

## A complete $^1\text{H}$ and $^{13}\text{C}$ NMR data assignment for two substituted fluorenylspirohydantoin

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This work presents the spectral characterization of 1',3'-bis(hydroxymethyl)-2'*H*,5'*H*-spiro[fluorene-9,4'-imidazolidine]-2',5'-dione and 2-bromo-2'*H*,5'*H*-spiro[fluorene-9,4'-imidazolidine]-2',5'-dione. The structures of the substituted fluorenylspirohydantoin were verified by  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR spectroscopy. In addition, 2D NMR spectra, including  $^1\text{H}$ - $^1\text{H}$  COSY, HMQC, HMBC and  $^1\text{H}$ - $^1\text{H}$  NOESY sequences, were used for a complete assignment of the  $^1\text{H}$  and  $^{13}\text{C}$  chemical shifts for each compound.

**Keywords:** 1',3'-bis(hydroxymethyl)-2'*H*,5'*H*-spiro[fluorene-9,4'-imidazolidine]-2',5'-dione, 2-bromo-2'*H*,5'*H*-spiro[fluorene-9,4'-imidazolidine]-2',5'-dione, NMR.

### INTRODUCTION

Hydantoin usually serve as a base for synthesis of numerous biologically active derivatives [1]. Such examples are the fluorenylspirohydantoin where the hydantoin ring shares a carbon (C-5(9'), i.e. spiro-carbon) with the fluorene substituent (**1** and **2**). The compounds from this class possess antitumor and antibacterial activity [2-6] which explains their use as constituents in various pharmaceutical products. Also, they were found to coordinate metal ions which led up to the synthesis of some biologically active copper (II), nickel (II), platinum (II) and ruthenium (III) complexes [7-11]. It should be mentioned that the fluorenylspirohydantoin can act as aldose reductase inhibitors [12-14]. In addition, the fluorene-containing compounds find application in the manufacturing of organic light emitting diodes (OLED) [15].

The aim of this work was the structure verification of two substituted fluorenylspirohydantoin (**1** and **2**) by means of one- and two-dimensional NMR techniques, including  $^1\text{H}$ - $^1\text{H}$  COSY, HMQC, HMBC and  $^1\text{H}$ - $^1\text{H}$  NOESY sequences. 2D NMR spectra were proved to be useful for the verification of large and complex organic structures showing the correlations (spin couplings) between nuclei of the same or different kind in a given molecule [16, 17]. Thus, valuable information can be extracted from the different types of 2D NMR spectra about the neighborhood and

bonding of nuclei in complex structures, as it was reported in [16]. For example, the  $^1\text{H}$ - $^1\text{H}$  COSY spectrum shows mainly the spin couplings between vicinal hydrogens separated by three bonds ( $^3J_{\text{HH}}$ ). Both HMQC (Heteronuclear Multiple Quantum Coherence) and HSQC (Heteronuclear Single Quantum Coherence) spectra provide information about the  $^1J_{\text{CH}}$  couplings between  $^{13}\text{C}$  nuclei and protons attached to the corresponding carbons. Although HSQC ensures better  $^{13}\text{C}$  resolution along the  $\nu_1$ -axis, it is used much less often, compared to HMQC, due to the following disadvantages [18-20]. Firstly, the HSQC experiment requires more than twice as many pulses as the basic HMQC sequence. Secondly, HSQC is more sensitive to variations in the inhomogeneity of the applied magnetic field and RF pulse to the sample and is more susceptible to artifacts than HMQC. Therefore, it is accepted that HMQC is more robust to experimental imperfections and changes than HSQC. As a result, it could be assumed that the single bond  $^1\text{H}$ - $^{13}\text{C}$  couplings in a given structural fragment that is present in similar complex organic structures can be detected with higher reproducibility when HMQC is used instead of HSQC.

