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1 **Probing intermolecular interactions in a diethylcarbamazine citrate salt by**  
2 **fast MAS  $^1\text{H}$  solid-state NMR spectroscopy and GIPAW calculations**

3  
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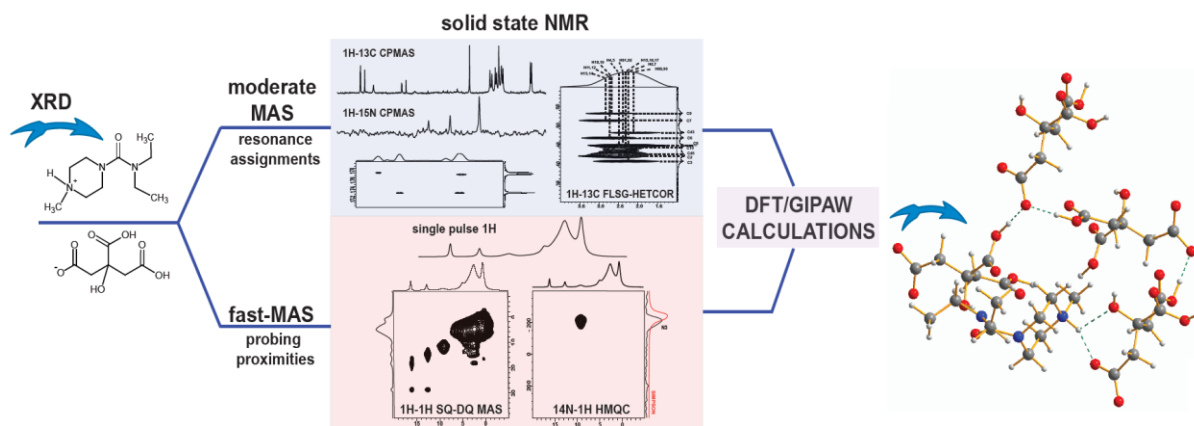
14  
15 **Abstract**

16  
17 Fast magic-angle spinning (MAS) NMR is used to probe intermolecular interactions in a  
18 diethylcarbamazine salt, that is widely used as a treatment against adult worms of *Wuchereria*  
19 *bancrofti* which cause a common disease in tropical countries named filariasis. Specifically, a  
20 dihydrogen citrate salt that has improved thermal stability and solubility as compared to the  
21 free form is studied. One-dimensional  $^1\text{H}$ ,  $^{13}\text{C}$  and  $^{15}\text{N}$  and two-dimensional  $^1\text{H}$ - $^{13}\text{C}$  and  $^{14}\text{N}$ -  
22  $^1\text{H}$  heteronuclear correlation NMR experiments under moderate and fast MAS together with  
23 GIPAW (CASTEP) calculations enable the assignment of the  $^1\text{H}$ ,  $^{13}\text{C}$  and  $^{14}\text{N}/^{15}\text{N}$  resonances.  
24 A two-dimensional  $^1\text{H}$ - $^1\text{H}$  double-quantum (DQ) –single-quantum (SQ) MAS spectrum  
25 recorded with BaBa recoupling at 60 kHz MAS identifies specific proton-proton proximities  
26 associated with citrate-citrate and citrate-diethylcarbamazine intermolecular interactions.

27  
28 **Keywords:**  $^1\text{H}$  NMR, diethylcarbamazine citrate, salt, GIPAW calculations, fast MAS NMR

1 **Graphical Abstract**

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3

4

5 **Highlights**

6

- 7 • Fast MAS NMR and GIPAW calculations provide chemical shift assignments
- 8 • 1D <sup>1</sup>H fast MAS gives well resolved resonances for hydrogen bonded protons
- 9 • Intermolecular interactions revealed by fast MAS 2D <sup>1</sup>H-<sup>1</sup>H DQ-SQ spectroscopy
- 10 • Strong citrate-citrate and weak diethylcarbamazine-citrate interactions are observed

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## 1 Introduction

2

3 Diethylcarbamazine (1-(N,N-diethylcarbamoyl)-4-methylpiperazine, DEC) is currently the  
4 first option drug for treating filariasis, a serious disease caused by *Wuchereria bancrofti*, a  
5 nematode worm transmitted by different types of mosquitoes, common in tropical countries  
6 [1]. Among many other symptoms, the main characteristic of this disease, if not-treated, is a  
7 massive and chronic swelling in the limbs, due to the presence of adult worms in the  
8 lymphatic vessels that cause serious inflammation – at this stage the disease is called  
9 elephantiasis. The use of the free form of DEC in tablet formulations is not possible because  
10 of its very low thermal stability, with the melting point being around 40-45 °C [2, 3]. To  
11 circumvent this limitation, a salt can be prepared by reacting citric acid with the DEC free  
12 base, resulting in the formation of a diethylcarbamazine dihydrogen citrate salt, i.e., a  
13 (DEC)<sup>+</sup>(citrate)<sup>-</sup> salt [4]. This compound is stable up to significantly higher temperatures and  
14 it is quite common for a table salt enriched with DEC-citrate to be prescribed, in order to  
15 improve the adherence of the patients to the treatment [3, 5, 6].

16 Although this compound has been used since 60 years ago (at least), the crystal structure was  
17 only recently determined by Silva et al. in 2010 [4], by using single-crystal and powder X-  
18 Ray diffraction (PXRD). The (DEC)<sup>+</sup>(citrate)<sup>-</sup> salt crystallizes in the centrosymmetric  
19 monoclinic P2<sub>1</sub>/c space group and the asymmetric unit contains one ionic pair of (DEC)<sup>+</sup> and  
20 (citrate)<sup>-</sup>. The characterization of this type of compound is interesting for pharmaceutical  
21 companies, since such use offers a strategy to improve the solubility and/or the thermal  
22 stability of an active pharmaceutical ingredient [7, 8]. In this context, the (DEC)<sup>+</sup>(citrate)<sup>-</sup>  
23 salt, as an example of a complex molecular packing, is a good target for the application of a  
24 combined experimental NMR and calculation approach [9-14]. Notably, this approach  
25 benefits greatly from the development since the late 1990s of NMR experiments under fast  
26 magic angle spinning (MAS). [15-18]

27 In this paper, <sup>1</sup>H MAS NMR techniques are used to investigate the solid-state structure of the  
28 (DEC)<sup>+</sup>(citrate)<sup>-</sup> salt. Specifically, a <sup>1</sup>H-<sup>1</sup>H double-quantum (DQ)- single-quantum (SQ)  
29 MAS NMR experiment [19-25] using BaBa recoupling experiment probes hydrogen-  
30 hydrogen proximities, mediated by homonuclear dipolar couplings. Additionally, fast MAS  
31 was employed to record a <sup>14</sup>N-<sup>1</sup>H HMQC NMR spectrum that probes the <sup>14</sup>N quadrupolar  
32 interaction of the protonated nitrogen [26-28]. A 2D <sup>1</sup>H-<sup>13</sup>C heteronuclear correlation

1 (HETCOR) MAS NMR spectrum recorded using cross polarisation (CP) transfer and  
2 Frequency Switched Lee-Goldburg (FSLG)  $^1\text{H}$ - $^1\text{H}$  homonuclear decoupling,  $^1\text{H}$ - $^{13}\text{C}$  CP-  
3 FSLG-HETCOR [29], was especially useful for assigning the DEC methylene protons and  
4 for probing proximities to non-protonated carbons in  $(\text{DEC})^+$  and  $(\text{citrate})^-$  ions. The solid-  
5 state NMR experiments are complemented by the calculation of NMR parameters using the  
6 GIPAW method.

