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Exploring the Spectral Enantiodiscrimination Potential of DNA-based Orienting Medium using Deuterium NMR Spectroscopy

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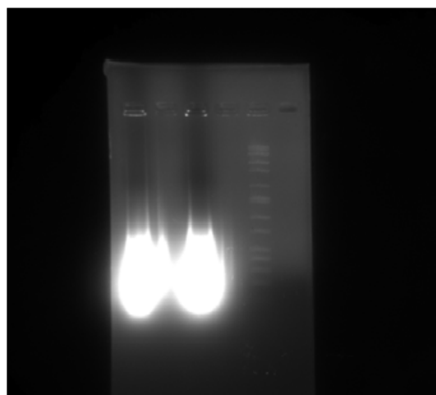
Additional details on preparation of DNA-based oriented samples

15 For the sample preparation, high molecular weight DNA (sigma-aldrich) from Salmon sperm testes is dissolved in autoclaved water as well as sonicated on ice between 1.5 and 2.5 hrs with 20s pulses and 10s of gap between pulses. The size distribution of sonicated DNA double helices is determined by 1% agarose gel electrophoresis in TAE (Tris-acetate EDTA) buffer with DNA marker (Novagen) as shown in Fig. SI-1. The size of fragmented DNA was found to be 150-300 base pairs and DNA sample was lyophilized overnight. Note that Sodium Cacodylate and Sodium azide are used in buffer 20 to inhibit bacterial growth, while Sodium EDTA was used to scavenge multivalent cations and inhibit DNA degradation by trace nucleases.

The buffer preparation is made as follows : Since small quantities of the compounds are to be weighted, for ease of preparation, 20 ml of buffer was prepared using 10.33 mg (9 mM) of Nacl, 1.6 mg (0.5 mM) of As(Nao(CH_3)$_2$), 0.39 mg (0.3 mM) of NaN_3 and 0.66 mg (0.1 mM) of Na_2EDTA. All these weighed compounds are added in 15 ml H_2O and then the 25 pH is adjusted at 6.5 with HCl or NaoH. Further another 5 ml of H_2O was added to make it 20 ml. Chemical structures of buffer components are reported in Fig. SI-2.

(a)



(b)

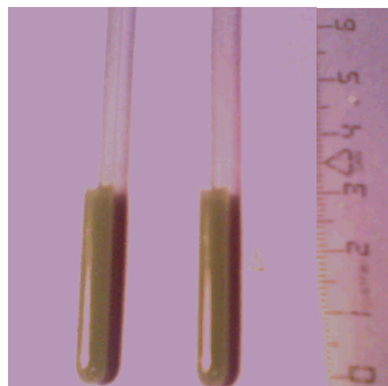


Fig. SI-1 (a) Distribution observed of different DNA fragments using electrophoresis on 1% Agarose gel. (b) Examples of DNA-based oriented (5-mm) NMR samples using the sample preparation described above and in the main text.

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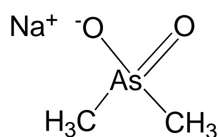

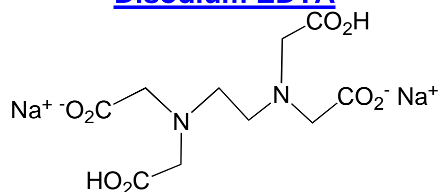

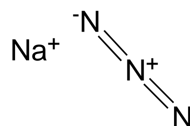

Fig. SI-2 Chemical structure of components of buffer used.

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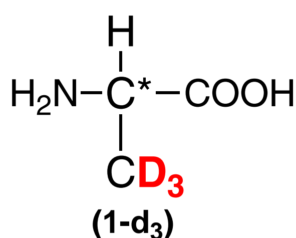
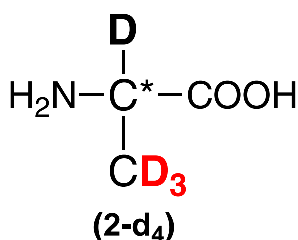
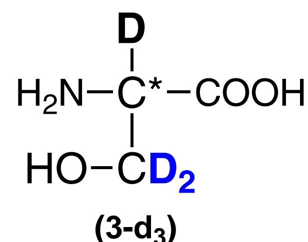
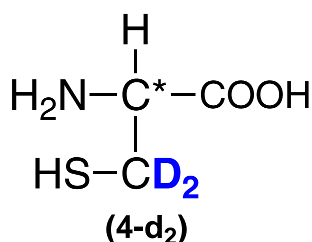
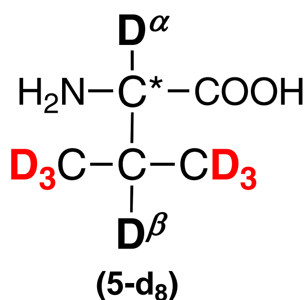
Structures and details on (pro)chiral amino acid studied

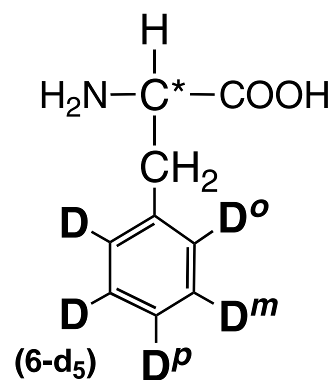


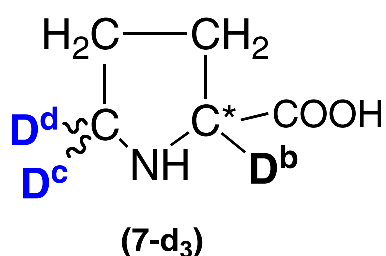


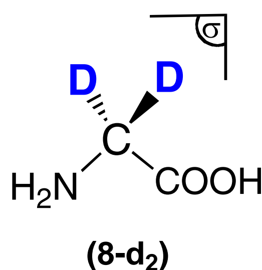












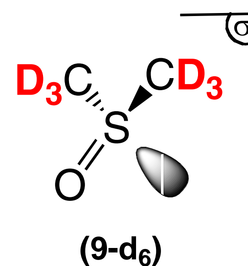


Fig. SI-3 Structures of seven deuterated AAs (**1** to **8**) investigated and DMSO (**9**). For clarity, the CD_3, CD_2 and CD
40 groups are colored in red, blue and black, respectively. **8** and **9** are prochiral molecules of C_s symmetry in average.