On the other hand, the HMBC (Heteronuclear Multiple-Bond Coherence) spectrum reveals the spin couplings between  $^1\text{H}$  and  $^{13}\text{C}$  nuclei separated by two or more chemical bonds. Finally, the  $^1\text{H}$ - $^1\text{H}$  NOESY (Nuclear Overhauser Effect Spectroscopy)

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spectrum shows the homonuclear correlations between protons spatially located from each other at a distance  $<5 \text{ \AA}$  [16]. All of this kind of various and valuable information, provided by the 2D NMR spectra, was used to fully assign the  $^1\text{H}$  and  $^{13}\text{C}$  NMR chemical shifts for 1',3'-bis(hydroxymethyl)-2'H,5'H-spiro[fluorene-9,4'-imidazolidine]-2',5'-dione (**1**) and 2-bromo-2'H,5'H-spiro[fluorene-9,4'-imidazolidine]-2',5'-dione (**2**).

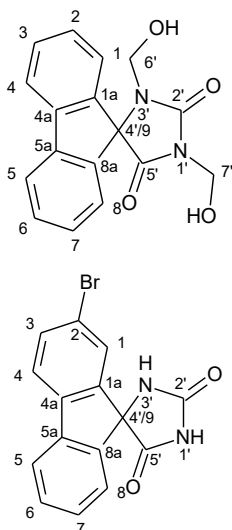


Fig. 1. Studied compounds **1**(top) and **2**(bottom)

## EXPERIMENTAL

### Instrumentation

Digital apparatus (SMP10) and Koffler apparatus were used for measuring the melting points of the synthesized compounds. The UV-Vis spectra were measured on a Perkin-Elmer Lambda 9 UV/Vis/NIR spectrophotometer from 200 nm to 1000 nm. The IR spectra were registered in KBr pellets on a VERTEX 70 FT-IR (Bruker Optics) spectrometer from 4000  $\text{cm}^{-1}$  to 400  $\text{cm}^{-1}$  at a resolution of 4  $\text{cm}^{-1}$  with 9 scans. The Raman spectra (the stirred crystals placed in aluminium disc) were measured on RAM II (Bruker Optics) with a focused laser beam of 50 mW power of a Nd:YAG laser (1064 nm) from 4000  $\text{cm}^{-1}$  to 51  $\text{cm}^{-1}$  at a resolution of 2  $\text{cm}^{-1}$  with 25 scans. The Attenuated Total Reflection FTIR (ATR) spectra were recorded with a VERTEX 70 FT-IR spectrometer (Bruker Optics). The ATR accessory is MIRacle™ with a one-reflection ZnSe element (PIKE Technology) and the stirred crystals were pressed by an anvil to the reflection element; the spectra are from 4000  $\text{cm}^{-1}$  to 600  $\text{cm}^{-1}$  at a resolution of 2  $\text{cm}^{-1}$  with 16 scans.

$^1\text{H}$ ,  $^{13}\text{C}$  and 2D NMR spectra of 1',3'-bis(hydroxymethyl)-2'H,5'H-spiro[fluorene-9,4'-imidazolidine]-2',5'-dione and 2-bromo-2'H,5'H-spiro[fluorene-9,4'-imidazolidine]-2',5'-dione were

recorded on a Bruker Avance II + 600MHz NMR spectrometer operating at 600.130MHz ( $^1\text{H}$ ) and 150.903MHz ( $^{13}\text{C}$ ), using TMS as internal standard and DMSO- $d_6$  as solvent. The temperature was kept at 293.0 K for all NMR experiments. Chemical shifts are expressed in ppm and coupling constants (J) in Hertz. 1D and 2D NMR spectra were recorded using the standard Bruker pulse programs.

### Materials

Puriss p.a. grade reagents purchased from Merck and Sigma-Aldrich were used in this study

#### Synthesis of 1',3'-bis(hydroxymethyl)-2'H,5'H-spiro[fluorene-9,4'-imidazolidine]-2',5'-dione

1',3'-Bis(hydroxymethyl)-2'H,5'H-spiro[fluorene-9,4'-imidazolidine]-2',5'-dione was obtained in crystal-like form by refluxing a mixture of (9'-fluorene)-spiro-5-hydantoin (5 g, 0.02 mol) and formalin (37%, 35  $\text{cm}^3$ ) for 6 hours. The product was filtered off and subsequently recrystallized from ethyl acetate/petroleum ether. The achieved yield was 4.60 g (82%). Melting point = 181–182°C;  $R_f$  = 0.65 (used mobile phases in a given volume ratio - ethyl acetate: petroleum ether = 1:2).

