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Deuterium Isotope Effects in ^{13}C NMR Spectra of Intramolecularly Hydrogen-Bonded Salicylaldehyde-4-phenylthiosemicarbazone*

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• NMR
• DFT

The structure of salicylaldehyde-4-phenylthiosemicarbazone (**1**) has been studied by using NMR and DFT methods. It was demonstrated that in DMSO-d_6 and CD_2Cl_2 solution the molecule existed in the hydroxy-thione tautomeric form and that there was no proton transfer at ambient temperature. There was not a conclusive evidence for the thiol form although it could not be excluded. Partially deuterated molecule was prepared and deuterium isotope effects (DIE) on ^{13}C chemical shifts were measured and analyzed. DIEs have also pointed to the hydroxy-thione form as the dominant form in solution. Moreover, changes in DIEs and in chemical shifts observed in different solvents have pointed to interactions of **1** and solvent molecules *via* intermolecular hydrogen bonding in addition to the existing intramolecular interactions.

INTRODUCTION

Isotope effects in ^{13}C NMR spectra have proven to be an effective tool for studying the structure and dynamics of organic and biological molecules.[1–3] Most published papers discuss deuterium isotope effects on nuclear shielding, but also other isotopes and effects on different NMR parameters (coupling constant, relaxation times) have been investigated.[2] Deuterium is interesting because of its largest change in mass upon isotopic (proton/deuterium) substitution and the relative ease of its incorporation into a molecule. Secondary deuterium isotope effects (DIE) on ^{13}C chemical shifts can be easily determinated as the difference between chemical shifts of the non-deuterated and deuterated molecules:

$$^{n}\Delta^{13}C(D) = \delta^{13}C(H) - \delta^{13}C(D) \qquad (1)$$

where n is the number of bonds between the site of deuteration and the observed carbon atom. The magnitude and sign of isotope effects might depend on various structural factors such as hybridization, conjugation, torsional angle, resonance, *etc*.[1–3] It is now generally accepted that the intrinsic isotope effects are of rovibrational origin[4,5] and are due to the anharmonicity of the potential curve of the X-H(D) bond. However, it has been demonstrated that in conjugated π-electron molecules isotopic perturbations can be observed many bonds away from the isotopic site (up to twelve, which was accounted for by subtle charge shifts throughout the molecule as a consequence of bond-length shortening and change in vibrational averaging upon isotopic substitution.[6–10] Isotope effects were also shown to be affected by the presence of hydrogen bonds and this feature can

* Dedicated to Professor Nikola Kallay on the occasion of his 65th birthday.

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be used to study both intra- and intermolecular H-bonding interactions.[1] In hydrogen bonded systems, isotope effects may have intrinsic and equilibrium effect contributions.

Owing to their interesting properties and practical application, isotope effects in Schiff bases of *o*-hydroxy aromatic types have been studied intensively over the last ten years.[11–16] These are to a large extent controlled by intramolecular H-bonds. It has been demonstrated that $^2\Delta^{13}$C-2 (deuterium isotope effect over two bonds on carbon atom linked to the OH group) can be related to proton transfer and tautomeric equilibrium.

Salicylaldehyde-4-phenylthiosemicarbazone (**1**) (Figure 1) is a Schiff base derived from salicylaldehyde and 4-phenylthiosemicarbazide and belongs to an important class of molecules possessing biological activity.

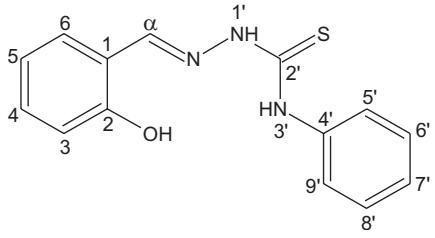

Figure 1. The structure and atom numbering of **1**.

Thiosemicarbazones and their metal complexes have been shown to possess significant anticancer, antiviral, antibacterial, anti-inflammatory, antiamoebic and antifungal activities.[16–22] Bioactivity is greatly influenced by structural properties and is commonly increased in complexes with transition metal ions. Thiosemicarbazones might exist in the tautomeric thione and thiol forms (Figure 2) and can therefore act both as neutral and anionic bidentate ligands (azomethine N and S as donors).