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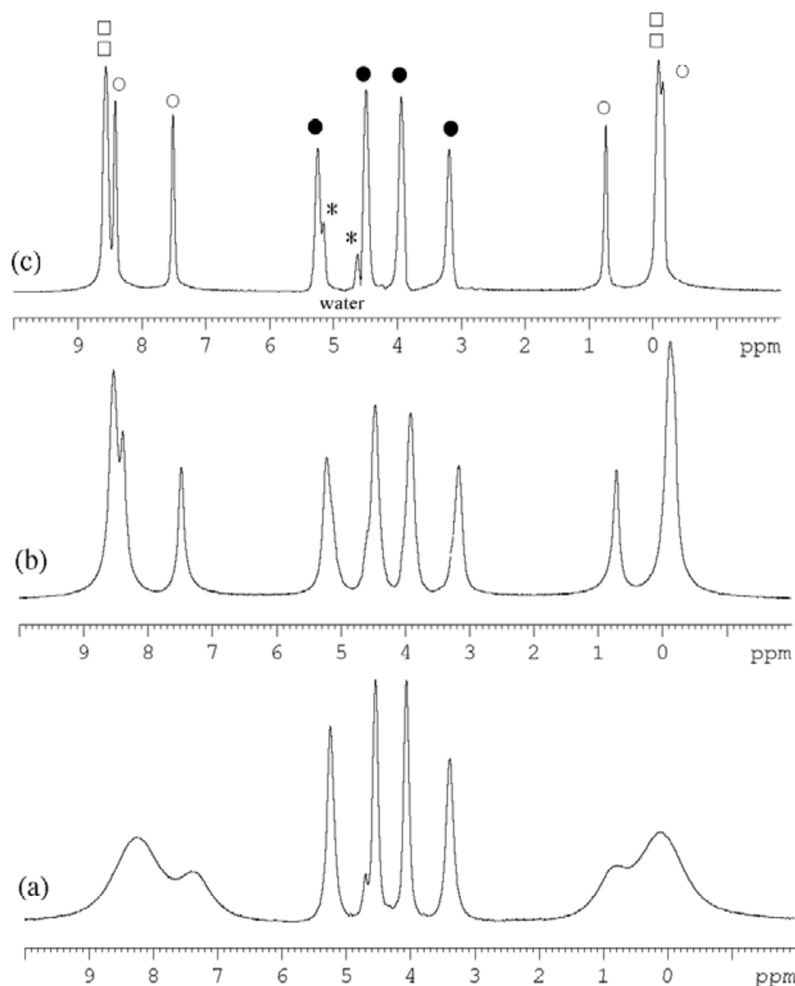
45 **Additional details or comments on deuterium 1D/2D-NMR experiments**

From NMR viewpoint, the orientation of DNA in the magnetic field is rather slow and can take one or two hours (at 14.1 T) to reach an homogeneous and uniform orientation, leading to optimal spectral quality. Recentrifugation of the NMR sample (repeated recycling, up and bottom) is also advised if
50 the sample homogeneity is unsufficient and provides low or medium resolution deuterium spectra. The evolution of mesophase inside the magnet can be monitored by the deuterium quadrupolar doublet of the solute. A typical example of spectral evolution quality is shown in Figure SI.4.



55 **Fig. SI-4** (a and b) Evolution of the sample homogeneity observed on the 92.1 MHz ^{2}H-{^{1}H} 1D spectrum of **(rac)-3-d$_3$** in DNA at 325 K. (a) Spectrum recorded while the sample was freshly prepared. (b) ^{2}H-{^{1}H} 1D spectrum recorded after numerous cycles of sample centrifugation (up/bottom) to homogeneize the DNA mesophase. Both 1D spectra are recorded by adding 128 scans. (c) Gaussian filtered ^{2}H-{^{1}H} 1D spectrum of **(rac)-3-d$_3$**. Signals marked by an asterisk correspond to HOD.

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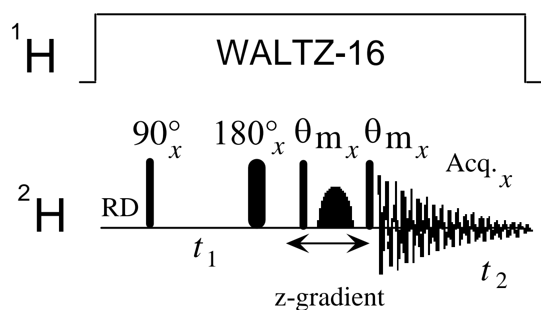
Table SI-1. Sample data, quadrupolar splittings and DOEs for all deuterated AAs investigated

Solute	**(rac)-1-d_3** (Alanine)	**(rac)-2-d_4** (Alanine)	**(rac)-3-d_3** (Serine)	**(rac)-4-d_2** (Cysteine)	**(rac)-5-d_8** (Valine)	**(rac)-6-d_5** (Phenylalanine)	**(*ee*)-7-d_3**[c] (Proline)	**8-d_2** (Glycine)
m /mg[a]	12	10	11	5	7	3	4	5
T /K	287	300	325	305	330	315	305	305
$\lvert\Delta\nu_Q$(HOD)$\rvert$ /Hz[b]	56	31	49	56	50	46	47	51
$\lvert\Delta\nu_Q$ (CD_3)$\rvert$ /Hz ($\Delta\Delta\nu_Q$ / DOE)	104 / 299(*L*) (195 / 0.97)	108 / 250 (142 / 0.79)	-	-	86 / 86 55 / 127 (72 / 0.79)	-	-	-
$\lvert\Delta\nu_Q$ (CD_2)$\rvert$ /Hz ($\Delta\Delta\nu_Q$ / DOE)	-	-	50 / 190 (140 / 1.16) 796 / 796	116 / 317 (201 / 0.92) 545 / 883 (338 / 0.47)	-	-	165 / 314(*L*) (149 / 0.62) 486 / 781(*L*) (295 / 0.46)	475 / 980 (505 / 0.69)
$\lvert\Delta\nu_Q$ (CD)$\rvert$ /Hz ($\Delta\Delta\nu_Q$ / DOE)	-	103 / 210 (107 / 0.68)	624 / 729 (105 / 0.16)	-	716 / 910 (α) (194 / 0.24) 373 / 550 (β) (177 / 0.38)	511 / 677 (*o*) (166 / 0.28) 515 / 674 (*m*) (159 / 0.27) 633 / 777 (*p*) (144 / 0.20)	591 / 660 (*D*) (69 / 0.11)	-

[a]Error : 1 mg. [b]NAD signal of HOD. [c]Sample enriched in *D*-isomer (*ee* = 40%).

