

. ¹³C CP/MAS study of matrices.

In the supplementary materials we present solid state NMR study of four matrices used in our MALDI TOF analysis. For three of them (DHBⁱ, DTⁱⁱ, HABⁱⁱⁱ) the X-ray crystal and molecular structure is reported. For each sample we have carried out series of measurements comparing ¹³C NMR spectra of matrix recrystallized in solvent (or solvents) as reported in X-ray paper, sample recrystallized from chloroform (solvent recommended for MALDI TOF), sample recrystallized from THF (used in our project) and sample recrystallized from chloroform and THF with 10% of NaI.

a) **DHB**- for this matrix two X-ray structures are deposited. DHB crystallized by slow evaporation from aqueous solution forms ordered phase while from mixture chloroform:acetone forms disordered structure. In both cases DHB crystallizes in monoclinic system with P2₁/a space group and one molecule in the asymmetric unit. Figure 4a shows ¹³C CP/MAS spectrum of sample crystallizes from mixture chloroform:acetone 3:1 v/v recorded with spinning rate 8 kHz at room temperature. In the unit cell DHB molecules form complex hydrogen bonded array with number of intra- and intermolecular contacts.

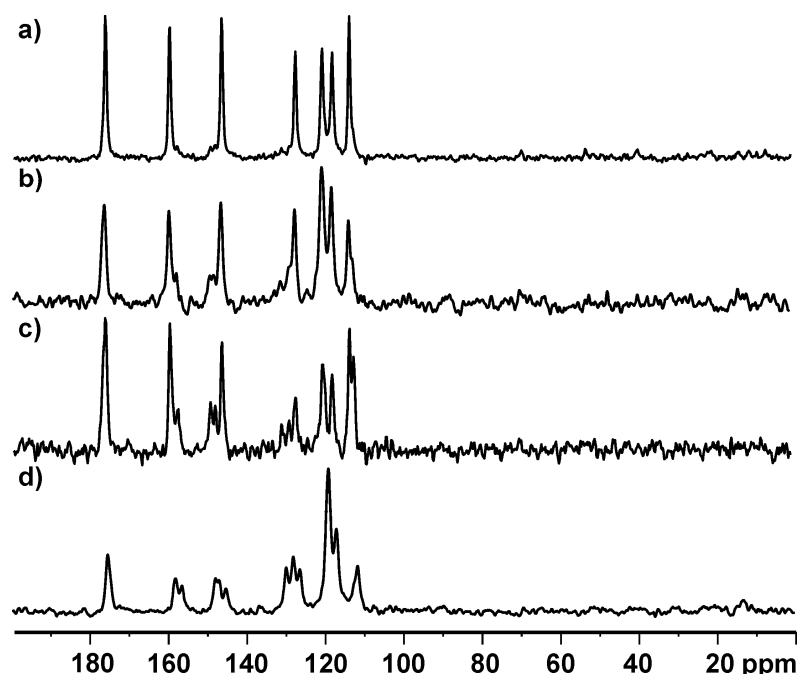


Figure 1

¹³C CP/MAS spectra of different crystallization forms of DHB recorded with 8 kHz spinning rate at room temperature: a) chloroform and acetone (3:1 v/v), b) THF, c) DHB : NaI (10:1); chloroform and acetone (3:1 v/v), d) DHB: NaI (10:1); THF.

The ^{13}C NMR signals are very sharp and well resolved. Number of signals is consistent with X-ray data and confirm that one molecule is an independent part of the unit cell. Such picture is typical for well defined crystalline samples and in this case assignment of signals is straightforward. Signals coming from carboxyl group ($\delta=174.47$ ppm) and quaternary carbons bonded to hydroxyl groups ($\delta=158.41$ ppm and 145.13 ppm) are very diagnostic. The DHB is not soluble in pure chloroform hence Figure 4b shows matrix crystallized from THF. Analysis of spectrum proves that during THF crystallization second polymorph appears. The new signals adjacent to carbons bonded to hydroxyl groups ($\delta=156.1$ ppm and 147.1 ppm) are well seen. This effect is even better visible when sample crystallized from mixture chloroform and few drops of acetone with 10% of sodium iodide is recorded (Figure 4c). Figure 4d displays spectrum of DHB crystallized from THF with 10% of NaI. The differences between spectrum 4d and those shown in Figure 4a are apparent. From ^{13}C CP/MAS measurements we can conclude that DHB is very sensitive to environmental effects, at least two polymorphs are formed, very likely because of different hydrogen bonding pattern. The proportion of polymorphs depends on solvent used for sample crystallization.

b) **DT**- crystallizes in monoclinic system with $P2_1/a$ space group and one molecule in the asymmetric unit. In case of DT two tautomeric forms can be considered. However in Cambridge Structural Database (CSD) only one of them; 1,8-dihydroksyantracen-9(10H)-on crystallized from mixture chloroform:acetone 3:1 v/v is deposited. According to X-ray data hydroxyl groups adjacent to carbonyl contribute to intra-molecular hydrogen bonding. Molecular packing is stabilized by number of $\text{CH}\cdots\text{O}$ intermolecular contacts. The CH_2 hydrogen of central ring is strongly involved in such interaction with carbonyl group of neighboring molecule.