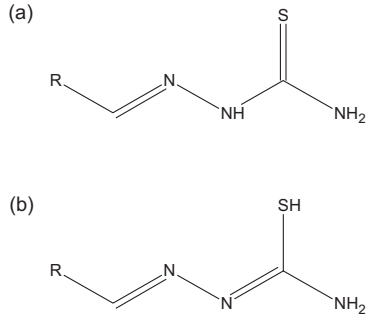

Figure 2. Thiosemicarbazone tautomers: (a) thione, (b) thiol.

Apart from their pharmacological importance, thiosemicarbazones have considerable analytical significance owing to their high selectivity for some transition metals. Applications of salicylaldehyde thiosemicarbazones as ionophores in mercury(II) ion-selective electrodes[23] as well as in adsorptive stripping voltammetry for determination of copper(II)[24] have been reported.

In the present paper, we report NMR structure elucidation and investigation of H-bonding interactions in **1**. DIEs were employed to gauge the proton transfer process and tautomerism. In order to explore the solvent and temperature dependence of DIE, factors found to affect the hydrogen bond equilibrium, we used two solvents with different proton donor or acceptor abilities and measured the effects at different temperatures. To relate the observed results with structure, we performed DFT calculations for geometry optimization of **1** and for prediction of its chemical shifts.

EXPERIMENTAL

Materials

The starting materials salicylaldehyde, 4-phenylthiosemicarbazide and methanol-d_4 were purchased from commercial sources and used without further purification. Methanol was dried using magnesium turnings and iodine and then distilled. Dichloromethane was dried using P_2O_5.

Salicylaldehyde-4-phenylthiosemicarbazone was prepared by the procedure published previously.[25] Salicylaldehyde-4-phenylthiosemicarbazone (150 mg) was refluxed in methanol-d_4 (4 mL) and, after standing overnight, the white precipitate was filtered off and dried over KOH. The percentage of deuteration, determined by mass spectrometry, was about 60 %.

Further, attempts to prepare monodeuterated (OD) salicylaldehyde-4-phenylthiosemicarbazone isotopomer included variation of the mentioned general procedure.[25] As a first step, we successfully prepared deuterated salicylaldehyde (confirmed by NMR) by dissolving salicylaldehyde (0.31 g; 2.5 mmol) in methanol-d_4 (1.4 mL) and then completely evaporating the solvent under vacuo. To deuterated salicylaldehyde, 20 mL of dichloromethane, molecular sieves (which were used to remove water released during the reaction of condensation of salicylaldehyde and 4-phenylthiosemicarbazide) and 4-phenylthiosemicarbazide (0.42 g; 2.5 mmol) were added. The reaction mixture was then stirred and gently warmed for approximately 4 h. After the reaction, molecular sieves were removed by filtration and the reaction mixture was left to stand overnight. The resulting white needle-like product was collected by filtration and dried over KOH. Unfortunately, instead of monodeuterated, a partially trideuterated sample was obtained owing to the fast exchange reaction with OHD formed during the reaction.

NMR Measurements

NMR spectra were recorded on a Bruker Avance DRX300 spectrometer equipped with *z*-gradient accessories and operating at 300.13 for ^{1}H and 75.47 for ^{13}C. Samples were

measured at 298 K in 5 mm NMR tubes. DMSO-d_6 (ε_r = 46.7) and CD_2Cl_2 (ε_r = 8.9) were used as solvents and TMS as internal standard. Sample concentration was 1.2×10^{-2} mol dm^{-3} both in DMSO-d_6 and CD_2Cl_2. Concentration dependent measurements in DMSO-d_6 were performed in the range 1.6×10^{-1}–3.7×10^{-3} mol dm^{-3}. Variable temperature measurements in DMSO-d_6 were performed in the range 298–373 K.