I) Deuterium 1D/2D NMR results for 1–d_3 and 2–d_4 (Alanine)

(a)



(b)

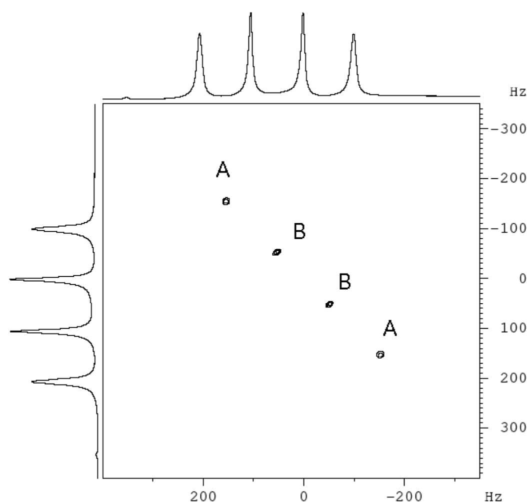


Fig. SI-5 (a) *Q*-COSY Fz sequence used. (b) Symmetrized 92.1 MHz ^{2}H-{^{1}H} *Q*-COSY Fz 2D spectrum of (***rac***)**-1-d_3** in DAN oriented solution recorded at 285 K. 2D matrix made of 1700 (t_2) * 256 (t_1) data points with NS = 4 scans, no exponential filtering. Experimental time: 20 min.

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Table SI-2. Spectral data of (***rac***)-**1-d$_3$** *versus* Te (/K) (step of 5 K)

Temp. / K	$\Delta\nu_Q$(HOD) / Hz	$\Delta\nu_{1/2}$ (HOD) / Hz	T_1 (HOD) / ms	$\Delta\nu_Q$ (*L*) /Hz	$\Delta\nu_{1/2}$ (*L*) / Hz	T_1 (*L-ala*) aniso / ms	$\Delta\nu_Q$ (*D*) / Hz	$\Delta\nu_{1/2}$ (*D*) / Hz	T_1 (*D-ala*) aniso /ms	$\lvert\Delta T_1\rvert$ aniso / ms	$\Delta\nu_{1/2}$ (iso) / Hz	T_1(ala) (iso) / ms
287	56.	6	194	299	10	150	104	6	150	< 1	-	-
290	57	5	211	297	9	158	106	6	160	1	-	-
295	57	8	256	292	10	181	107	6	181	< 1	-	-
300	57	7	294	287	9.	201	108	6.	201	< 1	-	-
305	57.	6.	340	281	9	223	109	6.	222	1	-	-
310	57	8	397	273	9	249	108	6.	251	2	- [a]	-
315	55	8	414	266	9	273	108	7	275	1	36	ND
320	56	8	423	260	10	298	106	7	301	3	23	324
325	57	9	445	257	10	320	106	7	322	2	20	330
330	58.	9	469	251	10	353	105	6	353	< 1	18	357
335	58	10	495	247	11	389	104	6	389	< 1	14	396
340	60.	10	530	241	12	412	103	6	413	1	9	427
345	59	11	561	236	12.	436	102	7	434	2	12	458

85 [a]Very broad line observed.

All T_1 values (in ms) reported in Table are the average of values measured both components of doublet and three ^{2}H-{^{1}H} inversion-recovery 1D experiments.

$\Delta\nu_{1/2}$ (at half height) values reported here is the average value measured on the two components of doublet.

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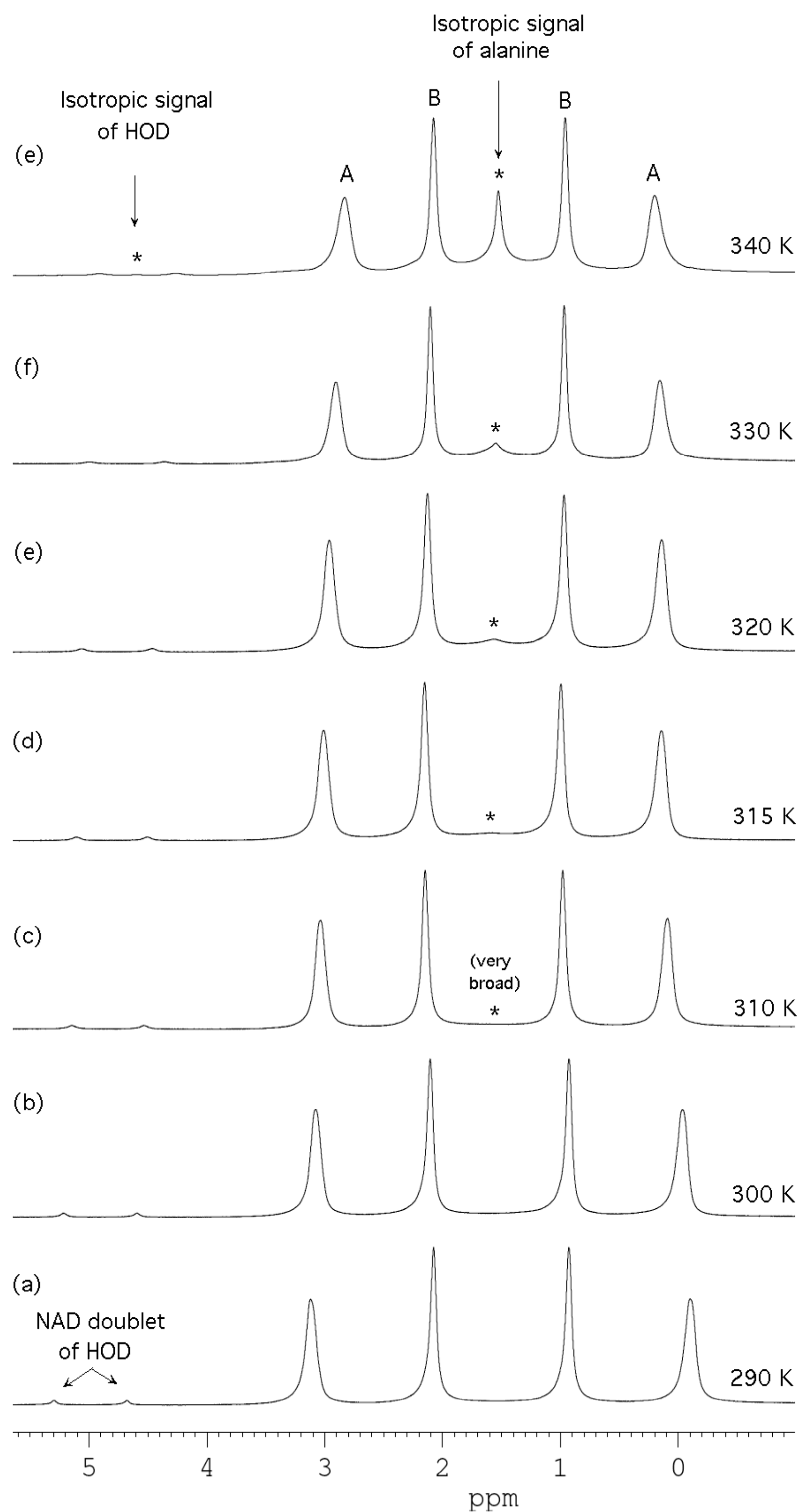

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Fig. SI-6 Evolution of 92.1 MHz ^{2}H-{^{1}H} 1D spectra (the spectra of complete series is not shown) of **(*rac*)-1-d$_3$** solution *versus* Temperature (287 to 345 K). 64 scans were added, no exponential filtering used prior to FT. Above 315K, isotopic part (central signal) starts to be visible.

