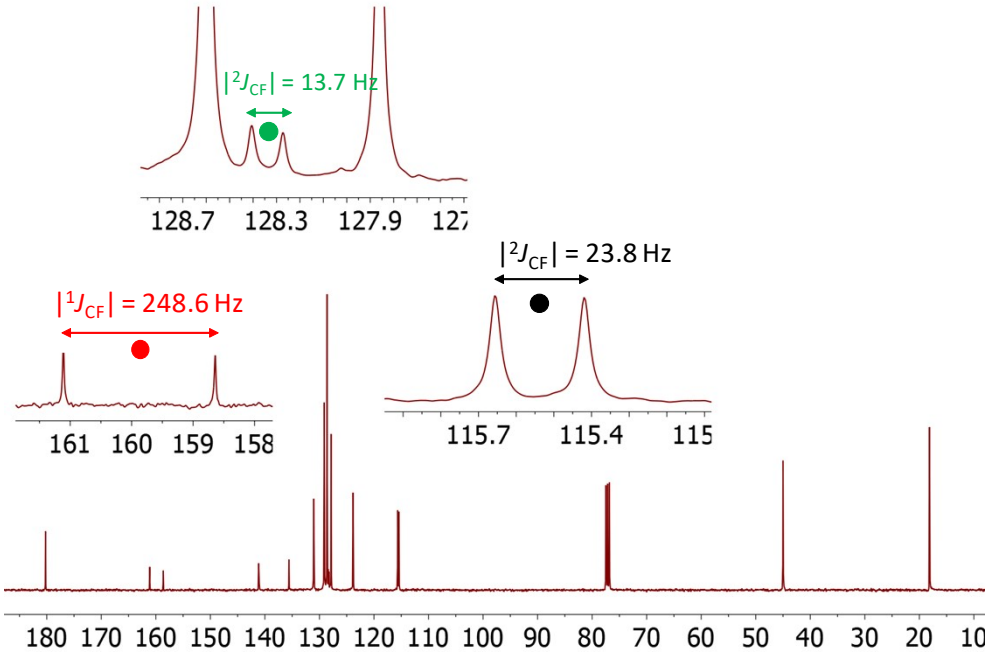

Figure SI-2. 100.1 MHz ^{13}C-{^{1}H} spectrum recorded on an isotropic sample of **FLU** (26.7 mg) in 4.75 h. The color code corresponds to the isotopomers presented in **Figure SI-1a**. Exponential filtering (LB = 3) was applied.

IV. ^{19}F-{^{1}H} spectra of Efavirenz (EFA) in PBLG/$CHCl_3$ with two different *ee*%

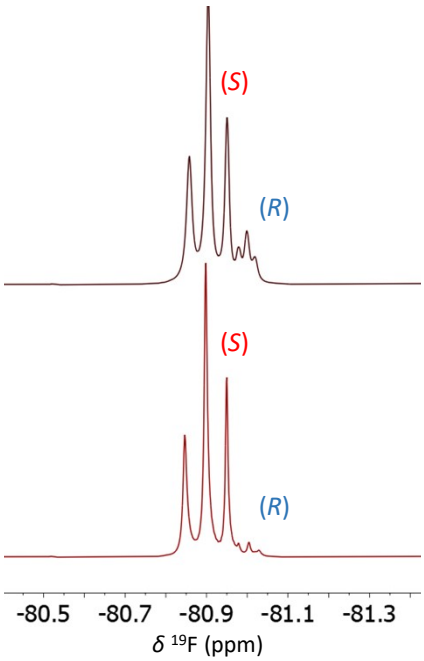

Figure SI-3: 282.4 MHz ^{19}F-{^{1}H} spectra of **EFA** (30 mg) dissolved in PBLG/$CHCl_3$, *ee*(*S*) = 71.5% (top) and *ee*(*S*) = 90.5% (bottom). The spectra were recorded at 297 K with NS = 8. No filtering applied.