Proton spectra with spectral width of 6200 Hz and a digital resolution of 0.09 Hz per point were measured with 8–16 scans.

DIEs on ^{13}C chemical shifts were measured in one-tube experiments. Narrow region APT and PENDANT spectra with spectral widths of 7000 Hz and digital resolution of 0.11 and 0.17 Hz per point, respectively, were collected. The number of scans was 1500-12000. Signs of isotope effects were determined by quantitative addition of non-deuterated **1** into the NMR tube with deuterated sample.

For complete assignment of the ^{1}H and ^{13}C NMR signals, two-dimensional homo- and heteronuclear correlation experiments (COSY, HSQC and HMBC) were performed. In the COSY experiment, 2046 points in the *f*2 dimension and 512 increments in the *f*1 dimension were used. For each increment, 8 scans and a spectral width of 4007 Hz were applied. Digital resolution was 1.97 and 7.82 Hz per point in *f*2 and *f*1 dimensions, respectively. Typical spectral conditions for HSQC and HMBC spectra were as follows. Spectral width was 3906 Hz in *f*2 and 18870 Hz in *f*1 dimension for both experiments. 2K data points were applied in the time domains and for each data set 157 and 246 increments were collected for HSQC and HMBC spectra, respectively. The resulting digital resolution was 3.81 Hz per point in *f*2 dimension and 34.3 and 36.9 Hz per point in *f*1 dimension in HSQC and HMBC spectra, respectively.

Calculations

Equilibrium geometries of thiosemicarbazone forms were calculated using the B3LYP density functional method[26,27] and 6–311++G(3df,3pd) basis set. The solvent effect of DMSO and dichloromethane was introduced in the calculations using the reformulation of PCM[28,29] known as integral equation formalism (IEFPCM) of Tomasi and coworkers.[30–33] All calculations were performed using GAUSSIAN 03 package.[34] The *in vacuo* and in solvent equilibrium geometries of tetramethysilane (TMS) were calculated using the same basis set as above. All the NMR shieldings *in vacuo* and in solvent were calculated using GIAO and PCM GIAO[35,36] methods on the previously optimized geometries.

RESULTS AND DISCUSSION

NMR Assignments and Structure Elucidation

Proton and carbon chemical shifts assignments were made by the combined use of one- (^{1}H, APT or PENDANT) and two-dimensional NMR experiments (COSY, HSQC and HMBC). Chemical shifts in CD_2Cl_2 and DMSO-d_6 solutions are given in Table I.

Table I. ^{1}H and ^{13}C chemical shifts (ppm)[(a)] of **1** in CD_2Cl_2 and DMSO–d_6

Atom	CD_2Cl_2		DMSO–d_6	
	^{1}H δ / ppm	^{13}C δ / ppm	^{1}H δ / ppm	^{13}C δ / ppm
1		117.52		120.15
2		157.74		156.49
3	6.99	117.20	6.88	115.92
4	7.37	132.92	7.23	131.20
5	6.98	120.72	6.84	119.10
6	7.33	132.07	8.08	129.96
α	8.08	147.45	8.48	139.95
1'	9.51		11.75	
2'		176.44		175.61
3'	8.43		10.03	
4'		138.15		139.04
5', 9'	7.57	125.60	7.57	125.56
6' 8'	7.43	129.26	7.36	127.90
7'	7.30	127.06	7.19	125.04
OH	9.33		9.96	

[(a)] $c = 1.2 \times 10^{-2}$ mol dm^{-3}

As already mentioned, thiosemicarbazones might exist in the two tautomeric forms shown in Figure 2. Additional tautomers are possible for **1**, *e.g.*, hydroxy-thione and keto-thione forms, owing to the proton transfer in the intramolecular OH· · ·N hydrogen bond (Figure 3).