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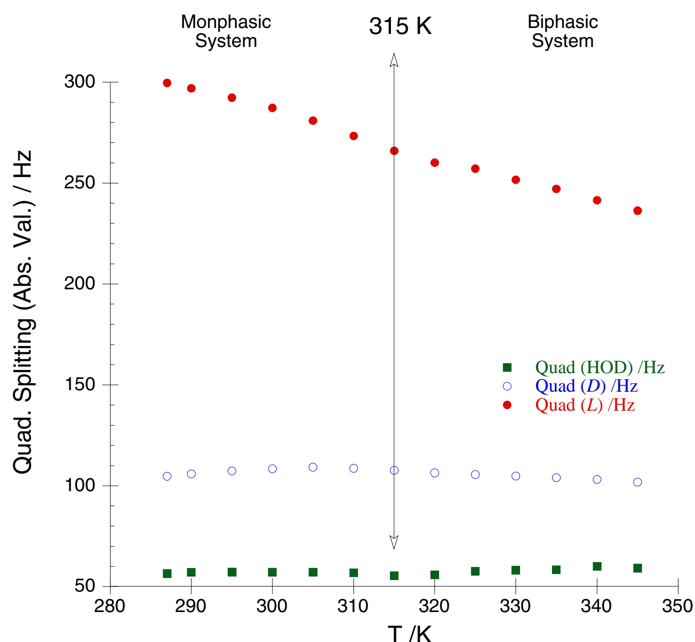

Fig. SI-7 Variation of $|\Delta\nu_Q(L/D)|$ in Hz of **(*rac*)-1-d**$_3$ *versus* T (287 – 345 K) as well as $|\Delta\nu_Q(\mathrm{HOD})|$. For *L* and *D* isomers, the variation of $|\Delta\nu_Q(L/D)|$ is monotonous and rather linear in range of T explored. The difference of slope could suggest a difference of affinities between the two isomers and the DNA fibers (to be confirmed).

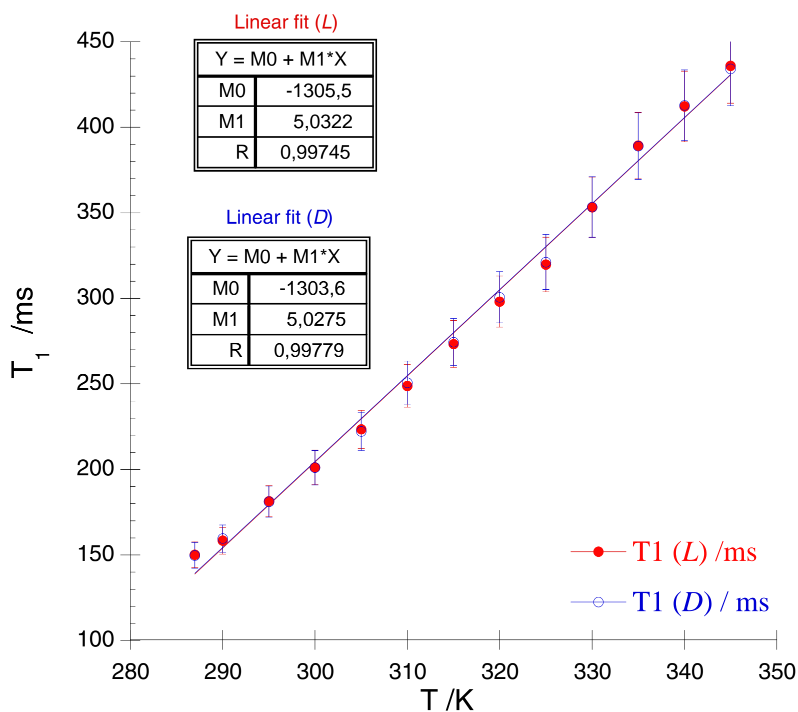

Fig. SI-8 Variation of $T_1(^2H)$ (*L/D*) of **(rac)-1-d$_3$** *vs.* T. (287 – 345 K) obtained from three inversion-recovery 1D experiments.

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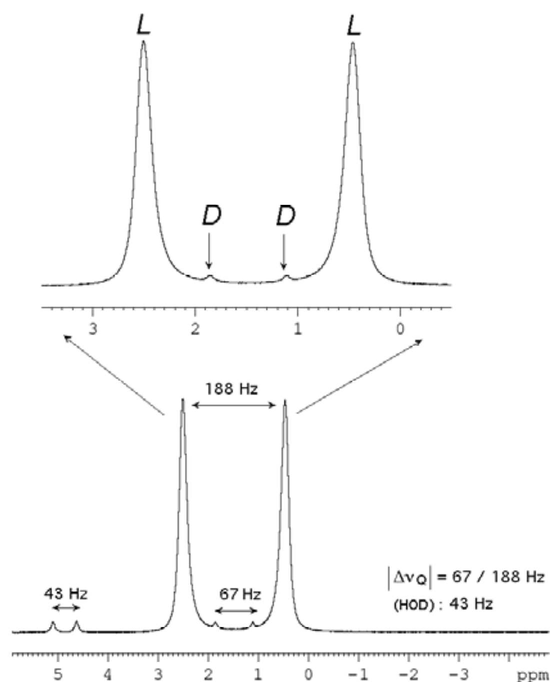

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Fig. SI-9 (bottom) 92.1 MHz ^{2}H-{^{1}H} 1D spectrum of ***(L)*-1-d$_3$** (solute : 12.5 mg) in DNA oriented solution recorded at 305 K (NS = 512). (top) Expansion of the ^{2}H-{^{1}H} 1D spectrum centered on the alanine signals. Note the compound has been sold as "enantiopur". Apparently, the ^{2}H-{^{1}H} 1D spectrum shows some traces of *D*-enantiomer (inner quadrupolar doublet).