## 8 **Experimental and computational methods**

### 10 **NMR Experiments**

12 **Sample and packing:** the active pharmaceutical ingredient, diethylcarbamazine citrate, was  
13 kindly donated by Fundação Oswaldo Cruz – Farmanguinhos, Rio de Janeiro, and used as  
14 received. Phase purity was verified by powder X-Ray diffraction and the data was recorded at  
15 room temperature using a Rigaku D/MAX 200 diffractometer (with a rotatory anode  
16 operating at 150 kV and 40 mA) operating with monochromatic Cu  $K\alpha$  radiation ( $K\alpha \lambda =$   
17  $1.5406 \text{ \AA}$ ). Approximately 1 and 60 mg of the powdered sample were packed into a 1.3 and 4  
18 mm zirconia MAS NMR rotor, respectively.

20 **Proton detected MAS NMR experiments:** experiments were performed using a triple  
21 resonance probehead (HXY) for 1.3 mm rotors at a spinning frequency of 60 kHz. The  
22 bearing and drive gases were at room temperature – taking into account sample heating due  
23 to MAS [30], we estimate the sample temperature to correspond to  $\sim 50 \text{ }^\circ\text{C}$ .

24 The data were collected using a Bruker Avance II+ spectrometer operating with a 14.1 T  
25 wide bore magnet (600 MHz for  $^1\text{H}$  resonance frequency). The  $^1\text{H}$   $90^\circ$  pulse duration was 2.5  
26  $\mu\text{s}$  corresponding to a nutation frequency of 100 kHz. Natural abundance *L*-alanine was  
27 employed for  $^1\text{H}$  chemical shift referencing with respect to tetramethylsilane using the methyl  
28 group resonance at 1.1 ppm that corresponds to 1.85 ppm for adamantane [31].

30 **2D  $^1\text{H}$  double quantum (DQ) MAS NMR experiment:** One rotor period of the back to back  
31 (BaBa) [32, 33] recoupling sequence was used for the excitation and reconversion of DQ  
32 coherences. A 16-step phase cycle was used in order to select  $\Delta p = \pm 2$  on the DQ excitation  
33 pulses (4 steps) and  $\Delta p = -1$  (4 steps) on the  $z$ -filter  $90^\circ$  pulse, where  $p$  is the coherence

1 order. 16 transients were coadded for each of 160  $t_1$  FIDs, using the States method to achieve  
2 sign discrimination in  $F_1$  with a rotor-synchronized  $t_1$  increment of 16.7  $\mu$ s. The total  
3 experimental time was 4.3 h using a recycle delay of 6 s.

4

## 5 **2D $^{14}\text{N}$ - $^1\text{H}$ heteronuclear multiple quantum correlation (HMQC) MAS NMR**

6 **experiment:** a modified version of the  $^{14}\text{N}$ - $^1\text{H}$  HMQC pulse sequence of Gan et al [34]  
7 employing rotary resonance recoupling ( $\text{R}^3$ ) [35] was used. The modification consists of  
8 applying a second  $^1\text{H}$   $90^\circ$  pulse ( $90^\circ$  out of phase with respect to the first  $90^\circ$  pulse)  
9 immediately after the first  $^1\text{H}$   $90^\circ$  pulse and using phase inversion (every rotor period) of the  
10  $n = 2$  ( $\nu_1 = 2 \nu_R$ ) rotary-resonance recoupling pulses [36]. A four-step nested phase cycle was  
11 used to select changes in coherence order  $\Delta p = \pm 1$  (on the first  $^1\text{H}$  pulse, 2 steps) and  $\Delta p =$   
12  $-1$  (on the last  $^{14}\text{N}$  pulse, 2 steps). The recoupling duration was  $4 \tau_R = 66.8 \mu\text{s}$ . The  $^{14}\text{N}$  pulse  
13 duration was 5  $\mu\text{s}$ . For each of 64  $t_1$  FIDs (using the States method to achieve sign  
14 discrimination in  $F_1$  with a rotor synchronized increment of 16.7  $\mu\text{s}$ ), 64 transients were co-  
15 added with a recycle delay of 6 s, corresponding to a total experimental time of 7 h.

16

17  **$^{13}\text{C}$  and  $^{15}\text{N}$  detected CP MAS NMR experiments:**  $^1\text{H}$ - $^{13}\text{C}$  and  $^1\text{H}$ - $^{15}\text{N}$  CPMAS experiments  
18 were performed using a Bruker Avance III spectrometer operating with a narrow bore 11.7 T  
19 magnet (500 MHz for  $^1\text{H}$  resonance frequency) and equipped with a HX probehead for 4 mm  
20 rotors. A MAS frequency of 5 kHz was used. A two-pulse phase-modulated TPPM-15  
21 scheme [37, 38] was used for  $^1\text{H}$  decoupling at a nutation frequency of 100 kHz. Cross  
22 polarization was applied by using a 90-100% amplitude ramp on  $^1\text{H}$  [39] during a contact  
23 time of 2 ms (for  $^{13}\text{C}$ ) or 4 ms (for  $^{15}\text{N}$ ). 256 (for  $^{13}\text{C}$ ) or 4096 (for  $^{15}\text{N}$ ) transients were  
24 coadded with a recycle delay of 3 s. For  $^{13}\text{C}$ , adamantane was used as an external reference  
25 for tetramethylsilane (TMS), setting the  $\text{CH}_2$  signal to 38.5 ppm [31, 40]. For  $^{15}\text{N}$ , glycine  
26 was used as an external reference ( $-347.4$  ppm, related to nitromethane) [41]. To convert to  
27 the chemical shift scale frequently used in protein NMR, where the alternative IUPAC  
28 reference [42] is liquid ammonia at  $-50^\circ\text{C}$ , it is necessary to add 379.5 to the given values  
29 [43].