V. Effect of temperature on the ^{19}F-{^{1}H} spectrum of Efavirenz (racemic and scalemic mixtures) in PBLG/$CHCl_3$

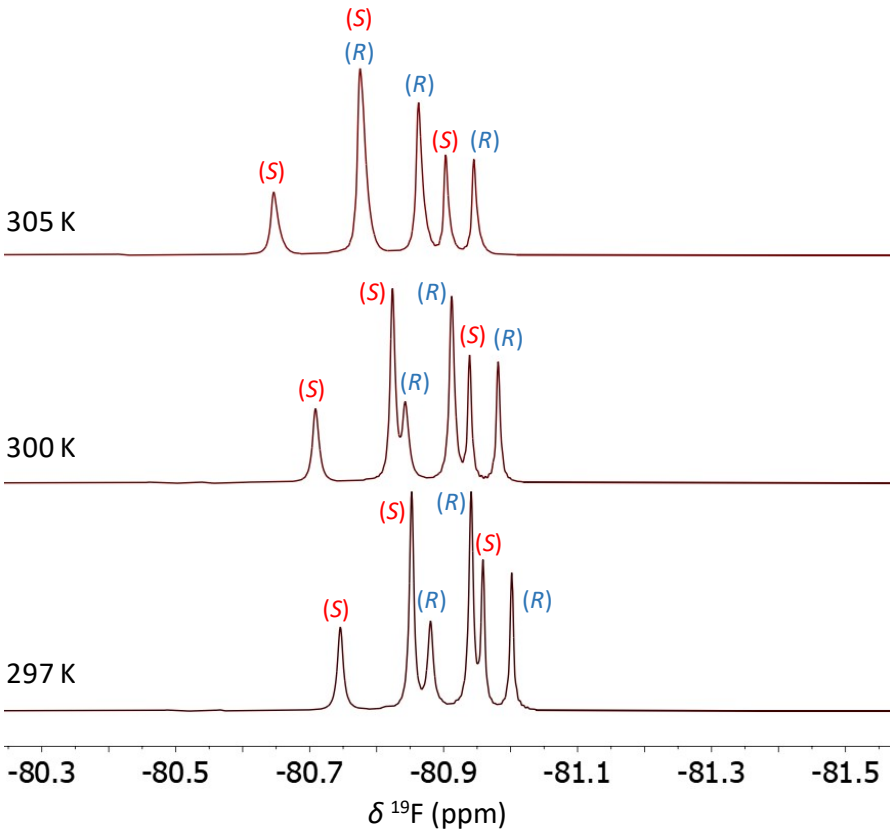

Figure SI-4. 282.4 MHz ^{19}F-{^{1}H} spectra of a racemic sample of **EFA** (20 mg) dissolved in PBLG/$CHCl_3$ recorded at different temperatures: 297 K (bottom), 300 K (middle) and 305 K (top) with NS = 8. No filtering applied.

Table SI-3. Variation of anisotropic parameters for a 30 mg of scalemic sample of EFA (*ee*(*S*) = 90.5%) dissolved in PBLG/$CHCl_3$. These values are related to the ^{19}F-{^{1}H} NMR spectra shown in **Fig. 6**.

Temperature (K)	^{19}F $\lvert\Delta\delta_{aniso}(R,S)\rvert$ (ppm)	$\lvert T_{FF}(S)\rvert$ (Hz)	$\lvert T_{FF}(R)\rvert$ (Hz)	$\lvert\Delta T_{FF}(R,S)\rvert$ (Hz)
300	0.1161	10.5	13.3	2.8
310	0.1051	19.2	1.0	18.2
320	0.0998	26.9	6.2	20.7

VI. Limits of detection (LOD)

After estimations based on the SNR values measured on ^{19}F-{^{1}H} NMR spectra of **FLU** and **EFA** at different concentrations, the LOD values were verified empirically. Here are the ^{19}F-{^{1}H}

experiments performed on **FLU** (at 42 µg/mL, *i.e.,* 0.17 µmol/mL) and **EFA** (at 50 µg/mL, *i.e.,* 0.16 µmol/mL). Bottom spectrum was recorded in the same conditions than for the *ee*% measurement (*i.e.*, NS = 8) and led to SNR > 3. Experiments with NS = 32 (top spectra) confirm the presence of the identified compounds.