#### Synthesis of 2-bromo-2'H,5'H-spiro[fluorene-9,4'-imidazolidine]-2',5'-dione

2.55  $\text{cm}^3$  (8 g, 0.05 mol) of  $\text{Br}_2$  in 25  $\text{cm}^3$  of glacial  $\text{CH}_3\text{COOH}$  was added dropwise to an aqueous suspension of (9'-fluorene)-spiro-5-hydantoin (5 g, 0.02 mol) in a hot glacial  $\text{CH}_3\text{COOH}$  (250  $\text{cm}^3$ ) and 2 g  $\text{FeCl}_3$ . The resulting mixture was stirred for 2.5 hours, heated once at 90–91 °C for 18 hours, then heated again at 100 °C for another 9 hours and diluted with water. The obtained crystals were filtered off and recrystallized from ethanol. The achieved yield was 4.60 g (70%). Melting point = 352–353 °C,  $R_f$  = 0.63 (used mobile phases in a given volume ratio - chloroform: methanol = 9:1). A modification of the procedure of Pan and Fletcher [2] was used to synthesize the compound 2-bromo-2'H,5'H-spiro[fluorene-9,4'-imidazolidine]-2',5'-dione. Thin layer chromatography, performed on Kieselgel 60 F<sub>254</sub>, 0.2 mm Merck plates, was applied to determine the purity of both compounds.

#### UV-Vis and IR spectral data for 1',3'-bis(hydroxymethyl)-2'H,5'H-spiro[fluorene-9,4'-imidazolidine]-2',5'-dione

UV-Vis (DMSO):  $\lambda_{\text{max}}$  = 273 nm.

IR (KBr,  $\text{cm}^{-1}$ ): 3377 ( $\nu(\text{CO-H})$ ), 3331 ( $\nu(\text{CO-H})$ ), 3063 ( $\nu(\text{C-H})$ , arom.), 3010 ( $\nu(\text{C-H})$ , arom.), 2948 ( $\nu_{\text{as}}(\text{CH}_2)$ ), 2925 ( $\nu_{\text{as}}(\text{CH}_2)$ ), 2900 ( $\nu_{\text{s}}(\text{CH}_2)$ ), 2853 ( $\nu_{\text{s}}(\text{CH}_2)$ ), 1770 ( $\nu(\text{C=O})$ ), 1704 ( $\nu(\text{C=O})$ ),

1585, 1465, 1450, 1435, 1370, 1344, 1333, 1290, 1244, 1214, 1179, 1164, 1081( $\nu(\text{CH}_2\text{-OH})$ ), 1066, 1042, 1032 ( $\nu(\text{CH}_2\text{-OH})$ ), 1000, 952, 924, 873, 868, 789, 775, 738, 722, 651, 627, 602, 544, 515, 494, 461, 418.

*Raman spectral bands,  $\nu_{\text{max}}$  ( $\text{cm}^{-1}$ ):* 3086 ( $\nu(\text{C-H})$ , arom.), 3071 ( $\nu(\text{C-H})$ , arom.), 3043 ( $\nu(\text{C-H})$ , arom.), 2949 ( $\nu_{\text{as}}(\text{CH}_2)$ ), 1769 ( $\nu(\text{C=O})$ ), 1606, 1585, 1488, 1357, 1299, 1242, 1215, 1184, 1159, 1151, 1106, 1080 ( $\nu(\text{CH}_2\text{-OH})$ ), 1023 ( $\nu(\text{CH}_2\text{-OH})$ ), 1001, 872, 790, 753, 689, 621, 603, 548, 515, 423.