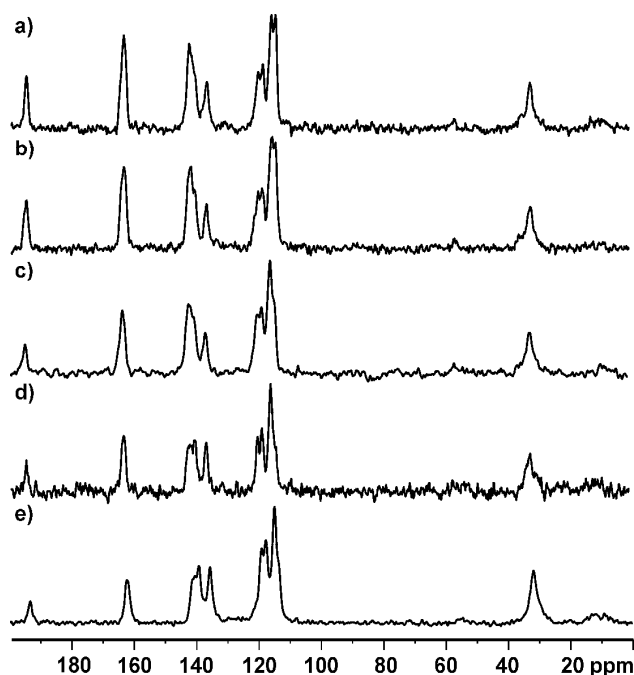


Figure 2

^{13}C CP/MAS spectra of different crystallization forms of DT recorded with 8 kHz spinning rate at room temperature: a) chloroform and acetone (3:1 v/v), b) chloroform, c) THF, d) DT : NaI (10:1); chloroform and acetone (3:1 v/v), e) DT : NaI (10:1); THF.

Figure 2a shows ^{13}C CP/MAS spectrum of sample crystallizes in chloroform:acetone (3:1 v/v) recorded with spinning rate 8 kHz at room temperature. The sample crystallized from chloroform (Figure 2b) and THF (Figure 2c) presents very similar pattern. Moreover, addition of sodium iodide in chloroform (Figure 2e) and THF (Figure 2f) does not cause visible changes of spectra. Hence it can be concluded that in conditions under investigation DT remains well defined crystalline sample. The formation of second tautomer is not observed.

c) **HABA** – in case of this matrix two isomers with *cis* and *trans* geometry with respect to N=N bond can be considered. In CSD crystal and molecular structure only *trans* isomer is deposited. X-ray single crystal determination indicated that HABA crystallizes in the $P2_12_12_1$ space group. Two phenyl rings are positioned in the *trans* form with respect to the azo double bond. The presence of the solvent water molecule plays a key role in its packing structure because it is involved in three kinds of O–H/O intermolecular hydrogen bonds. Both of the first two are found between phenolic oxygen atom in HABA and water molecule forming an infinite helical structure.