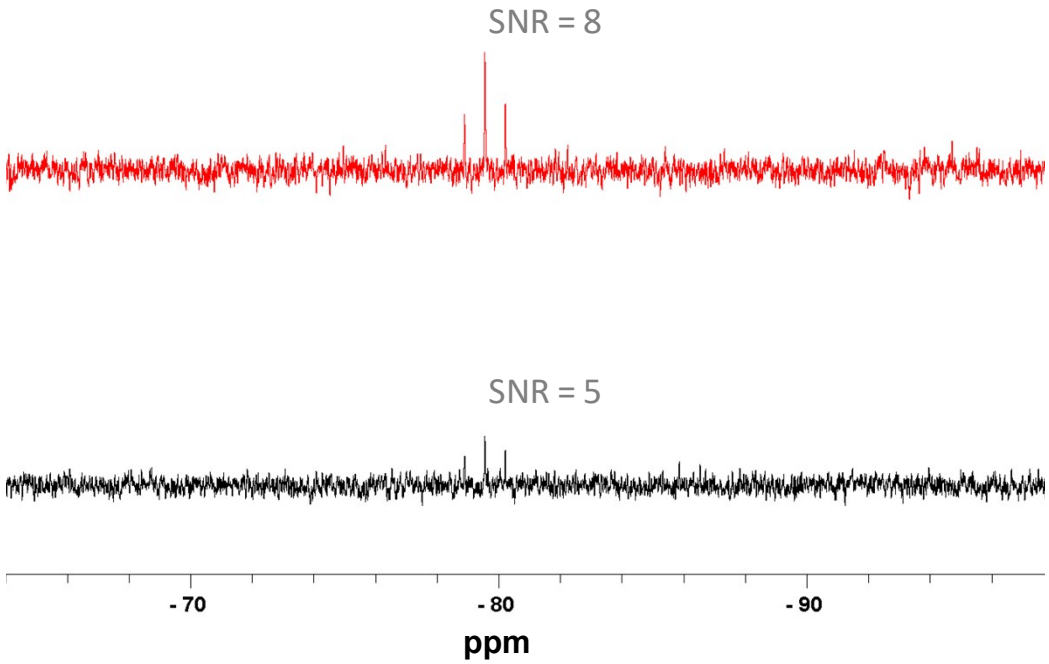

Figure SI-4. 282.4 MHz ^{19}F-{^{1}H} spectra of (*S*)-**EFA** (50 µg/mL, *i.e.,* 0.16 µmol/mL) dissolved in PBLG/$CHCl_3$ recorded at 300 K with NS = 8 (bottom) and NS = 32 (top). The SNR is here measured on the central line of the triplet.

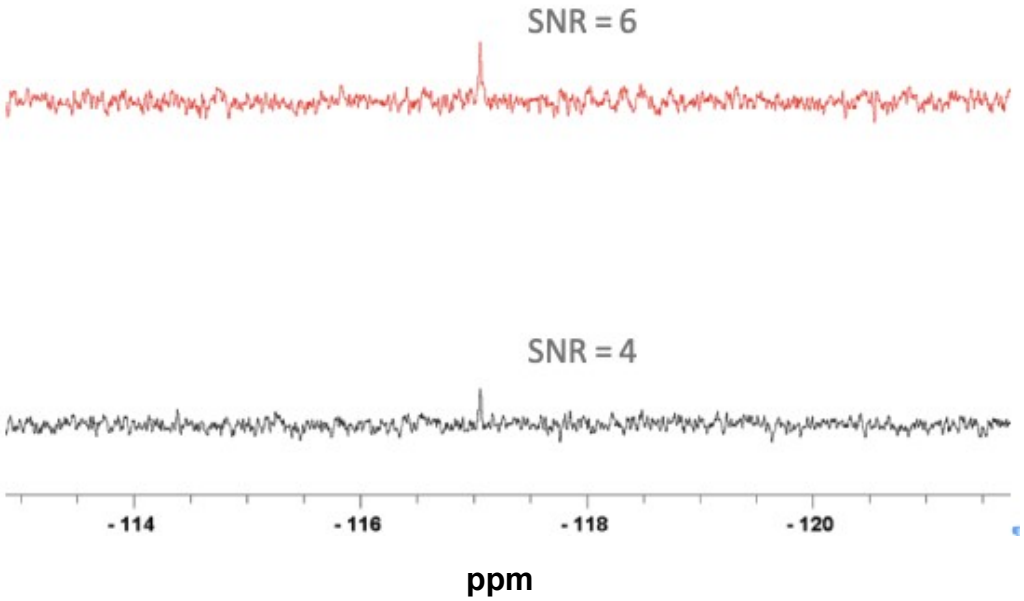

Figure SI-5. 282.4 MHz ^{19}F-{^{1}H} spectra of (*R*)-**FLU** (42 µg/mL, *i.e.*, 0.17 µmol/mL) dissolved in PBLG/$CHCl_3$ recorded at 300 K with NS = 8 (bottom) and NS = 32 (top).

VII. Analysis of a possible self-aggregation of EFA in solution

A possible self-aggregation process occurring in the concentration range used in the case of **EFA** has been monitored by comparing ^{1}H diffusion NMR experiments (^{1}H-DOSY) on two **EFA** isotropic samples (*i.e.,* in $CDCl_3$) and at two concentrations: 0.05 and 0.16 mol.L^{-1}. In practice, any molecular aggregation would lead to an increase of the hydrodynamic radius involving an import shift of the analyte diffusion coefficient.