X-ray data[37] have shown that in the solid state **1** has adopted the hydroxy-thione form, as depicted in Figure 1. Chemical shift values (Table I) also indicate that **1** existed in the hydroxy-thione form in both DMSO-d_6 and CD_2Cl_2 solutions at ambient temperature. Hence, the chemical shifts of C-2 and C-2' atoms have been assigned at ≈157 and ≈176 ppm, respectively. The later chemical shift value is an intermediate one between the thione and thiol forms usually observed for thiourea derivatives, and hence the thiol form could not be completely excluded. The C-2 site has been reported to be very sensitive to the

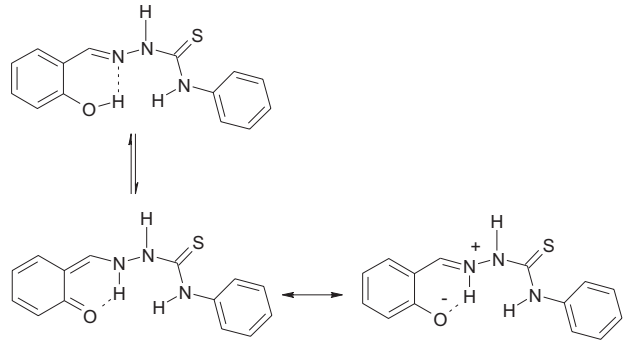

Figure 3. Resonance and proton transfer forms of the OH· · ·N hydrogen bonded **1**.

Table II. Concentration effects on some proton chemical shifts (ppm) in DMSO–d_6 at 298 K

Atom	c / mol dm^{-3}						
	1.6×10^{-1}	1.1×10^{-1}	5.5×10^{-2}	2.5×10^{-2}	1.5×10^{-2}	7.4×10^{-3}	3.7×10^{-3}
H-α	8.529	8.499	8.492	8.489	8.484	8.486	8.486
NH1'	11.788	11.767	11.762	11.759	11.746	11.757	11.756
NH3'	10.065	10.048	10.044	10.042	10.032	10.040	10.040
OH	9.986	9.974	9.971	9.968	9.957	9.939	9.962

position of the proton transfer equilibrium.[13,15] In the proton transferred NH-forms, C-2 was found to be much more deshielded (≈180 ppm).[15,38] This is in accord with our DFT calculations (see later in the text). C-α chemical shifts of ≈147 ppm and ≈140 ppm were observed in CD_2Cl_2 and DMSO-d_6, respectively, reflecting the predominant population of the hydroxy tautomer. The OH signal was broad and was found at ≈ 9–10 ppm, which was lower than the usual values observed for similar Schiff bases (13–16 ppm)[11–16] and was mostly due to the shielding effect of the thiourea group and extended π-electron delocalization in the molecule. No splitting of H-α was observed as it could be expected for the proton transferred NH-forms.[12,13] The thiourea H-1' protons were found at 9.51 and 11.75 ppm while H-3' were at 8.43 and 10.03 ppm in CD_2Cl_2 and DMSO-d_6, respectively (Table I). These are the values usually found for similar thiosemicarbazones.[39] The HMBC spectrum revealed correlation peaks between H-1' and C-α as well as between H-3' and C-5',9', which were only possible for the thione form. The COSY spectrum exhibited correlation peaks characteristic of the hydroxy-thione form as shown in Figure 1, including a long-range correlation between the imino-proton H-α and NH1' proton, thus further corroborating the presence of the thione form.