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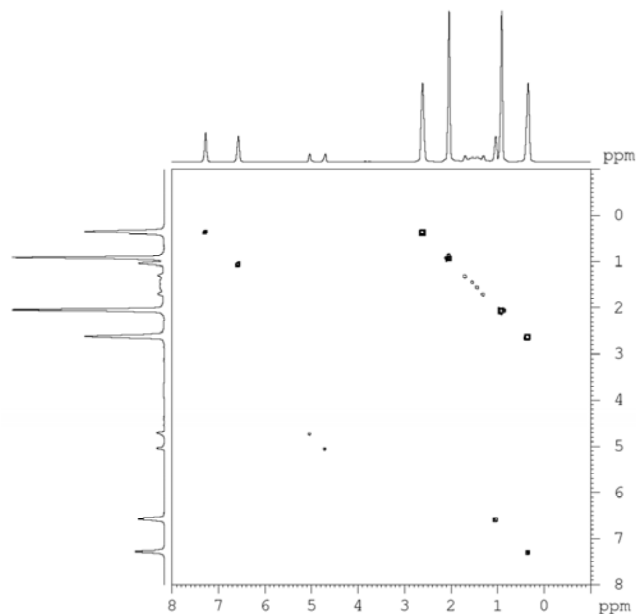
(a)



(b)

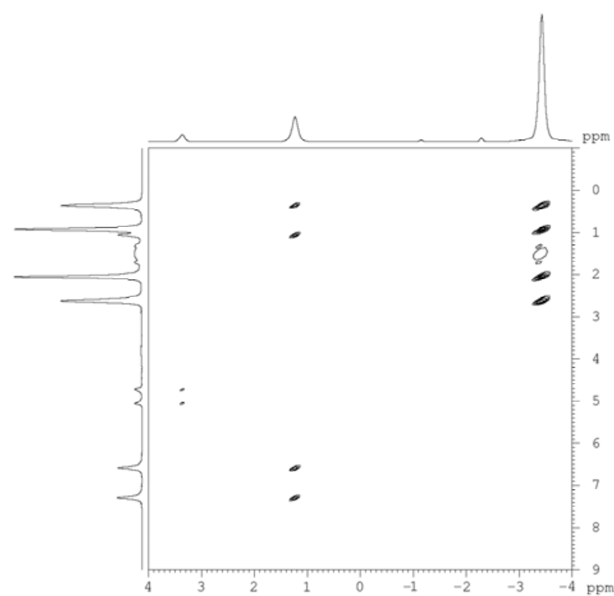


Fig. SI-10 (a) Symmetrized 92.1 MHz ^{2}H-{^{1}H} *Q*-COSY Fz 2D spectrum of **(*rac*)-2-d$_4$** in DNA at 300 K. The 2D matrix was recorded with 1700 * 512 data points (8 scans per FID). (b) Tilted 2D map plotted at high-intensity level.

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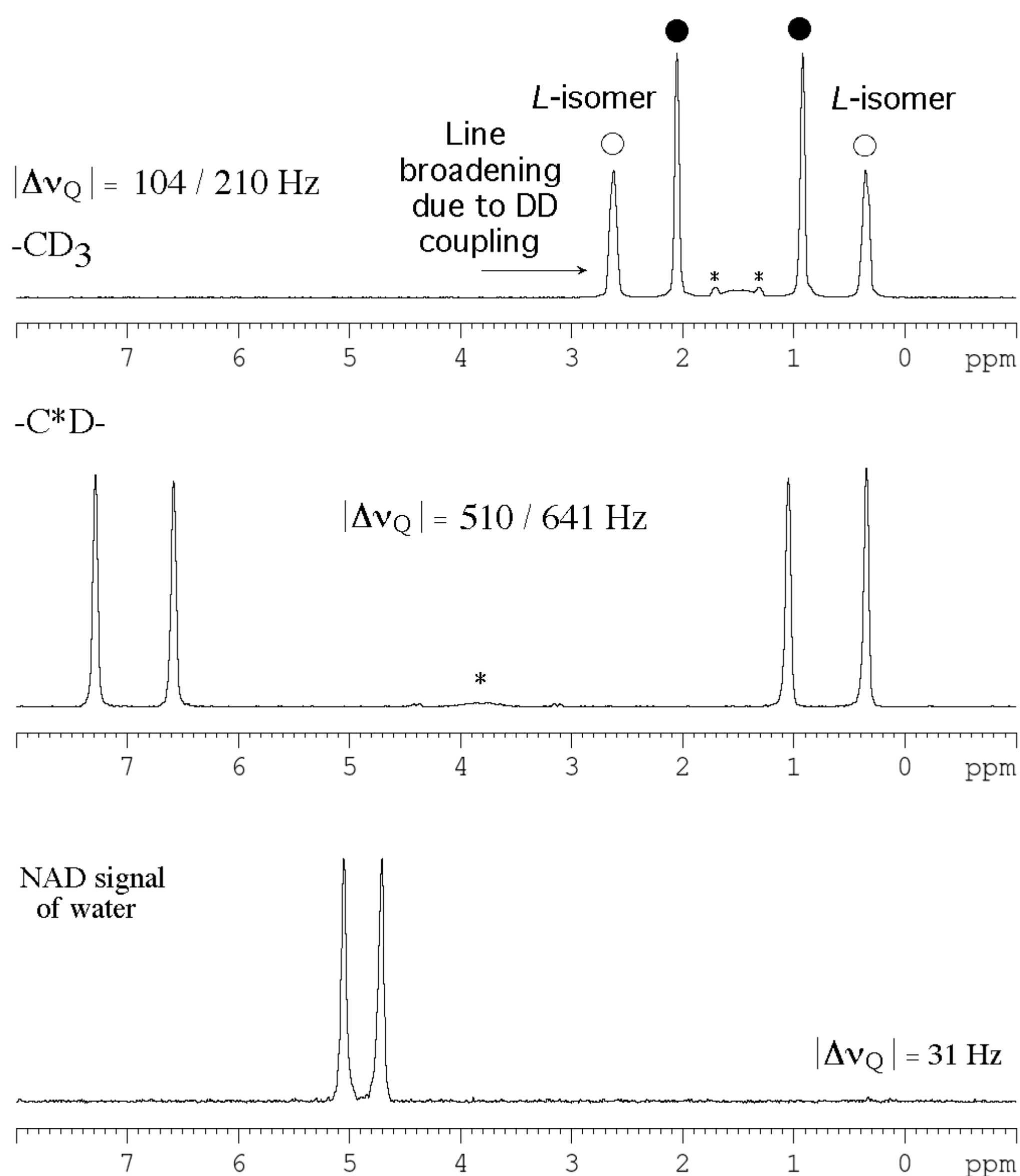

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Fig. SI-11 1D subspectra of exctracted from 92.1 MHz ^{2}H-{^{1}H} *Q*-COSY Fz map of **(*rac*)-2-d$_4$** in DNA at 300 K. For each sub-spectra are reported the quadrupolar splittings of *D*/*L* enantiomers and the HOD as well. The difference of peak intensity for the two enantiomers on the methyl group originates from the non resolved geminal ^{2}H-^{2}H dipolar couplings that broadening peaks. The stereochemical assignment (*L*/*D*) of doublets associated to the methyl group results from the
150 comparison with the previous results obtained for **(*ee*)-1-d$_3$**.