30

31 **2D CP -  $^1\text{H}$  (FSLG)- $^{13}\text{C}$  Heteronuclear Correlation MAS NMR [29]:** the experiment was  
32 performed using a Bruker Avance III spectrometer operating with a wide bore 11.7 T magnet  
33 (500 MHz for  $^1\text{H}$  resonance frequency) and using a 4 mm HXY probehead at a MAS

1 frequency of 12.5 kHz. Both homonuclear (FSLG, [44, 45]) and heteronuclear SPINAL-64  
2 [46]  $^1\text{H}$  decoupling employed a nutation frequency of 100 kHz. For each of 128  $t_1$  FIDs  
3 (using the States method to achieve sign discrimination in  $F_1$  with a rotor synchronized  
4 increment of 80  $\mu\text{s}$ ), 16 transients were coadded with a recycle delay of 11 s corresponding to  
5 a total experimental time of 6.5 h. For CP (contact time of 200  $\mu\text{s}$ ), a 90-100% amplitude  
6 ramp on  $^1\text{H}$  was employed.  $^{13}\text{C}$  chemical shifts were referenced using *L*-alanine as an external  
7 reference (using the  $\text{CH}_3$  signal centred at 20.5 ppm), corresponding to the same adamantane  
8 reference referred to above. The FSLG scaling factor in the  $^1\text{H}$  chemical shift axis was 0.56  
9 with the  $^1\text{H}$  chemical shifts being referenced according to the fast MAS spectrum.

10

### 11 **DFT GIPAW Calculations:**

12

13 Calculations were performed by employing a plane-wave based DFT approach as  
14 implemented in the CASTEP code, UK academic release version 8.0 [47]. Initial atomic  
15 coordinates were taken from the published crystal structure [4] for which the “.cif” file is  
16 available on the Crystallography Open Database  
17 <http://www.crystallography.net/cod/cod/4501669.html>, with code 4501669: Space Group  
18  $P2_1/c$ ,  $Z = 4$ ,  $Z' = 0$ , 224 atoms in the unit cell, cell dimensions ( $\text{\AA}$ ):  $a = 13.8050$ ,  $b = 10.2581$ ;  
19  $c = 13.9890$ , cell angles ( $^\circ$ ):  $\alpha = 90.00$ ,  $\beta = 93.689$  and  $\gamma = 90.00$ ; cell volume  $V = 1976.92$   
20  $\text{\AA}^3$ . A new “.cif” file was created from the original one to describe only the conformer with  
21 the higher probability (70%, conformer 1), i.e., the C7A', C8A', H8A1', H8A2', H8A3',  
22 H7A1' and H7A2' atoms were manually deleted.

23 The unit cell parameters were fixed, space group symmetry was imposed, and periodic  
24 boundary conditions were applied during the geometry optimization. NMR shielding  
25 calculations were performed using the gauge-including projector-augmented wave (GIPAW)  
26 approach [48, 49]. Both geometry optimizations and NMR chemical shift calculations used a  
27 plane-wave basis set and the PBE exchange correlation functional [49, 50] at a basis cut-off  
28 energy of 700 eV with integrals taken over the Brillouin zone by using a Monkhorst–Pack  
29 grid of minimum sample spacing  $0.1 \times 2\pi \text{\AA}^{-1}$ . A semi empirical dispersion correction was  
30 applied using the TS scheme [51] during both geometry optimization and NMR shielding  
31 calculations, employing ultrasoft pseudopotentials generated on the fly (OTF) [52]. After  
32 geometry optimization, the forces, energies and displacements were better than  $0.05 \text{ eV } \text{\AA}^{-1}$ ,

1 0.000002 eV and 0.0002 Å, respectively. Distances stated in this paper are for the geometry-  
2 optimised structure. GIPAW calculated NMR shielding were visualized, processed, and  
3 tabulated through the CCP-NC output files visualization tool, MagresView version 1.6 [53],  
4 running on Mozilla Firefox web browser version 49.0.2.