*ATR spectral bands,  $\nu_{\text{max}}$  ( $\text{cm}^{-1}$ ):* 3372 ( $\nu(\text{CO-H})$ ), 3319 ( $\nu(\text{CO-H})$ ), 3063 ( $\nu(\text{C-H})$ , arom.), 3011 ( $\nu(\text{C-H})$ , arom.), 2948 ( $\nu_{\text{as}}(\text{CH}_2)$ ), 1768 ( $\nu(\text{C=O})$ ), 1699 ( $\nu(\text{C=O})$ ), 1584, 1464, 1450, 1424, 1368, 1343, 1332, 1289, 1243, 1213, 1179, 1164, 1108, 1078 ( $\nu(\text{CH}_2\text{-OH})$ ), 1065, 1041, 1030 ( $\nu(\text{CH}_2\text{-OH})$ ), 1000, 951, 924, 873, 868, 788, 774, 736, 649, 620.

*UV-Vis and IR spectral data for 2-bromo-2'H,5'H-spiro[fluorene-9,4'-imidazolidine]-2',5'-dione*

*UV-Vis (DMSO):*  $\lambda_{\text{max}} = 282 \text{ nm}, 255 \text{ nm}$ .

*IR (KBr,  $\text{cm}^{-1}$ ):* 3349 ( $\nu(\text{N-H})$ ), 3194 ( $\nu(\text{N-H})$ ), 3069 ( $\nu(\text{C-H})$ , arom.), 2975 ( $\nu_{\text{as}}(\text{CH}_2)$ ), 2753, 1782 ( $\nu(\text{C=O})$ ), 1756 ( $\nu(\text{C=O})$ ), 1717 ( $\nu(\text{C=O})$ ), 1607, 1483, 1467, 1449, 1398, 1294, 1242, 1204, 1179, 1156, 1130, 1113, 1086, 1052, 1023, 982, 953, 884, 826, 793, 782, 750, 734, 723, 693, 661, 655, 638, 564, 527, 510, 476, 447, 428.

*Raman spectral bands,  $\nu_{\text{max}}$  ( $\text{cm}^{-1}$ ):* 3071 ( $\nu(\text{C-H})$ , arom.), 3050 ( $\nu(\text{C-H})$ , arom.), 1763 ( $\nu(\text{C=O})$ ), 1606, 1576, 1484, 1349, 1295, 1265, 1230, 1202, 1157, 1128, 1114, 1063, 1029, 989, 941, 762, 730, 692, 644, 529, 441.

*ATR spectral bands,  $\nu_{\text{max}}$  ( $\text{cm}^{-1}$ ):* 3354 ( $\nu(\text{N-H})$ ), 3185 ( $\nu(\text{N-H})$ ), 3073 ( $\nu(\text{C-H})$ , arom.), 2716, 2369, 1951, 1779 ( $\nu(\text{C=O})$ ), 1713 ( $\nu(\text{C=O})$ ), 1606, 1576, 1507, 1484, 1466, 1449, 1395, 1293, 1264, 1235, 1202, 1179, 1156, 1129, 1113, 1087, 1062, 1015, 988, 953, 868, 823, 759, 742, 732, 718, 692, 654, 638.

*Discussion of structure verification of 1 presented in Table 1*

The  $^{13}\text{C}$  NMR spectrum of 1',3'-bis(hydroxymethyl)-2'H,5'H-spiro[fluorene-9,4'-imidazolidine]-2',5'-dione (**1**) showed 11 signals – 6 pairs of atoms were magnetically equivalent (Table 1). The two signals with the highest chemical shifts