Hydrogen Bonding

X-ray analysis has revealed the existence of two intramolecular OH· · ·N=C-α and NH3'· · ·N=C-α hydrogen bonds with donor-acceptor bond lengths of 2.726(2) and 2.675(2) Å, respectively.[37] Comparison of proton and carbon chemical shifts in non-polar CD_2Cl_2 and polar DMSO-d_6 reveals some important and significant differences (Table I). The largest chemical shift difference was observed for the C-α amounting to –7.5 ppm (shielding effect) on going from CD_2Cl_2 to DMSO-d_6. The C-6 atom was also shielded (up-field shift) by –2.11 ppm, whereas C-1 underwent a down-field shift of 2.63 ppm. Both NH1' and NH3' protons in DMSO-d_6 were shifted to lower fields by 2.24 ppm and 1.60 ppm compared to CD_2Cl_2 and were observed at 11.75 ppm and 10.03 ppm, respectively. The chemical shift change for the OH proton was much smaller (0.63 ppm). These changes in shielding are a consequence of perturbations in the hydrogen bonding network caused by the influence of the solvent. Namely, significant deshielding effects observed for NH1', NH3' and H-α, protons point to interactions of these protons and DMSO-d_6 *via* intermolecular hydrogen bonds in addition to the existing intramolecular interactions. Further, the H-α resonance signal was significantly broadened in DMSO-d_6 compared to CD_2Cl_2, which supports the above conclusion. As a consequence of these interactions, electron density redistribution occurred and subsequently C-α exhibited a noticeable up-field shift in DMSO-d_6 (Table I). It is seen from Table II that slight changes in proton chemical shifts are observed for different concentrations of **1**, being the largest for H-α, and NH1'. Further, raising the temperature from 298 to 373 K caused up-field shifts and had the most prominent effect on NH1'(Table III). These findings provide further evidence for intermolecular hydrogen bonds with DMSO revealing H-α, and NH1' as the most probable interactive sites. More detailed theoretical studies aimed at better description and understanding of intermolecular hydrogen-bonding interactions between **1** and solvent molecules have been undertaken and are in progress.[37]

Table III. Temperature effects on some proton chemical shifts (ppm)[(a)] in DMSO-d_6

Atom	^{1}H δ / ppm		$\Delta^{(b)}$ / ppm
	298 K	373 K	
H-α	8.484	8.481	–0.003
NH1'	11.746	11.351	–0.395
NH3'	10.032	9.760	–0.272
OH	9.957	9.662	–0.295

[(a)] $c = 1.5 \times 10^{-2}$ mol dm^{-3}
[(b)] $\Delta = \delta_H$ (373 K) – δ_H (298K)

Geometry Optimization and Calculation of Chemical Shifts

Ground-state geometries for the hydroxy-thione and keto-thione forms of **1** were calculated using the density functional theory (DFT) with B3LYP functional and 6–311++G(3df,3pd) basis set. The optimized geometries

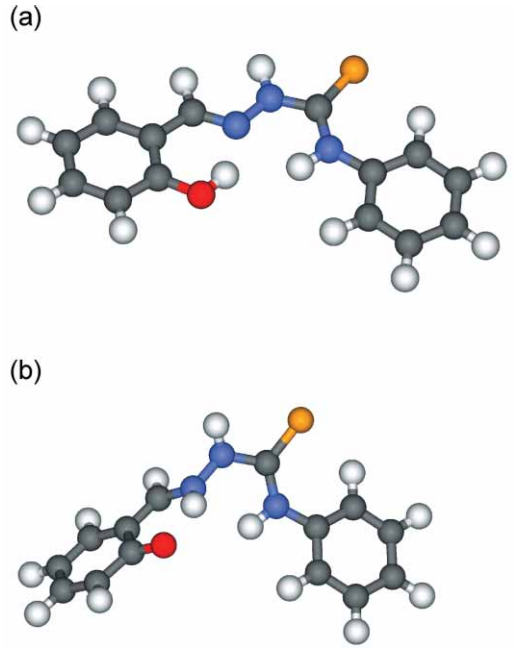

Figure 4. Geometries of (a) hydroxy- and (b) keto-thione forms of **1** optimized at the B3LYP/6–311++G(3df,3pd) level of theory.

of the hydroxy-thione and keto-thione forms of **1** are depicted in Figure 4.

The geometrical parameters obtained for the hydroxy-thione form are in a good agreement with experimental data[37] as far as bond lengths are considered, while the torsional angles exhibit deviations. The calculated parameters show that the hydroxy-thione form is almost planar while the experimental data reveal some twisting of the salicylaldehyde and phenyl rings.[37] Deviations of the calculated values from the experimental ones are caused by crystal packing.