## 5 **Results and discussion**

### 6 **Chemical shift assignments**

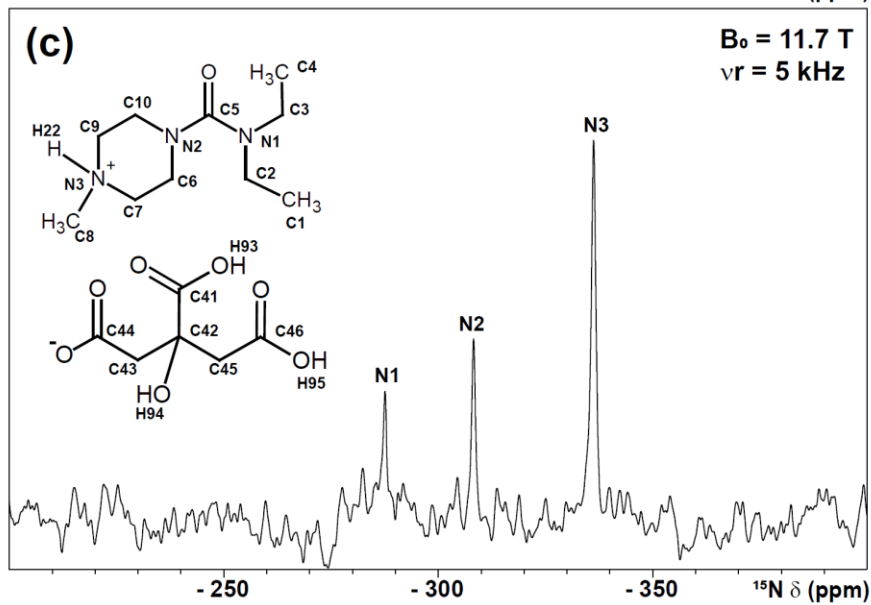
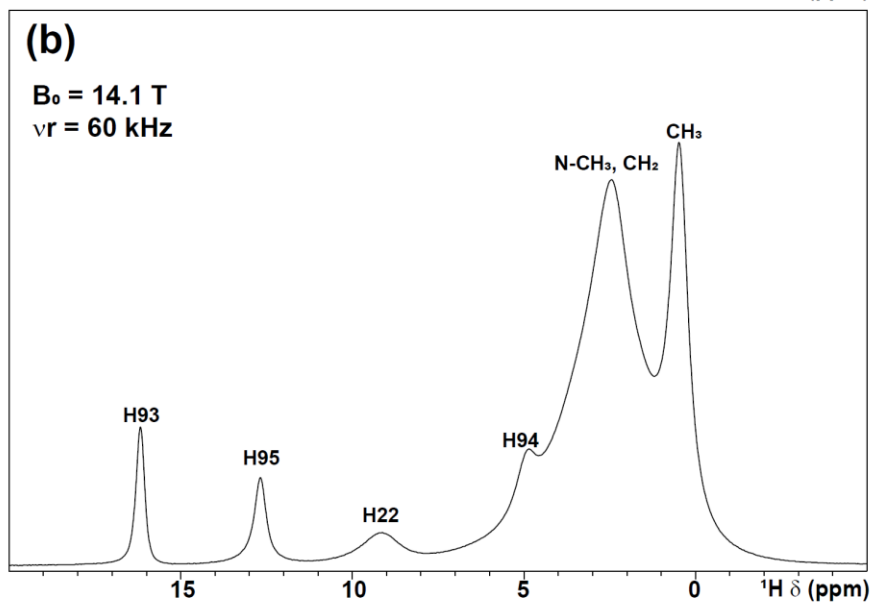
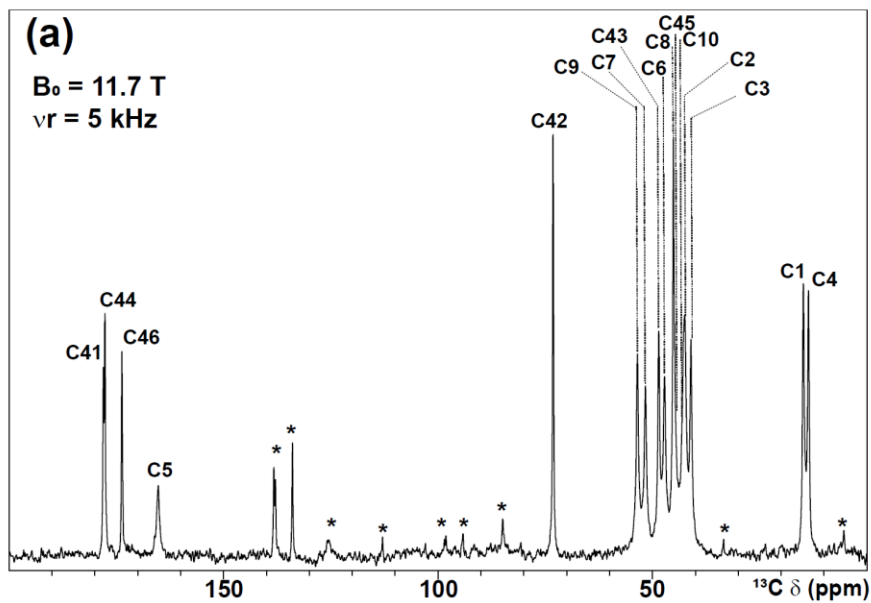
7  
8  
9  
10 One-dimensional  $^1\text{H}$  (one-pulse),  $^{13}\text{C}$  and  $^{15}\text{N}$  (CP MAS) spectra of diethylcarbamazine  
11 citrate are presented in Figure 1, while Table 1 compares experimental and calculated  
12 (GIPAW)  $^1\text{H}$ ,  $^{13}\text{C}$  and  $^{15}\text{N}$  chemical shifts. Note that the geometry optimisation within  
13 CASTEP causes a relabelling of the atoms – in this paper, we use the CASTEP numbering;  
14 see Table 1 below for a comparison with the numbering employed in the crystallographic cif  
15 file. It is a well known phenomenon [10, 14] that the gradient of a plot of experimental  $^{13}\text{C}$   
16 chemical shift against calculated shielding deviates slightly from minus one [54]. Thus, in  
17 this work, to enable a clearer comparison between experimental and GIPAW calculated  $^{13}\text{C}$   
18 chemical shifts, we use an approach previously employed in Ref. [55], whereby there are two  
19 different reference shieldings for calculated  $^{13}\text{C}$  isotropic chemical shifts above and below 70  
20 ppm.

21 The assignment of the experimental  $^1\text{H}$  chemical shifts is based on a 2D  $^1\text{H}$ - $^{13}\text{C}$   
22 correlation spectrum in Figure 2b (expanded in Figure 3) that was recorded with a CP-  
23 HETCOR experiment employing FSLG  $^1\text{H}$  decoupling. The use of CP to establish a  
24 heteronuclear correlation based on through-space  $^1\text{H}$ - $^{13}\text{C}$  dipolar couplings is beneficial for  
25 the observation of cross peaks for  $\text{CH}_2$  moieties that usually have low sensitivity in a  $J$ -based  
26  $^1\text{H}$ - $^{13}\text{C}$  refocused INEPT spectrum[55, 56]. Such a refocused INEPT  $^1\text{H}$ - $^{13}\text{C}$  correlation  
27 spectrum is (for a short spin-echo duration) usually selective for one-bond C-H  
28 connectivities. By contrast, we observe that the use here of CP, even for a relatively short  
29 contact time of 200  $\mu\text{s}$ , results in the observation of albeit low-intensity cross peaks  
30 corresponding to longer-range C-H proximities in Figure 2b and 3. In this way, cross peaks  
31 for the intramolecular longer-range C-H proximities involving the carboxylic acid (C41 and  
32 C46), carboxylate (C44) and quaternary (C42) citric acid carbons are revealed. Specifically,  
33 as shown in Figure 3, cross peaks are observed for the COOH groups, C41 with H93 (1.98 Å)



1 and C46 with H95 (1.93 Å). For the carboxylate C44, cross peaks are observed with the C43  
2 CH<sub>2</sub> protons (distances of 2.13 (H89) and 2.15 (H90) Å), while for the central quaternary  
3 carbon C42, cross peaks are observed to the OH proton (H94, 1.98 Å) and the C43 and C45  
4 CH<sub>2</sub> groups (H89 to H92, distances between 2.14 and 2.18 Å).

5 As well as the <sup>15</sup>N CP MAS spectrum presented in Figure 1, a <sup>14</sup>N-<sup>1</sup>H HMQC  
6 spectrum is presented in Figure 2c. This two-dimensional <sup>14</sup>N-<sup>1</sup>H spectrum was recorded with  
7 a short duration of rotary resonance recoupling such that a cross peak is only observed for the  
8 protonated N3 nitrogen. For the spin  $I = 1$  nucleus, <sup>14</sup>N, there is line broadening due to the  
9 second-order quadrupolar interaction and the <sup>14</sup>N shift depends on the sum of the isotropic  
10 chemical shift and the isotropic second-order quadrupolar shift. The red spectrum to the right  
11 of Figure 3c corresponds to a simulation using the calculated (GIPAW) quadrupolar  
12 parameters (see Table S1); good agreement to experiment is evident.