in  $^{13}\text{C}$  NMR spectrum,  $\delta_{\text{C}}$  155.34 ppm and  $\delta_{\text{C}}$  171.10 ppm, were assigned to the carbonyl carbons,  $\text{C}^2=\text{O}$  and  $\text{C}^5=\text{O}$ , respectively. The signal at  $\delta_{\text{C}}$  73.71 ppm was for the spiro-carbon, C-4'/9. The DEPT 135 spectrum showed two negative signals at  $\delta_{\text{C}}$  63.83 ppm and 61.89 ppm confirming the presence of methylene groups in the structure. The HMQC spectrum showed correlations between the corresponding pairs of signals, ( $\delta_{\text{H}}$  4.40 ppm –  $\delta_{\text{C}}$  63.83 ppm) and ( $\delta_{\text{H}}$  4.91 ppm –  $\delta_{\text{C}}$  61.89 ppm). Additionally, two HMBC correlations were found for each of the signals at  $\delta_{\text{H}}$  4.40 ppm (with  $\delta_{\text{C}}$  73.71 ppm and  $\delta_{\text{C}}$  155.34 ppm) and  $\delta_{\text{H}}$  4.91 ppm (with  $\delta_{\text{C}}$  155.34 ppm and  $\delta_{\text{C}}$  171.10 ppm). The protons in the  $\text{C}^6\text{H}_2$  group are separated by three bonds only from the spiro-carbon, C-4'/9 ( $\delta_{\text{C}}$  73.71 ppm), compared to the protons in the  $\text{C}^7\text{H}_2$  group which are located at three bonds away from each of the two carbonyl carbons. Consequently, the signals at  $\delta_{\text{H}}$  4.40 ppm and  $\delta_{\text{C}}$  63.83 ppm were assigned to the protons and carbon in the  $\text{C}^6\text{H}_2$  group, respectively, while the signals at  $\delta_{\text{H}}$  4.91 ppm and  $\delta_{\text{C}}$  61.89 ppm were for the protons and carbon in the  $\text{C}^7\text{H}_2$  group. The COSY spectrum showed the following correlations, ( $\delta_{\text{H}}$  4.40 ppm –  $\delta_{\text{H}}$  5.77 ppm) and ( $\delta_{\text{H}}$  4.91 ppm –  $\delta_{\text{H}}$  6.57 ppm). Also, two HMBC correlations were found, ( $\delta_{\text{H}}$  5.77 ppm –  $\delta_{\text{C}}$  63.83 ppm) and ( $\delta_{\text{H}}$  6.57 ppm –  $\delta_{\text{C}}$  61.89 ppm). The lack of HMQC correlations for the signals at  $\delta_{\text{H}}$  5.77 ppm and  $\delta_{\text{H}}$  6.57 ppm showed that the protons of the hydroxyl groups are bonded to atoms that are different from C. Therefore, the signal with the chemical shift  $\delta_{\text{H}}$  5.77 ppm was assigned to the proton in the hydroxyl group which is bonded to the methylene group,  $\text{C}^6\text{H}_2$ . Analogously, the signal with the chemical shift  $\delta_{\text{H}}$  6.57 ppm was assigned to the proton in the other hydroxyl group which is bonded to the methylene group,  $\text{C}^7\text{H}_2$ .

The strong HMBC correlation, ( $\delta_{\text{H}}$  7.37 ppm –  $\delta_{\text{C}}$  73.71 ppm), showed the 3-bond coupling of H-1/8 with the spiro-carbon, C-4'/9. It is less likely to find HMBC correlations of H-2/7, H-3/6 or H-4/5 with C-4'/9, because these protons are separated from the spiro-carbon by more than three bonds. In addition, there is a COSY correlation, ( $\delta_{\text{H}}$  7.37 ppm –  $\delta_{\text{H}}$  7.35 ppm), showing the coupling of H-1/8 with H-2/7. Due to the fact that only the meta (vicinal) coupling ( $^3J_{\text{CH}}$ ) in benzene rings is usually resolved, it is possible to assign the quaternary carbons, C-1a/8a and C-4a/5a [21, p. 27].

**Table 1.**  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectral data and  $^1\text{H}$ - $^1\text{H}$  COSY and HMBC correlations for **1** [600.130 MHz ( $^1\text{H}$ ) and 150.903 MHz ( $^{13}\text{C}$ )]<sup>a</sup>.

Atom	$\delta$ ( $^{13}\text{C}$ ), ppm	DEPT	$\delta$ ( $^1\text{H}$ ), ppm	Multiplicity (J, Hz)	$^1\text{H}$ - $^1\text{H}$ COSY <sup>b</sup>	HMBC <sup>b</sup>
2' (C=O)	155.34	C				
5' (C=O)	171.10	C				
6'	63.83	CH <sub>2</sub>	4.40	d (6.7)	OH (C-6')	2'. 4'/9
7'	61.89	CH <sub>2</sub>	4.91	d (7.0)	OH (C-7')	2'. 5'
4'/9	73.71	C				
1/8	124.27	CH	7.37 <sup>c</sup>	m	2	3. 4a . 4'/9
1a/8a	140.68	C				
2/7	128.15	CH	7.35 <sup>c</sup>	m	1.3. 4 <sup>e</sup>	1 <sup>e</sup> , 1a, 3 <sup>d</sup> , 4
3/6	130.08	CH	7.51	td (7.4; 1.4)	2, 4	1, 2 <sup>d</sup> , 4 <sup>e</sup> , 4a
4/5	120.86	CH	7.93	d (7.6)	2 <sup>e</sup> , 3	1 <sup>e</sup> , 2, 1a
4a/5a	141,15	C				
OH (C-7')			6,57	t (7,1)	7'	7'
OH (C-6')			5,77	t (6,8)	6'	6'