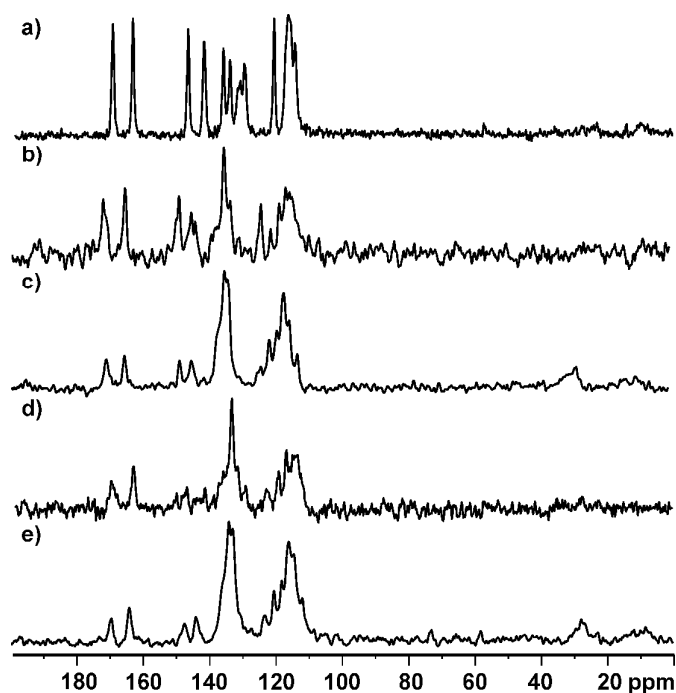


Figure 3

^{13}C CP/MAS spectra of different crystallization forms of HABA recorded with 8 kHz spinning rate at room temperature: a) ethanol and water (1:1 v/v), b) chloroform, c) THF, d) HABA : NaI (10:1); chloroform and acetone (3:1 v/v), e) HABA : NaI (10:1); THF.

Figure 3a shows ^{13}C CP/MAS spectrum of sample crystallized from mixture ethanol:water. The solubility of HABA in chloroform is very poor, only small amount of sample was available for NMR measurement (Figure 3b). In consequence, signal to noise ratio is much worse compared to sample a. On the other hand, it is apparent that lack of water in the crystal lattice of matrix has significant influence of molecular packing.

d) **IAA**- in contrast to matrices described above there is no X-ray data for IAA. From chemical point of view, two isomers of IAA with *cis* and *trans* geometry around C=C bond can be considered however there is no clear evidence confirming the presence of both isomers in the solid state.

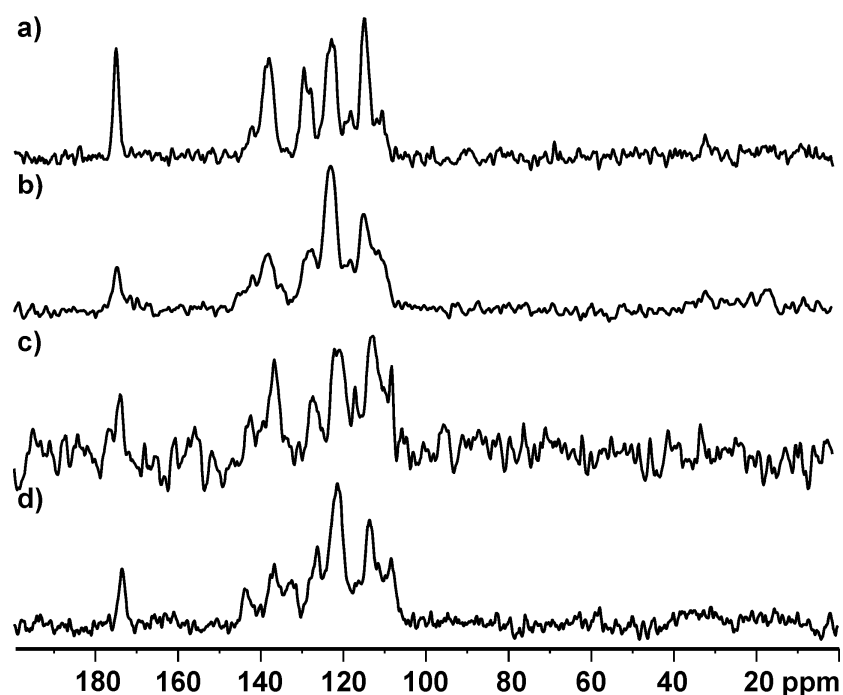


Figure 4

^{13}C CP/MAS spectra of different crystallization forms IAA recorded with 8kHz spinning rate at room temperature: a) chloroform and small amount of methanol, b) THF, c) IAA : NaI (10:1); chloroform:acetone and small amount of methanol, d) IAA : NaI (10:1); THF.

Our attempt to find the optimal solvent for growing up the monocrystals failed. Figure 4a shows the ^{13}C CP/MAS spectrum of IAA crystallized from chloroform with few drops of methanol. The resonance lines are rather broad, characteristic for amorphous phases. With exception of carboxyl group the assignment of vinyl and aromatic signals is unequivocal. The spectrum of IAA sample recrystallized from THF (Figure 4b) is very poor quality. Shown in Figure 4c IAA with NaI cationization agent crystallized from mixture of solvents is typical for disordered phases. Little bit better picture was recorded for sample crystallized from THF (Figure 4d).

ⁱ Haisa M., Kashino S., Handa S-H, Tanaka K., Okazaki S., Shibagaki M. *Acta Crystallogr B* 1982;38:1480;

ⁱⁱ Ahmed F.R. *Acta Crystallogr B* 1980;36:3184;

ⁱⁱⁱ Qiana H., Huang W., *J Mol Struct* 2005;743:191;