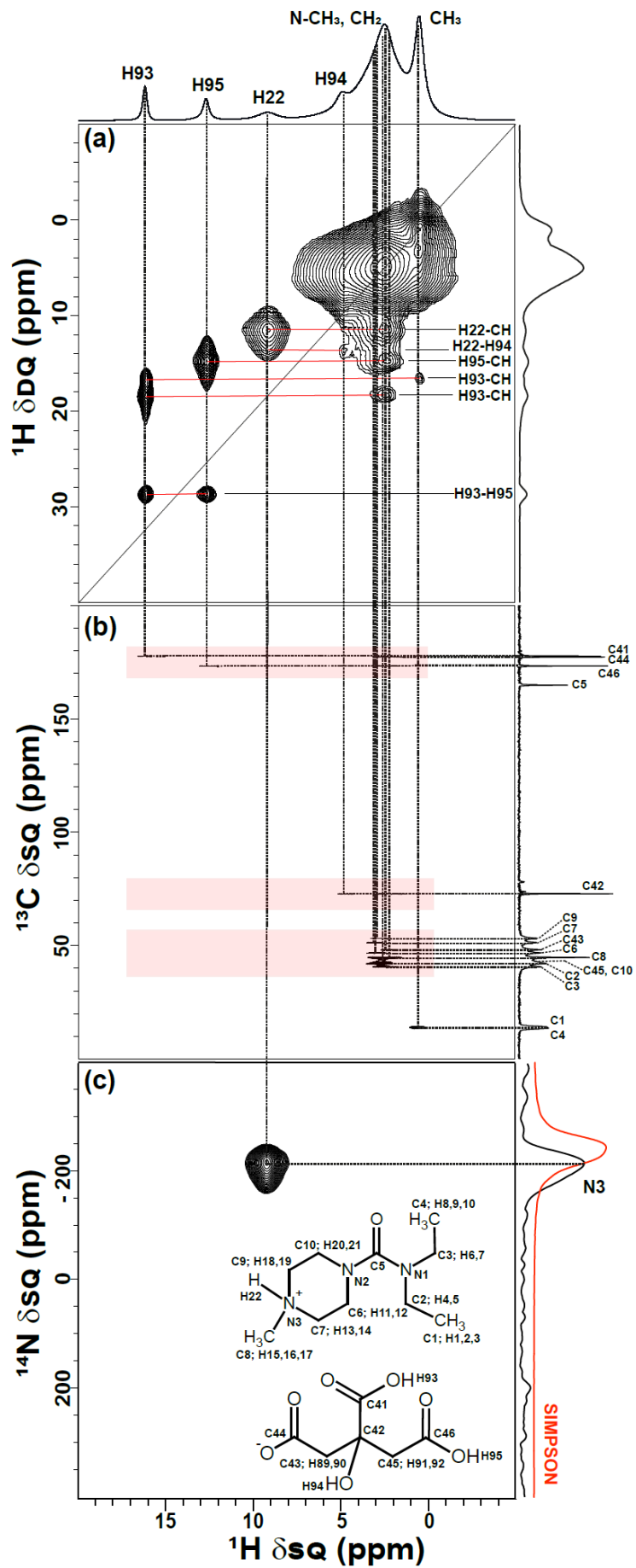
1 **Figure 1:** One-dimensional solid-state MAS NMR spectra of the diethylcarbamazine citrate  
 2 salt: (a) a  $^1\text{H}$  (500 MHz)- $^{13}\text{C}$  CP MAS (5 kHz) spectrum; (b) a  $^1\text{H}$  (600 MHz) single-pulse  
 3 MAS (60 kHz) spectrum (8 co-transients were added for a recycle delay of 6 s); (c) a  $^1\text{H}$  (500  
 4 MHz)- $^{15}\text{N}$  CP MAS (5 kHz) spectrum. Asterisks denote spinning sidebands in (a).

5  
 6 **Table 1:** Experimental and calculated (GIPAW) isotropic chemical shifts (in ppm) for the  
 7 diethylcarbamazine citrate salt.

LABELLING		Atom descriptor	GIPAW calculation <sup>a</sup>	Experimental
CASTEP	X-Ray [4]		$\delta_{\text{iso}}$	$\delta_{\text{iso}}$
C1	C10A	CH <sub>3</sub>	10.9	14.2
C2	C9A	CH <sub>2</sub>	42.0	42.1
C3	C7A	CH <sub>2</sub>	40.5	40.7
C4	C8A	CH <sub>3</sub>	8.6	13.6
C5	C6A	C=O	163.0	164.9
C6	C4A	CH <sub>2</sub>	49.3	48.2
C7	C3A	CH <sub>2</sub>	50.9	51.3
C8	C5A	CH <sub>3</sub>	44.2	44.8
C9	C2A	CH <sub>2</sub>	53.0	53.2
C10	C1A	CH <sub>2</sub>	43.4	44.5
C41	C6C	COOH	180.9	177.6
C42	C3C	C <sub>quat</sub>	74.2	72.8
C43	C2C	CH <sub>2</sub>	49.1	46.8
C44	C1C	COO <sup>-</sup>	179.3	177.3
C45	C4C	CH <sub>2</sub>	42.6	42.4
C46	C5C	COOH	176.3	173.3
H1,H2,H3	H10A1,H10A2,H10A3	CH <sub>3</sub>	0.4	0.5
H4	H9A1	CH <sub>2</sub>	2.5	2.3
H5	H9A2	CH <sub>2</sub>	2.3	
H6	H7A1	CH <sub>2</sub>	2.1	
H7	H7A2	CH <sub>2</sub>	2.5	
H8, H9, H10	H8A1, H8A2, H8A3	CH <sub>3</sub>	0.1 <sup>b</sup>	0.5
H11	H4A1	CH <sub>2</sub>	2.7	2.7
H12	H4A2	CH <sub>2</sub>	2.8	
H13	H3A1	CH <sub>2</sub>	2.9	
H14	H3A2	CH <sub>2</sub>	3.3	2.8
H15, H16, H17	H5A1, H5A2, H5A3	CH <sub>3</sub>	2.1 <sup>b</sup>	2.3
H18	H2A1	CH <sub>2</sub>	2.4	2.7
H19	H2A2	CH <sub>2</sub>	3.0	