<sup>a</sup> In DMSO-*d*<sub>6</sub> solution. All these assignments were in agreement with COSY, HMQC and HMBC spectra.

<sup>b</sup> For brevity these correlations are given only in one of the benzene rings.

<sup>c</sup> Data from HMQC.

<sup>d</sup> These correlations are weak.

<sup>e</sup> These correlations are extremely weak.

In this case, there are two strong HMBC correlations, ( $\delta_{\text{H}}$  7.37 ppm -  $\delta_{\text{C}}$  141.15 ppm) and ( $\delta_{\text{H}}$  7.35 ppm -  $\delta_{\text{C}}$  140.68 ppm), indicating the coupling of the protons, H-1/8 and H-2/7, with the quaternary carbons, C-4a/5a and C-1a/8a.

It must be mentioned that the DEPT 135 spectrum didn't show any peaks at  $\delta_{\text{C}}$  140.68 ppm and  $\delta_{\text{C}}$  141.15 ppm supporting the hypothesis that the corresponding chemical shifts can be assigned to C-1a/8a and C-4a/5a. On the other hand, the multiplet structure of the signals at  $\delta_{\text{H}}$  7.51 ppm (triplet of doublets) and  $\delta_{\text{H}}$  7.93 (doublet) in addition to the following strong HMBC correlations, ( $\delta_{\text{H}}$  7.51 ppm -  $\delta_{\text{C}}$  141.15 ppm) and ( $\delta_{\text{H}}$  7.93 ppm -  $\delta_{\text{H}}$  140.68 ppm), showed that the signals with the chemical shifts  $\delta_{\text{H}}$  7.51 ppm and  $\delta_{\text{H}}$  7.93 were for H-3/6 and H-4/5, respectively. In addition, the COSY spectrum showed two strong correlations of H-3/6 ( $\delta_{\text{H}}$  7.51 ppm) with both H-2/7 ( $\delta_{\text{H}}$  7.35 ppm) and H-4/5 ( $\delta_{\text{H}}$  7.93 ppm) as well as one weak correlation between H-2/7 and H-4/5.

#### Discussion of structure verification of **2** presented in Table 2

The  $^{13}\text{C}$  NMR spectrum of 2-bromo-2'*H*,5'*H*-spiro[fluorene-9,4'-imidazolidine]-2',5'-dione (**2**) showed 15 signals corresponding to the number of

carbons in the compound (Table 2). Analogously to the previous structure, the signals at  $\delta_{\text{C}}$  72.16 ppm,  $\delta_{\text{C}}$  157.52 ppm and  $\delta_{\text{C}}$  173.56 ppm were assigned to the spiro- and carbonyl carbons, C-4'/9, C<sup>2</sup>=O and C<sup>5</sup>=O, respectively. The  $^1\text{H}$  NMR spectrum showed two singlet peaks at  $\delta_{\text{H}}$  8.61 ppm and  $\delta_{\text{H}}$  11.31 ppm corresponding to the protons in the NH groups. In comparison with the proton in the N<sup>3</sup>H group, the proton in the N<sup>1</sup>H group is located closer to the carbonyl group, C<sup>5</sup>=O, thus, it would be more strongly deshielded by the magnetic anisotropic effect of the two carbonyl groups [21, page 58]. Therefore, the singlet with the highest chemical shift ( $\delta_{\text{H}}$  11.31 ppm) can be assumed to correspond to the proton in the N<sup>1</sup>H group. Based on the following HMBC correlations, ( $\delta_{\text{H}}$  8.61 ppm -  $\delta_{\text{C}}$  72.16 ppm;  $\delta_{\text{H}}$  8.61 ppm -  $\delta_{\text{C}}$  157.52 ppm;  $\delta_{\text{H}}$  8.61 ppm -  $\delta_{\text{C}}$  173.56 ppm), the signal with the chemical shift  $\delta_{\text{H}}$  8.61 ppm was assigned to the proton in the N<sup>3</sup>H group, separated by two bonds only from the spiro-carbon, C-4'/9 ( $\delta_{\text{C}}$  72.16 ppm), compared to the proton in the N<sup>1</sup>H group. On the other hand, no HMBC and COSY correlations were found for the singlet at  $\delta_{\text{H}}$  11,31 ppm, assigned to the proton in the N<sup>1</sup>H group. A possible reason could be the participation of the proton in the N<sup>1</sup>H group in an intermolecular or intramolecular proton exchange