^{1}H DOSY experiments were performed with bipolar pulse-field gradient stimulated echoes, followed by a LED block to limit discrepancies from eddy currents (Bruker pulse-program "ledbpgp2s"). The subsequent pseudo-2D maps were treated with the Dynamics Center (version 2.8.3 from Bruker) by a univariate processing, in which diffusion coefficient (DC) are extracted by fitting with an adapted Stejskal-Tanner equation. The diffusion coefficient of **EFA**, noted DC(EFA) corresponds to the average DC obtained on five ^{1}H NMR signals of this compound. DC(EFA) is then normalized by the diffusion coefficient of $CHCl_3$: (DC(EFA) / DC($CHCl_3$) measured with the same DOSY experiment to circumvent any bias between the two diffusion experiments (convection, slight viscosity variation with concentration).

The superimposed DOSY maps are shown below in **Figure SI-6**. The normalized DC(**EFA**) value varies from 0.368 to 0.372 at 0.05 and 0.16 mol.L^{-1}, respectively (see **Table SI-4**). Such a tiny residual variation of **EFA** diffusion rules out a concentration dependent aggregation.

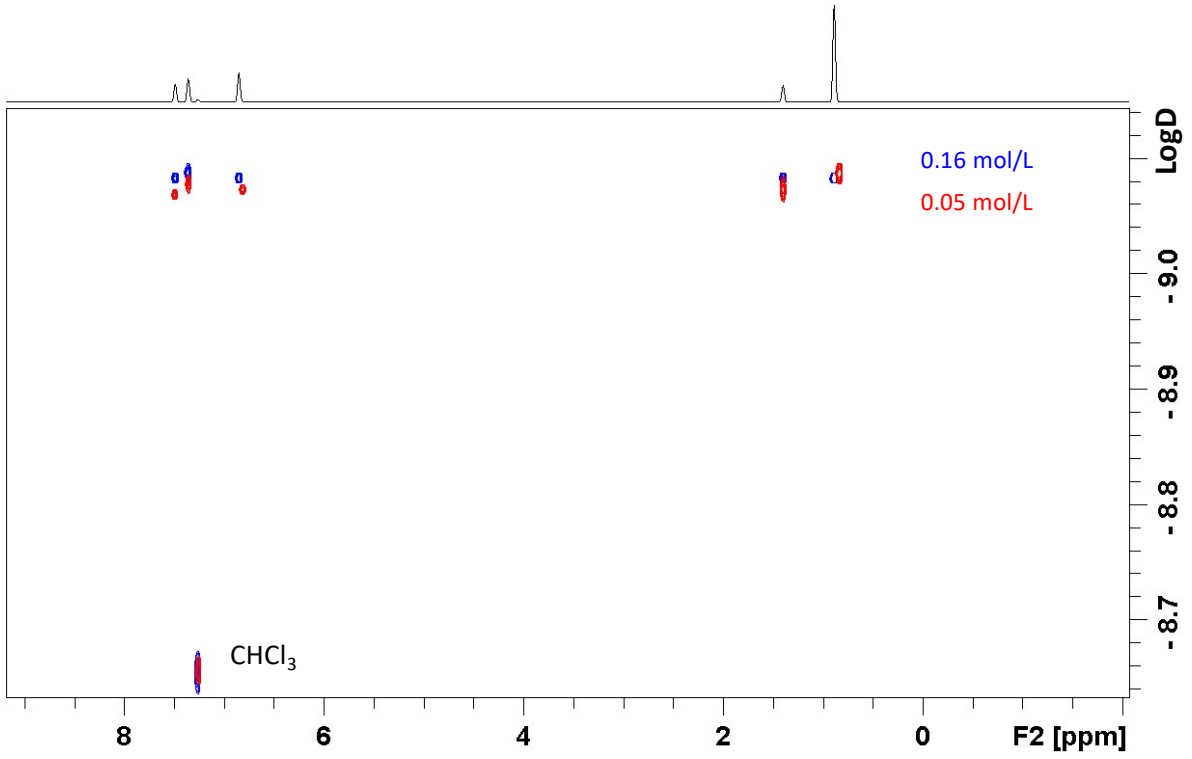

Figure SI-6. Superimposed (400 MHz) ^{1}H DOSY 2D map of EFA in $CDCl_3$ recorded at 0.05 mol/L (red) and 0.16 mol/L (blue) at 300 K. The two spectra are manually aligned with the $CHCl_3$ diffusion coefficient along the vertical dimension. Experimental parameters are given in the **Table SI-4**.