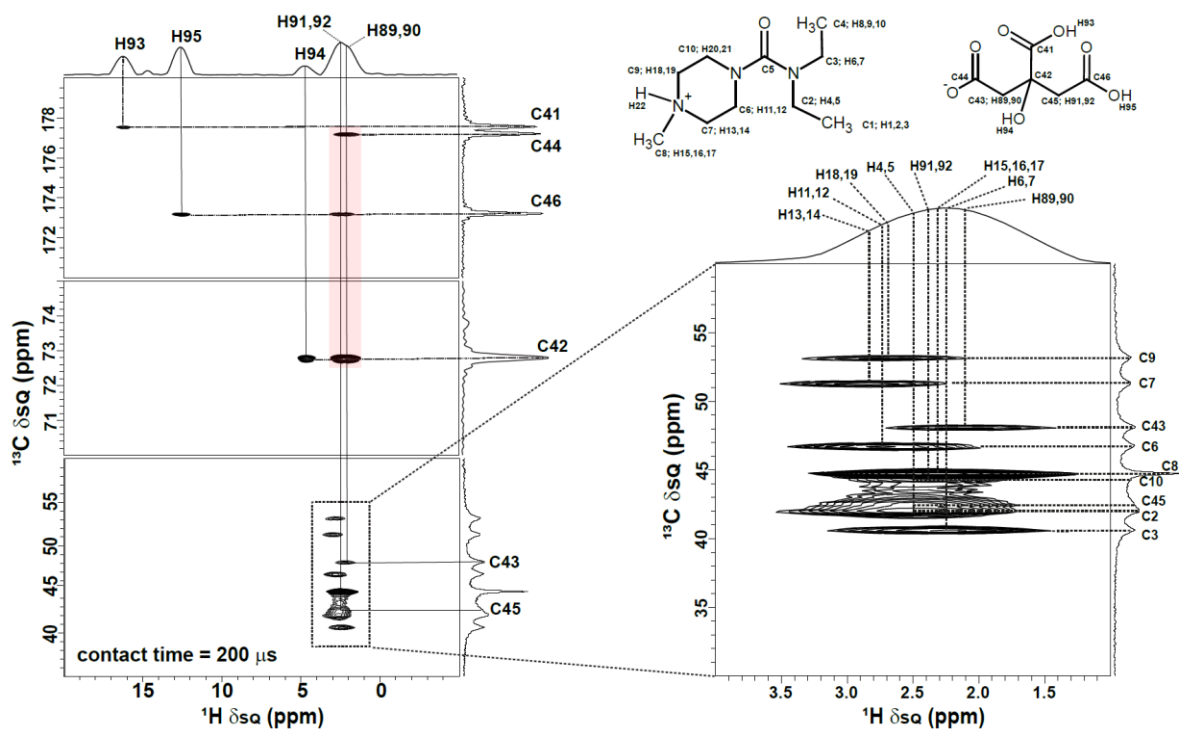
H20	H1A1	CH <sub>2</sub>	3.6	2.9
H21	H1A2	CH <sub>2</sub>	2.7	
H22	H2A	N <sup>+</sup> H	9.4	9.1
H89	H2C1	CH <sub>2</sub>	1.6	2.1
H90	H2C1	CH <sub>2</sub>	2.3	
H91	H4C1	CH <sub>2</sub>	2.5	2.7
H92	H4C2	CH <sub>2</sub>	2.1	
H93	H6C	COOH	16.7	16.2
H94	H3C	OH	4.2	4.8
H95	H7C	COOH	14.3	12.8
N1	N2A	N(C=O)N	-286.8	-287.7
N2	N1A	N(C=O)N	-309.4	-308.4
N3	N3A	N <sup>+</sup> H	-328.6	-336.4

- 1 <sup>a</sup> Calculated isotropic chemical shifts are given by  $\delta_{\text{iso}}^{\text{calc}} = \sigma_{\text{ref}} - \sigma_{\text{calc}}$ , where  $\sigma_{\text{ref}}$  is 30 ppm for <sup>1</sup>H and -153
- 2 ppm for <sup>14</sup>N/<sup>15</sup>N. For <sup>13</sup>C two shielding references were used:  $\sigma_{\text{ref}} = 170$  ppm for  $\delta_{\text{iso}} > 70$  ppm and  $\sigma_{\text{ref}} = 173$
- 3 ppm for  $\delta_{\text{iso}} < 70$  ppm [55].
- 4 <sup>b</sup> For CH<sub>3</sub> groups, the stated calculated isotropic <sup>1</sup>H chemical shift corresponds to the average for the three
- 5 protons.



1 **Figure 2:** 2D solid-state MAS NMR spectra of the diethylcarbamazine citrate salt: (a) a  $^1\text{H}$ -  
 2  $^1\text{H}$  (600 MHz) DQ-SQ MAS (60 kHz) correlation spectrum with skyline projections recorded  
 3 using one rotor period of BaBa recoupling; (b) a  $^1\text{H}$  (500 MHz)- $^{13}\text{C}$  HETCOR MAS (12.5  
 4 kHz) spectrum (together with a  $F_1$  skyline projection) recorded using FSLG  $^1\text{H}$  decoupling  
 5 and a CP contact time of 200  $\mu\text{s}$  (red shaded rectangles indicate the regions for which  
 6 zoomed-in views are presented in Figure 3); (c) a  $^{14}\text{N}$ - $^1\text{H}$  (600 MHz) HMQC MAS (60 kHz)  
 7 spectrum recorded using four rotor periods of rotary resonance recoupling – on the right-hand  
 8 side, the skyline projection is compared to a spectrum simulated using the GIPAW calculated  
 9 NMR parameters (see a SIMPSON [57] input file (in the Supporting Information) for N3).  
 10 The base contour is at (a) 10%, (b) 20% and (c) 20% of the maximum intensity.

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**Figure 3:** Zoomed-in regions for the  $^1\text{H}$ - $^{13}\text{C}$  HETCOR spectrum of the diethylcarbamazine citrate salt presented in Figure 2b, together with skyline projections.