[22, p. 104]. As can be seen from Fig. 10, the widened shape of the singlet at  $\delta_{\text{H}}$  11.31 ppm indicates that the proton in the  $\text{N}^1\text{H}$  group could participate in an intermediate exchange [22, pp. 99-101].

The HMBC spectrum showed one strong and one weak correlation, ( $\delta_{\text{H}}$  7.48 ppm –  $\delta_{\text{C}}$  72.16 ppm) and ( $\delta_{\text{H}}$  7.75 ppm –  $\delta_{\text{C}}$  72.16 ppm), respectively. While there were not any COSY correlations for the singlet at  $\delta_{\text{H}}$  7.75 ppm, one weak COSY correlation was found between the doublet at  $\delta_{\text{H}}$  7.48 ppm and the triplet at  $\delta_{\text{H}}$  7.40 ppm. Additionally, the following HMBC correlations were found – ( $\delta_{\text{H}}$  7.75 ppm –  $\delta_{\text{C}}$  121.08 ppm), ( $\delta_{\text{H}}$  7.75 ppm –  $\delta_{\text{C}}$  132.76 ppm), ( $\delta_{\text{H}}$  7.75 ppm –  $\delta_{\text{C}}$  140.00 ppm), ( $\delta_{\text{H}}$  7.48 ppm –  $\delta_{\text{C}}$  130.00 ppm) and ( $\delta_{\text{H}}$  7.48 ppm –  $\delta_{\text{C}}$  139.52 ppm). It should be mentioned that the DEPT 135 spectrum didn't show any peaks at the chemical shifts ( $\delta_{\text{C}}$ ) 121.08 ppm, 139.52 ppm, 140.00 ppm, 142.85 ppm and 145.12 ppm. As it was mentioned previously, only the meta (vicinal) coupling ( $^3J_{\text{CH}}$ ) is usually resolved in the benzene rings [21, page 27]. Thus, the signals at  $\delta_{\text{H}}$  7.48 ppm and  $\delta_{\text{H}}$  7.75 ppm were assigned to the protons, H-8 and H-1, separated by three bonds only from the spiro-carbon, C-4'/9 ( $\delta_{\text{C}}$

72.16 ppm), while the peaks at  $\delta_{\text{C}}$  130.00 ppm,  $\delta_{\text{C}}$  132.76 ppm,  $\delta_{\text{C}}$  139.52 ppm and  $\delta_{\text{C}}$  140.00 ppm were assigned correspondingly to C-6, C-3, C-5a and C-4a. Meanwhile, the triplet at  $\delta_{\text{H}}$  7.40 ppm was assigned to H-7, as one additional HMBC correlation was found, ( $\delta_{\text{H}}$  7.40 ppm –  $\delta_{\text{C}}$  142.85 ppm), showing the coupling of H-7 with C-8a.