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II) Deuterium 1D/2D NMR results for 3–d3 (Serine)

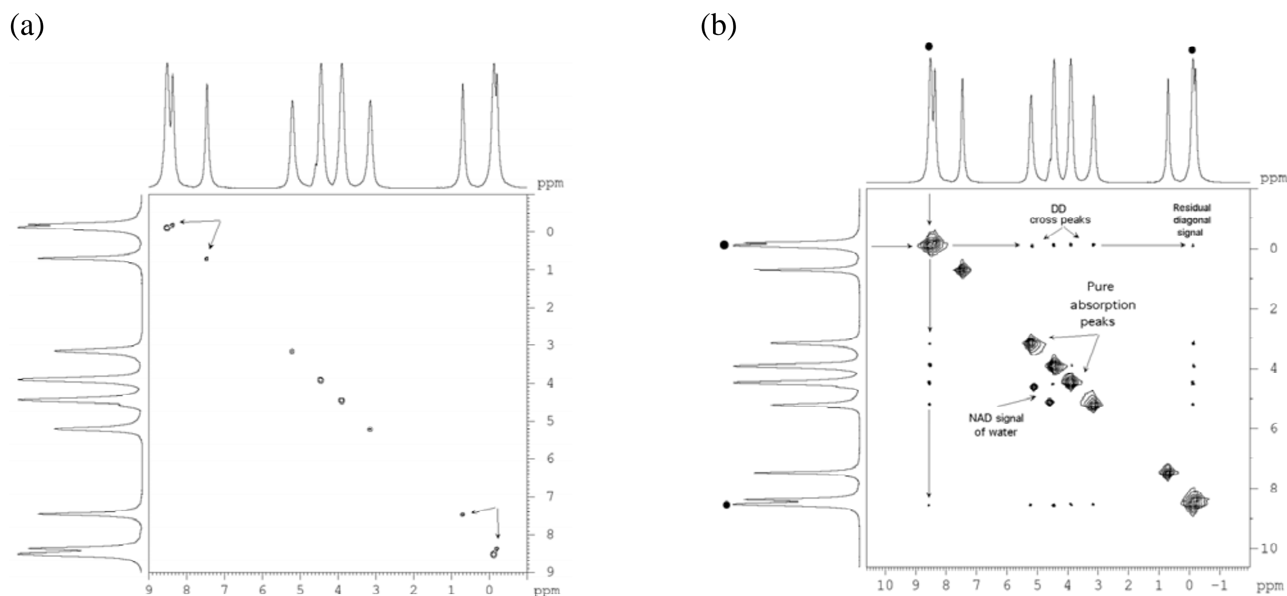

Fig. SI-12 (a) Symmetrized 92.1 MHz ^{2}H-{^{1}H} *Q*-COSY Fz 2D spectrum of **(*rac*)-3-d$_3$** at 325 K. (a) map plotted at high intensity level. (b) Map plotted at low h intensity level. The low intensity peaks visible on the 2D map correspond to ^{2}H-^{2}H peak correlations (due to geminal ^{2}H-^{2}H dipolar and scalar coupling). The ^{2}H-^{2}H correlation peaks allow to pair all doublets belonging to the same enantiomer.

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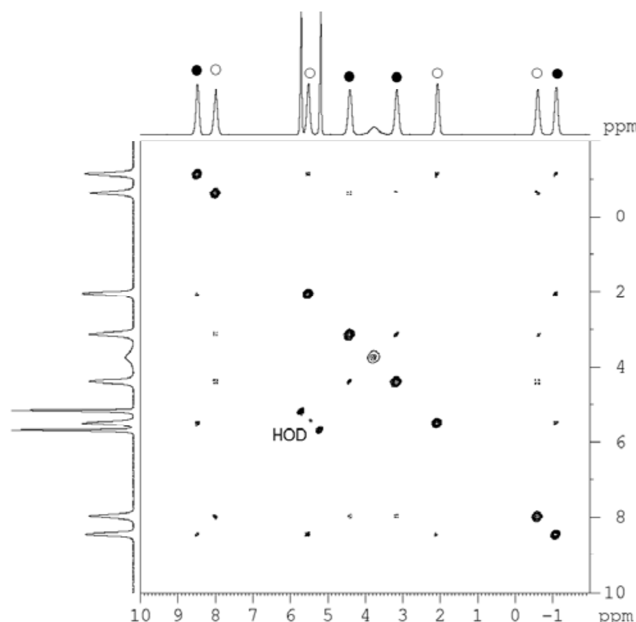
III) Deuterium 1D/2D NMR results for 4–d2 (Cysteine)



170 **Fig. SI-13** Symmetrized 92.1 MHz ^{2}H-{^{1}H} *Q*-COSY Fz 2D spectrum of **(*rac*)-4-d$_2$** at 305 K. The ^{2}H-^{2}H dipolar correlations between the two geminal enantiotopic C-D directions are detected, here. The ^{2}H-^{2}H correlation peaks allow to pair up the signals belonging to the same enantiomer (see symbols on the F_2 projection).