## 1 Proton-proton proximities

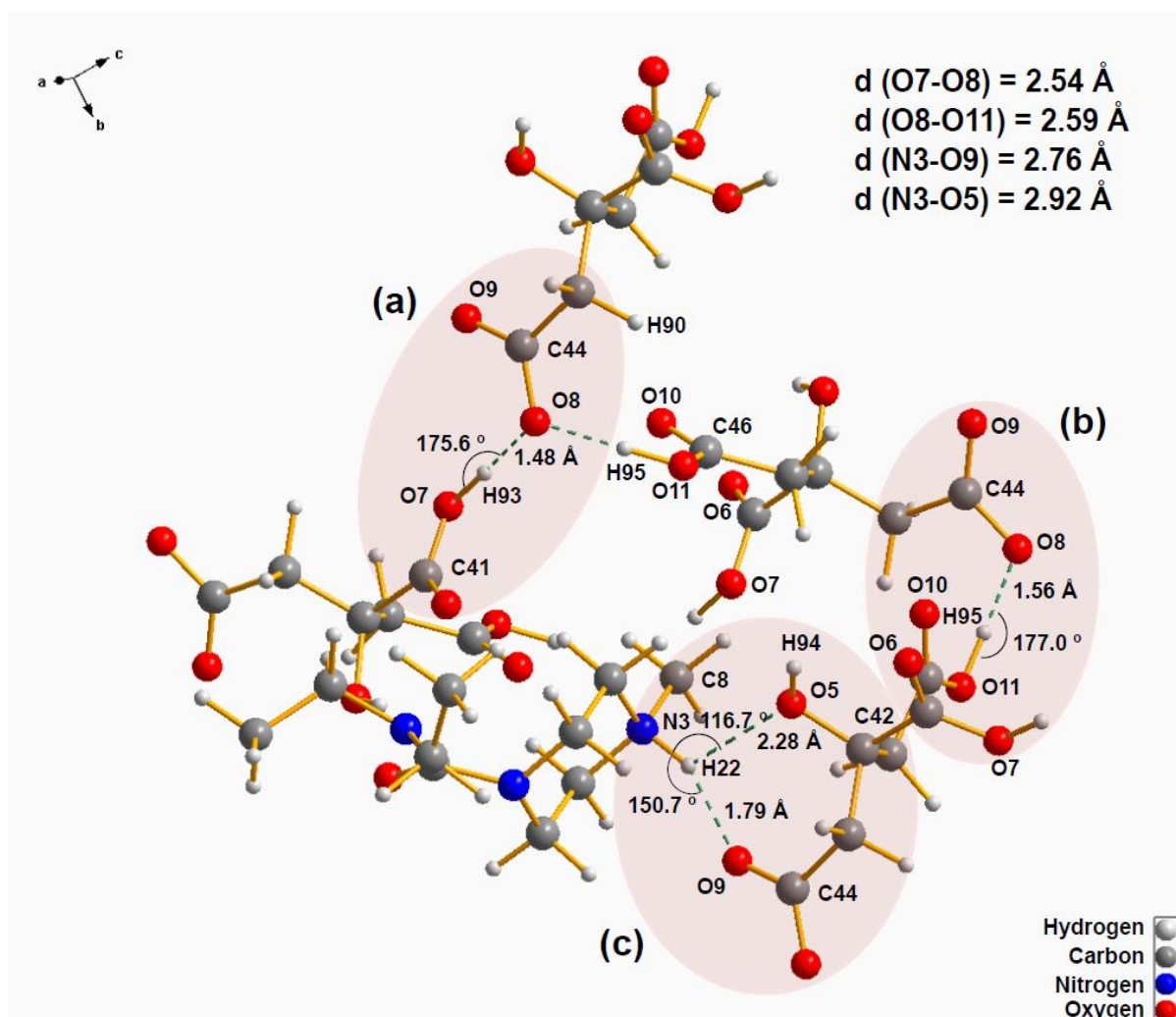
2  
3  $^1\text{H}$  solid-state NMR experiments performed under fast MAS are a powerful probe of  
4 intermolecular hydrogen bonding arrangements, with the  $^1\text{H}$  chemical shift being a sensitive  
5 indicator of hydrogen-bonding strength. In particular, two-dimensional  $^1\text{H}$  DQ MAS spectra  
6 provide valuable insight into proton-proton proximities of such hydrogen-bonded protons. A  
7 2D  $^1\text{H}$ - $^1\text{H}$  DQ-SQ spectrum of the diethylcarbamazine citrate salt recorded at fast MAS is  
8 presented in Figure 2a. There are two  $^1\text{H}$  resonances with chemical shifts above 10 ppm in  
9 Figure 1b, at 16.2 and 12.8 ppm. On the basis of the  $^1\text{H}$ - $^{13}\text{C}$  HETCOR spectrum (see Figure  
10 2b and 3) and the GIPAW chemical shielding calculations, these are assigned to the H93  
11 (16.2 ppm) and H95 (12.8 ppm) protons of the C41 and C46 COOH groups. As shown in  
12 Figure 4 (views a and b), both COOH protons form an intermolecular OH...O hydrogen  
13 bonding to the same oxygen atom (O8) of the C44 carboxylate group, i.e., the O8 oxygen  
14 atom exhibits bifurcated hydrogen bonding. Both OH...O hydrogen bonds are close to linear  
15 ( $175.6^\circ$  and  $177.0^\circ$ ): for H93 which has the  $^1\text{H}$  higher chemical shift, the hydrogen bond has  
16 slightly shorter H...O (1.48 Å compared to 1.56 Å) and O...O (2.54 Å compared to 2.59 Å)  
17 distances than that of H95. Note that the difference in experimental  $^1\text{H}$  chemical shifts of 3.4  
18 ppm (16.2 as compared to 12.8 ppm) is slightly bigger than that for the calculated (GIPAW)  
19  $^1\text{H}$  chemical shifts (2.4 ppm, 16.7 as compared to 14.3 ppm, see Table 1). In this respect, it is  
20 known that experimental  $^1\text{H}$  chemical shifts of hydrogen-bonded protons increase upon  
21 decreasing temperature [58] further noting that GIPAW calculations correspond to 0 K. Note  
22 that there is no evident hydrogen bonding for the two other oxygen atoms (O6 or O10)  
23 attached to the same carbon atoms (C41 and C46) as the H93 or H95 OH groups that could  
24 explain the large difference in the  $^1\text{H}$  higher chemical shifts of H93 or H95.

25 The bifurcated hydrogen bonding of the carboxylate O8 oxygen with the two COOH groups  
26 leads to a close proximity of the H93 and H95 COOH protons (2.50 Å, see Table 2), with a  
27 cross peak being observed at a  $^1\text{H}$  DQ frequency of  $16.2 + 12.8 = 29.0$  ppm in Figure 2a. DQ  
28 peaks are also observed for H93 and H95 with aliphatic protons (see Figure 2a and Table 2),  
29 notably, H95 has a close intermolecular proximity (2.29 Å) to the citrate  $\text{CH}_2$  H90 proton  
30 (see Figure 4a).