The HMQC spectrum showed that the carbon, C-3, is bonded to the proton, H-3, with the chemical shift  $\delta_{\text{H}}$  7,70 ppm. In this case, only one COSY correlation, ( $\delta_{\text{H}}$  7.70 ppm –  $\delta_{\text{H}}$  7.88 ppm), was found for H-3. Also, one of the HMBC correlations of H-3 is with C-4a, ( $\delta_{\text{H}}$  7.70 ppm –  $\delta_{\text{C}}$  140.00 ppm). Two HMBC correlations were found, ( $\delta_{\text{H}}$  7.88 ppm –  $\delta_{\text{C}}$  139.52 ppm) and ( $\delta_{\text{H}}$  7.88 ppm –  $\delta_{\text{C}}$  145.12 ppm), showing the 3-bond coupling between H-4 and the quaternary carbons, C-5a and C-1a. In addition, there was one NOESY correlation between H-3 and H-4, ( $\delta_{\text{H}}$  7.70 ppm –  $\delta_{\text{H}}$  7.88 ppm) (Fig. 16), supporting the hypothesis that the bromine is bonded to the carbon, C-2, with the chemical shift ( $\delta_{\text{C}}$ ) 121.08 ppm. Another weak COSY correlation of H-7 ( $\delta_{\text{H}}$  7.40 ppm) with H-6 ( $\delta_{\text{H}}$  7.50 ppm) was found as there was a strong HMBC correlation between H-7 and C-5 ( $\delta_{\text{C}}$  121.05 ppm).

**Table 2.**  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectral data and  $^1\text{H}$ - $^1\text{H}$  COSY and HMBC correlations for **2**) [600.130 MHz ( $^1\text{H}$ ) and 150.903 MHz ( $^{13}\text{C}$ )]<sup>a</sup>

Atom	$\delta$ ( $^{13}\text{C}$ ), ppm	DEPT	$\delta$ ( $^1\text{H}$ ), ppm	Multiplicity (J, Hz)	$^1\text{H}$ - $^1\text{H}$ COSY	HMBC
3' (NH)			8,61	s		2', 5', 4'/9
2' (C=O)	157,52	C				
1' (NH)			11,31	s		
5' (C=O)	173,56	C				
4'/9	72,16	C				
1	126,83	CH	7,75	s		2, 3, 4a, 4'/9 <sup>b</sup>
1a	145,12	C				
2	121,08	C				
3	132,76	CH	7,70	dd (8,2; 1,2)	4	1, 2, 4a
4	122,63	CH	7,88	d(8,1)	3	1a, 2, 3 <sup>b</sup> , 5a
4a	140,00	C				
5	121,05	CH	7,92	d (7,5)	6	4a, 8a, 7
5a	139,52	C				
6	130,00	CH	7,50	d(7,5)	5, 7 <sup>b</sup>	5a, 8
7	128,81	CH	7,40	t(7,4)	6 <sup>b</sup> , 8 <sup>b</sup>	5, 8a
8	123,54	CH	7,48	d(7,5)	7 <sup>b</sup>	5a, 6, 4'/9
8a	142,85	C				

<sup>a</sup> In DMSO- $d_6$  solution. All these assignments were in agreement with COSY, HMQC and HMBC spectra.

<sup>b</sup> These correlations are weak.

Moreover, the COSY spectrum showed a strong correlation of H-6 ( $\delta_{\text{H}}$  7.50 ppm) with H-5 ( $\delta_{\text{H}}$  7.92 ppm) while three strong HMBC correlations of H-5 with C-7 ( $\delta_{\text{C}}$  128.81 ppm), C-4a ( $\delta_{\text{C}}$  140.00 ppm) and C-8a ( $\delta_{\text{C}}$  142.85 ppm) were additionally found.

### CONCLUSION

This work highlighted the importance of the 2D NMR spectroscopy in the structure verification of organic compounds. The thorough analysis of the <sup>1</sup>H-<sup>1</sup>H COSY, HMQC, HMBC and <sup>1</sup>H-<sup>1</sup>H NOESY spectra ensured the accurate and complete assignment of the <sup>1</sup>H and <sup>13</sup>C NMR chemical shifts for the two substituted fluorenylspirohydantoin - 1',3'-bis(hydroxymethyl)-2'H,5'H-spiro[fluorene-9,4'-imidazolidine]-2',5'-dione and 2-bromo-2'H,5'H-spiro[fluorene-9,4'-imidazolidine]-2',5'-dione.

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