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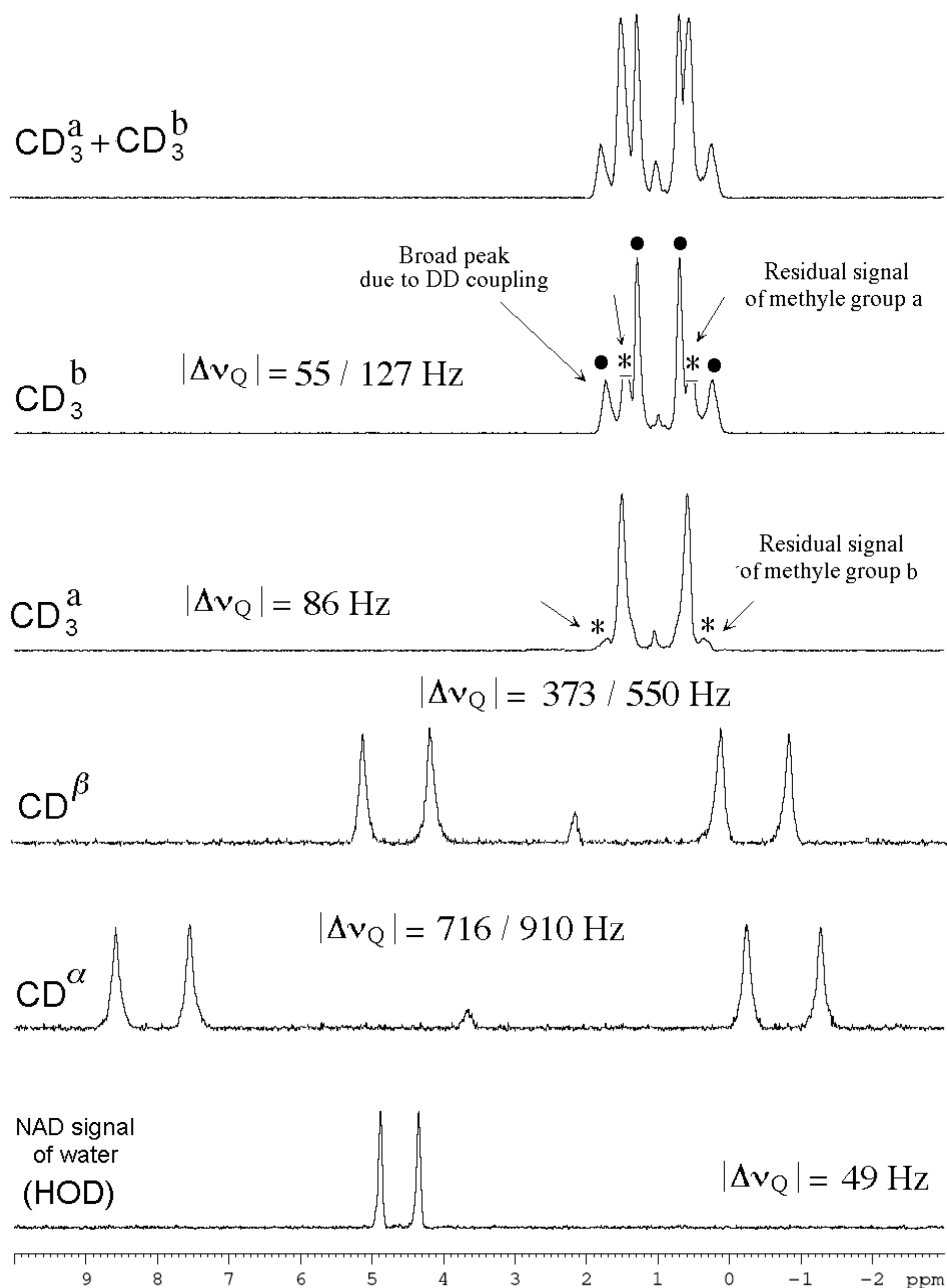
IV) Deuterium 1D/2D NMR results for 5–d_8 (Valine)



180 **Fig. SI-14** 1D subspectra of exctracted from 92.1 MHz ^{2}H-{^{1}H} *Q*-COSY Fz map of **(*rac*)-5-d_8** in DNA at 330 K. For each sub-spectra are reported the quadrupolar splittings of *D*/*L* enantiomers and the HOD as well. The difference of peak intensity for the two enantiomers for one the diastereotopic methyl groups probably originated from the non-resolved geminal ^{2}H-^{2}H dipolar couplings, thus increasing significantly the linewidths. As seen, only one of both diastereotopic methyl groups (noted b) shows spectral enantiodiscrimination.

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V) Deuterium 1D/2D NMR results for 6–d_5 (Phenylalanine)

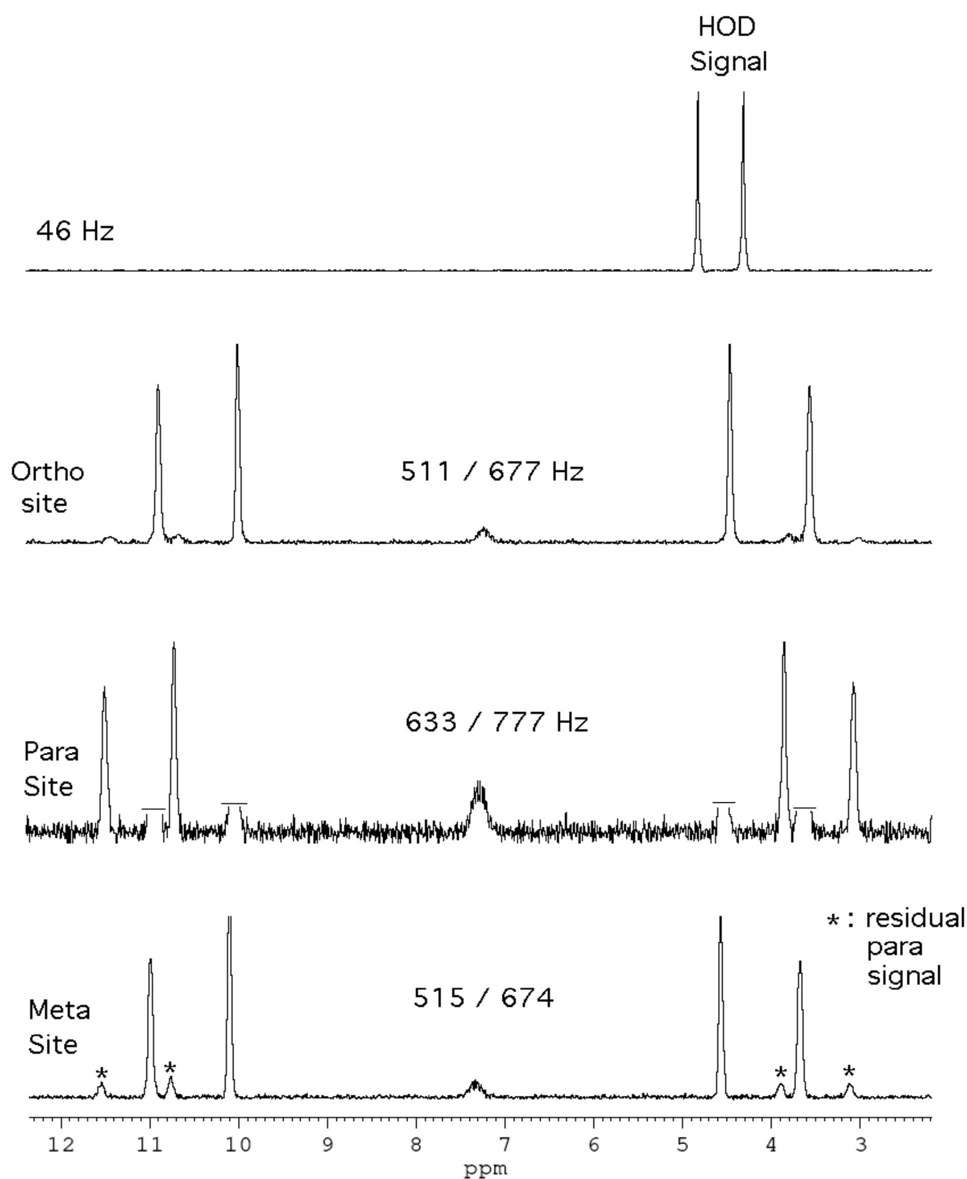

Fig. SI-15 1D subspectra of extracted from 92.1 MHz ^{2}H-{^{1}H} *Q*-COSY Fz map of **(*rac*)-6-d_5** in DNA at 315 K. For 195 each sub-spectra are reported the quadrupolar splittings of *D*/*L* enantiomers and HOD as well. All aromatic inequivalent deuterium sites show a spectral enantiodiscrimination. The broad signals observed in the aromatic region located at the middle of doublets (but not for HOD) correspond to no solubilised solute (phenylalanine is a very low soluble compound).