31 Consider the diethylcarbamazine  $\text{NH}^+$  H22 proton. As shown in Figure 4c, this has a close  
32 proximity to two hydrogen bond donor atoms, namely the other oxygen (O9) of the citrate  
33 carboxylate group and the oxygen (O5) of the citric acid OH group. While the N3...O5

1 distance (2.92 Å) is only slightly longer than the N3...O9 distance (2.76 Å), the NHO angle  
 2 is an unfavourable 116.7° for the OH O5 as compared to 150.7° for the carboxylate O9,  
 3 resulting in a significantly longer H..O distance of 2.28 Å as compared to 1.79 Å. The  
 4 experimental <sup>1</sup>H chemical shift for the diethylcarbamazine NH<sup>+</sup> H22 proton of 9.1 ppm is in  
 5 good agreement with the calculated (GIPAW) value of 9.4 ppm. Looking at the <sup>1</sup>H DQ MAS  
 6 spectrum in Figure 2a, it is interesting to observe a resolved weak cross peak at the OH  
 7 single-quantum frequency between the H22 and the OH H94 proton at a <sup>1</sup>H DQ frequency of  
 8 9.1 + 4.8 = 13.9 ppm, corresponding to a H-H distance of 3.05 Å. The low <sup>1</sup>H chemical shift  
 9 for the citric acid OH moiety of 4.8 ppm is consistent with it forming a hydrogen bond to the  
 10 DEC carbonyl oxygen with a relatively long O...O and H...O distance of 2.81 and 1.95 Å,  
 11 respectively (OHO bond angle of 144.3°).

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14 **Figure 4:** A representation of the geometry-optimised (CASTEP) crystal structure of the  
 15 diethylcarbamazine citrate salt, focusing on the intermolecular hydrogen-bonding  
 16 interactions. X...Y and H...Y distances and XHY angles are stated for the XH...Y



- 1 intermolecular hydrogen bonding exhibited by the (a, b) citrate COOH and (c)  
 2 diethylcarbamazine NH<sup>+</sup> groups.  
 3  
 4 **Table 2.** H-H proximities (< 3.5 Å) and corresponding <sup>1</sup>H DQ shifts (see Fig. 2a) for the NH, OH,  
 5 COOH and CH protons in the diethylcarbamazine citrate salt.

atom	H-H proximity	$\delta_{\text{iso}}^{\text{exp}}$ SQ / ppm	$\delta^{\text{exp}}$ DQ / ppm	H-H distance <sup>a</sup> (Å)
<b>H93</b> , C(41)OOH	<i>H95 (C(46)OOH)</i>	12.8	29.0	2.50
16.2 ppm	<i>H3 (C1, CH<sub>3</sub>)</i>	0.5	16.7	2.78
	<i>H2 (C1, CH<sub>3</sub>)</i>	0.5	16.7	3.01
	<i>H16 (C8, CH<sub>3</sub>)</i>	2.3	18.5	3.03
	<i>H15 (C8, CH<sub>3</sub>)</i>	2.3	18.5	3.14
	H90 (C43, CH <sub>2</sub> )	2.5	18.7	3.37
<b>H95</b> , C(46)OOH	<i>H90 (C43, CH<sub>2</sub>)</i>	2.5	15.3	2.29
12.8 ppm	<i>H93 (C(41)OOH)</i>	16.2	29.0	2.50
	<i>H20 (C10, CH<sub>2</sub>)</i>	2.9	15.7	3.03
	<i>H10 (C4, CH<sub>3</sub>)</i>	0.5	(13.3)	3.34
	<i>H15 (C8, CH<sub>3</sub>)</i>	2.3	15.1	3.45
	<i>H89 (C43, CH<sub>2</sub>)</i>	2.5	15.3	3.46
	<i>H13 (C7, CH<sub>2</sub>)</i>	2.8	15.6	3.49
<b>H22</b> , N(3) <sup>+</sup> H	H18 (C9, CH <sub>2</sub> )	2.7	11.8	2.34
9.1 ppm	H17 (C8, CH <sub>3</sub> )	2.3	11.4	2.38
	H16 (C8, CH <sub>3</sub> )	2.3	11.5	2.39
	H14 (C7, CH <sub>2</sub> )	2.8	11.9	2.40
	H21 (C10, CH <sub>2</sub> )	2.9	12.0	2.53
	H11 (C6, CH <sub>2</sub> )	2.7	11.8	2.56
	<i>H91 (C45, CH<sub>2</sub>)</i>	2.7	11.8	2.72
	H19 (C9, CH <sub>2</sub> )	2.7	11.8	2.96
	H15 (C8, CH <sub>3</sub> )	2.3	11.4	2.97
	H13 (C7, CH <sub>2</sub> )	2.8	11.9	2.98

	<i>H94 (OH)</i>	4.8	13.9	3.05
<b>H94, O(5)H</b>	<i>H14 (C7, CH<sub>2</sub>)</i>	2.8	7.6	2.39
4.8 ppm	<i>H19 (C9, CH<sub>2</sub>)</i>	2.7	7.5	2.67
	<i>H7 (C3, CH<sub>2</sub>)</i>	2.3	7.1	2.80
	H89 (C43, CH <sub>2</sub> )	2.5	7.3	2.82
	<i>H22 (N<sup>+</sup>H)</i>	9.1	13.6	3.05
	<i>H17 (C8, CH<sub>3</sub>)</i>	2.3	7.1	3.12
	<i>H11 (C6, CH<sub>2</sub>)</i>	2.7	7.5	3.34
	H91 (C45, CH <sub>2</sub> )	2.7	7.5	3.46
	<i>H20 (C10, CH<sub>2</sub>)</i>	2.9	7.7	3.47

1 <sup>a</sup> H-H distances are taken from the DFT (CASTEP) optimized structure. Intermolecular proximities  
2 are denoted using italic font.

3

#### 4 **Conclusions**

5

6 Fast MAS <sup>1</sup>H NMR experiments have been used in conjunction with GIPAW calculation to  
7 probe intermolecular interactions in a diethylcarbamazine citrate salt. Notably, 1D and 2D <sup>1</sup>H  
8 solid state experiments recorded under fast MAS (60 kHz) or at moderate MAS with <sup>1</sup>H  
9 homonuclear decoupling (FSLG) combined with the GIPAW calculation of NMR parameters  
10 enabled an assignment of the <sup>1</sup>H, <sup>13</sup>C and <sup>15</sup>N chemical shifts. These findings reinforce the  
11 use of NMR crystallography to characterize pharmaceutical supramolecular complexes, with  
12 special attention to co-crystals and salts, whose use has been growing more recently. The  
13 design of new drugs and formulations as well as the co-formulation of two (or more) drugs  
14 offers many challenges in terms of packing complexity and molecular dynamics.

15

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17

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## 6 **Supporting Information**

7

8 Experimental and calculated PXRD for the diethylcarbamazine citrate salt; GIPAW DFT  
9 calculated electric field gradient tensors, quadrupolar interaction parameters and calculated  
10 and experimental isotropic shifts for  $^{14}\text{N}$ ; input parameters for  $^{14}\text{N}$  lineshape SIMPSON  
11 simulations as well as SIMPSON simulated  $^{14}\text{N}$  lineshapes for the diethylcarbamazine citrate  
12 salt (pdf). The “.cif” and “.magres” files are also available.

13

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