Table SI-4: Absolute and normalized diffusion coefficients measured on two different concentrations in EFA in $CDCl_3$ at 300 K. Diffusion curves were obtained with a diffusion delay of Δ = 40 ms, and bipolar pulse-field-gradient of δ = 3 ms incremented via a 16-step-ramp from 2 to 85% of the maximum gradient strength (*i.e.*, 53 G/cm). 8 scans were accumulated per increment.

[EFA] (mol/L)	**DC (EFA)[a]** (10^{-10} m^2/s)	**DC ($CHCl_3$)** (10^{-10} m^2/s)	**Normalized DC(EFA)**
0.05	8.160	21.9	**0.368**
0.16	7.210	19.6	**0.372**

[a]: Average of DC values extracted from five ^1H NMR signals of EFA

VIII. Representative ^2H NMR spectrum of the mesophase

A representative ^2H NMR spectrum recorded on PBLG-based liquid crystal containing the API is presented in **Figure SI-7**. This anisotropic ^2H spectrum shows a quadrupolar doublet for the chloroform signal with a residual quadrupolar coupling of $|\Delta\nu_Q|$ = 679 Hz and a linewidth of 2.8 Hz at half-maximum (linewidth measurement without filtering). The doublet pattern and the symmetry between the two components highlight the nematic property of the mesophase as well as its spatial uniformity within the sensitive volume of the NMR probe. It should also be noted that there are no isotropic ^2H signal visible.

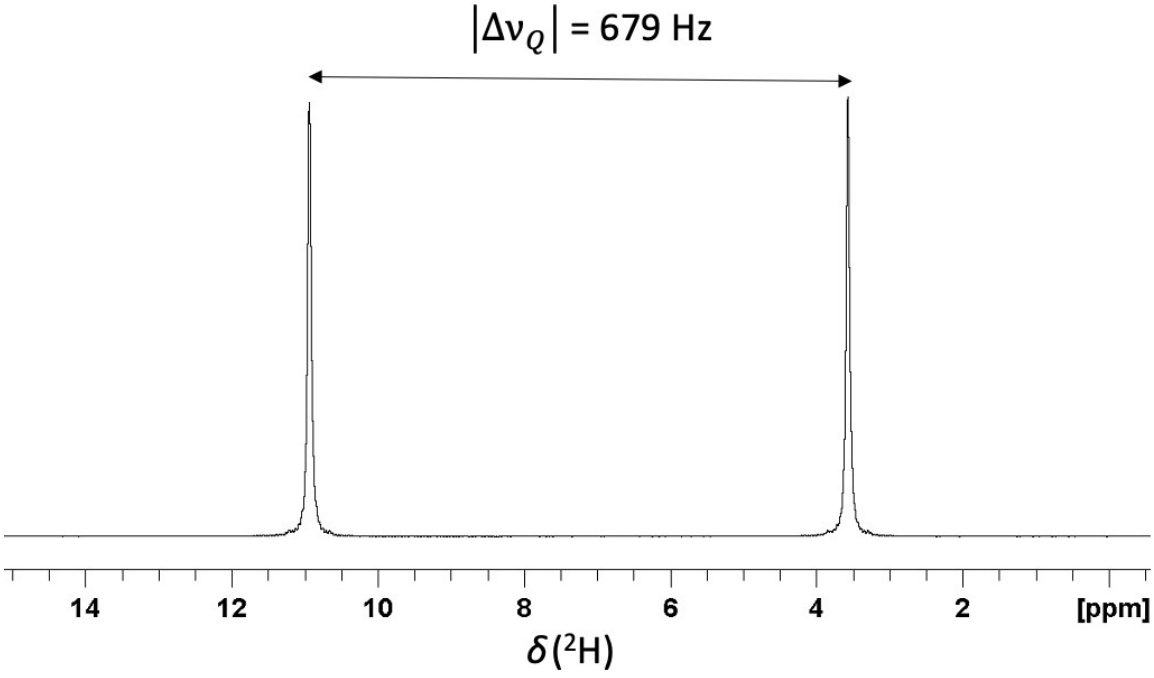

Figure SI-7. 92.1 MHz ^2H spectrum of (*S*)-**EFA** (11 mg), dissolved in a PBLG-based liquid crystal (102 mg) with a mixture of $CHCl_3/CDCl_3$ (480/120 mg) as organic co-solvent, recorded in eight scans at 300 K with a $^1H/^{13}C/^2H$ cryogenic probe. The spectrum is displayed here after an exponential filtering (LB = 3 Hz).