To obtain calculated results comparable with the experimental data, we have transformed the absolute shieldings returned by the program in chemical shifts subtracting to the absolute shielding of TMS the absolute shieldings of the molecule examined.

$$\delta_{rel} = \sigma_{TMS} - \sigma_{abs}$$

The same methodology was adopted in calculations of the chemical shifts in DMSO and dichloromethane.

The calculated isotropic chemical shifts for hydroxy-thione and keto-thione forms are displayed in Table IV. The calculated shifts in dichloromethane and DMSO for the hydroxy-thione form are in better agreement with those experimentally observed, especially for C-2, C-α and C-2' carbons.

Deuterium Isotope Effects

DIEs were determined from the mixtures of partially deuterated **1** prepared as described above and the non-deuterated sample, using equation (1). The sign of DIE was determined by quantitative addition of the non-deuterated sample in the NMR tube of the deuterated sample, as demonstrated in Figure 5. DIEs are given in Table V. In a series of Schiff bases derived from *ortho*-hydroxy aromatic aldehydes, high positive values of DIE at C-2 (≈ 300–500 ppb) and a linear dependence of ln $^2\Delta$C-2(D) *vs.* δOH were observed, indicating the absence of proton transfer equilibrium and the presence of the localized OH· · ·N hydrogen bond.[11–13] On the other hand, increasing the acidity of OH and basicity of the imine-N atom as well as lowering the temperature facilitated the proton transfer equilibrium. For the proton transferred forms, high positive and negative values of ΔC-2(D) were observed

Table IV. Calculated GIAO and PCM GIAO ^{13}C chemical shifts (δ in ppm from TMS) for hydroxy-thione (HT) and keto-thione (KT) forms of **1**

Atom	HT[(a)]	HT in DMSO	HT in CH_2Cl_2	KT[(a)]	KT in DMSO	KT in CH_2Cl_2
1	123.01	124.75	124.29	121.36	122.45	122.33
2	167.44	167.45	167.32	190.73	191.44	191.33
3	121.48	122.88	122.84	130.81	130.53	130.81
4	138.74	140.77	140.51	145.00	147.96	147.50
5	124.03	125.84	125.63	120.05	120.45	120.46
6	137.45	140.07	139.55	139.93	145.24	144.28
α	147.03	156.37	154.87	167.71	173.35	172.44
2'	178.72	186.33	184.92	187.90	192.13	192.64
4'	146.67	146.06	144.90	145.34	146.66	145.36
5'	123.80	132.86	132.44	125.89	136.91	136.77
6'	134.35	135.81	136.18	134.12	135.86	136.32
7'	129.83	134.17	133.62	130.23	136.41	135.98
8'	133.44	137.14	136.80	133.69	136.87	136.96
9'	124.27	129.44	128.58	124.41	134.39	132.31

[(a)] *in vacuo*

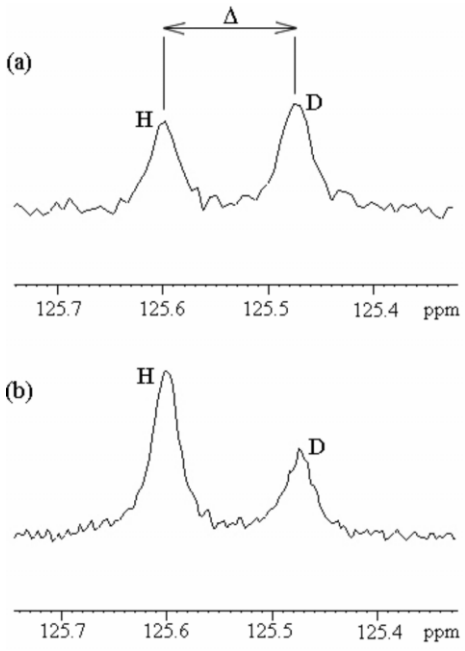

Figure 5. Determination of the deuterium isotope effect at the C-5',9' position. The narrow region APT spectrum of the partially deuterated **1** (a) before and (b) after addition of the non-deuterated **1**.

and no linear dependence of ln $^2\Delta$C-2(D) on δOH was found.[11–14] Also, DIEs were temperature dependent with an increase in the population of the proton transferred NH-form at lower temperatures.