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VI) Deuterium 1D/2D NMR results for 7–d_3 (Proline)

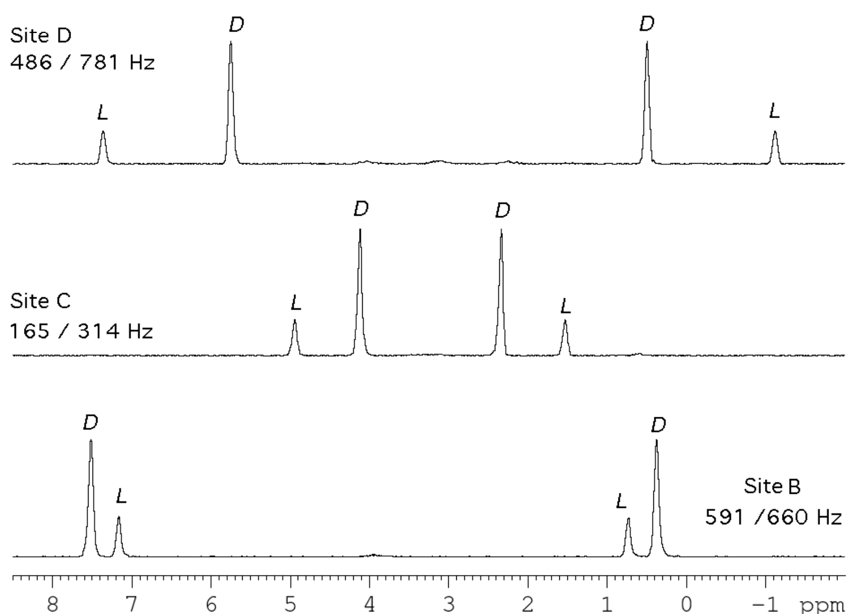

Fig. SI-16 1D subspectra of exctracted from 92.1 MHz ^{2}H-{^{1}H} *Q*-COSY Fz map of **(*ee*)-7-d$_3$** in DNA at 305 K. The
210 sample is enriched in *D* enantiomer. For each sub-spectra are reported the quadrupolar splittings of *L/D*-enantiomers and HOD as well. The three inequivalent deuterium sites show a spectral enantiodiscrimination.

VII) Deuterium 1D/2D NMR results for 8–d_2 (Glycine)

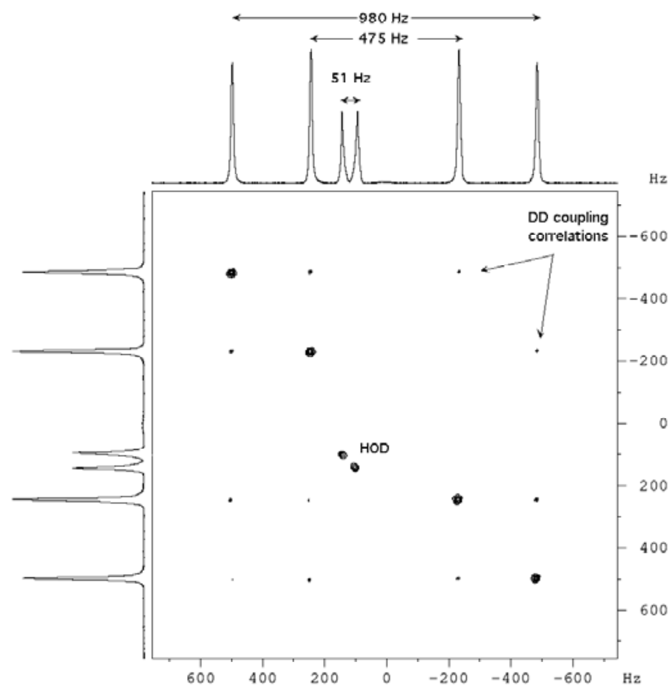

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Fig. SI-17 Symmetrized 92.1 MHz ^{2}H-{^{1}H} *Q*-COSY Fz 2D spectrum of **8-d$_2$** at 305 K. The ^{2}H-^{2}H dipolar correlations (low intensity peaks) between the two geminal enantiotopic C-D directions are detected, here.

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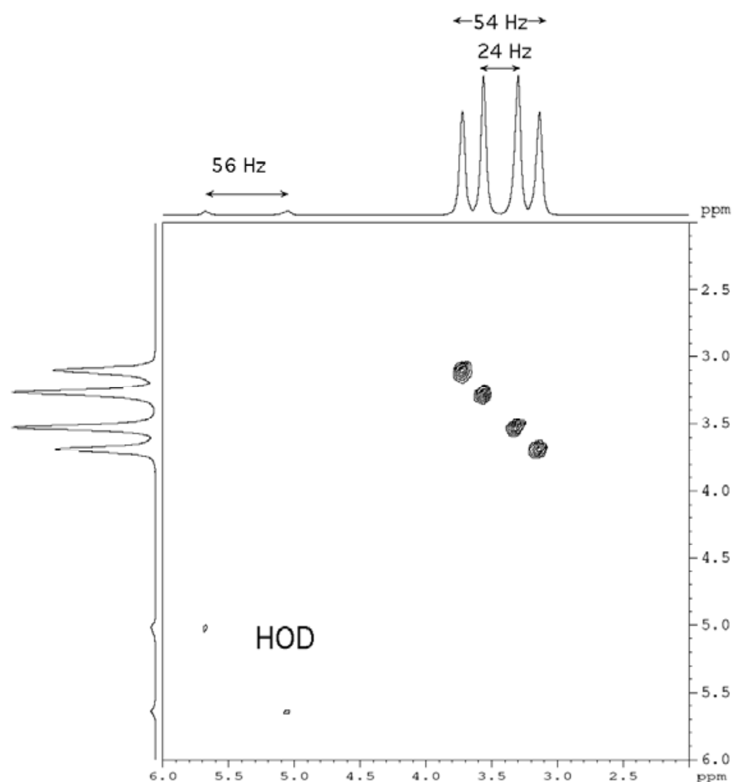
220 # VIII) Deuterium 1D/2D NMR results for DMSO-d_6



225 **Fig. SI-18** Symmetrized 92.1 MHz ^{2}H-{^{1}H} *Q*-COSY Fz 2D spectrum of DMSO (**9-d_6**) at 320 K. The DOE factor is 0.72. Note the small magnitude of quadrupolar splittings for both enantiotopic CD_3 groups compared to the values measured for amino acids.