As seen in Table V, DIEs are all positive in **1**, except for that observed at C-5, which was negative, as already found for some substituted salicylideneanilines.[11] The DIE at C-α in DMSO-d_6 could not be determined due to substantial line broadening. $^2\Delta$C-2(D) was positive in both CD_2Cl_2 and DMSO-d_6 and amounted to 140 ppb and 170 ppb, respectively. Comparison of DIEs in **1** with those obtained in similar systems[11–15] clearly indicates that **1** exists in the hydroxy-thione form with the intramolecular OH· · ·N hydrogen bond (Figure 3), which is in agreement with the observed chemical shift values and X-ray analysis. However, $^2\Delta$C-2(D) was found not to correlate with δOH as was reported for Schiff bases with localized OH-forms.[11] This is likely to be due to the intermolecular hydrogen bonding with solvent molecules, especially in the case of DMSO-d_6 being a strong hydrogen bond acceptor. Changes in chemical shifts and in DIEs observed in DMSO-d_6 with respect to those in CD_2Cl_2 support this suggestion. For more precise determination of this dependence, a correction for the diamagnetic effects should be taken into account.[11] Furthermore, the largest changes in DIE were observed for C-2' and C-α (the C-α signal was much narrower in CD_2Cl_2 than in DMSO-d_6 enabling the DIE to be determined) as a consequence of intermolecular interactions with solvent molecules. Variable temperature NMR experiments (283-328 K) have shown slight changes in DIEs, which supports the above conclusion.

Table V. Deuterium isotope effects Δ (ppb) on ^{13}C chemical shifts of **1** in CD_2Cl_2 and DMSO-d_6 and their difference $\Delta\Delta^{(a)}$ (ppb)

Atom	Δ / ppb		$\Delta\Delta^{(a)}$ / ppb
	CD_2Cl_2	DMSO-d_6	
1	56	68	12
2	140	170	30
3	84	105	21
4	–	–	–
5	–27	–34	–7
6	–	–	–
α	62	Broad	–
2'	81	122	41
4'	105	130	25
5',9'	135	125	–10
6',8'	–	–	
7'	35	–	–

[(a)] $\Delta\Delta = \Delta(\text{DMSO-d}_6) - \Delta(CD_2Cl_2)$

Finally, some caution should be exercised concerning the determination of DIEs. Namely, DIEs obtained in **1** were cumulative, *i.e.*, they arose from the simultaneous influence of three deuterium atoms present in the molecule (OD, N1'D and N3'D). Although a contribution of long-range effects (those transmitted over more than three bonds away from the isotopic site) is usually small, it can be both positive and negative and can therefore increase or decrease the measured effect. Unfortunately, attempts to prepare a monodeuterated OD isotopomer failed, as described in the experimental part.

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SAŽETAK

Deuterijski izotopni efekti u ^{13}C NMR spektrima salicilaldehid-4-feniltiosemikarbazona s intramolekulskom vodikovom vezom

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Struktura salicilaldehid-4-feniltiosemikarbazona (**1**) istražena je primjenom NMR i DFT metoda. Dokazano je, da u otopini DMSO-d_6 i CD_2Cl_2 molekula postoji u hidroksi-tionskom tautomernom obliku te da izostaje prijenos protona pri sobnoj temperaturi. Nije pronađen direktan dokaz o prisutnosti tiolne forme u otopini, premda se njezino postojanje ne treba u potpunosti isključiti. Pripravljen je djelomično deuterirani spoj te su izmjereni i analizirani deuterijski izotopni efekti (DIE) na ^{13}C kemijske pomake. DIE također ukazuju na hidroksi-tionski tautomer kao dominantni oblik u otopini. Štoviše, promjene u kemijskim pomacima i DIE, opaženi u različitim otapalima, upućuju na postojanje kako intramolekulskih interakcija tako i intermolekulskih vodikovih veza između **1** i molekula otapala.