



Regioisomers of 2,5,6,7,8-Pentaaryl-1*H*-Azepino[3,2,1-*ij*]Quinazoline-1,3(2*H*)-Dione Containing Various Aryl Substituents in the Azepine Ring: Structure Determination Using NMR Methods

Julia A. Pronina¹ · Darya D. Komolova² · Vitali M. Boitsov³ · Alexander V. Stepanov^{1,2} · Stanislav I. Selivanov^{1,2,4}

Received: 16 July 2022 / Revised: 1 August 2022 / Accepted: 1 September 2022 /
Published online: 12 October 2022

© The Author(s), under exclusive licence to Springer-Verlag GmbH Austria, part of Springer Nature 2022

Abstract

NMR spectroscopy methods were used to prove the structures of two similar regioisomers of 2,5,6,7,8-pentaaryl-1*H*-azepino[3,2,1-*ij*]quinazoline-1,3(2*H*)-dione containing various aryl substituents in the azepine ring which were obtained as reaction products and existed in CDCl₃ as inseparable mixture of two compounds with almost equal (56:44) relation between them. Complete signal assignment in ¹H and ¹³C spectra of each compound was made by using some homo- and heteronuclear NMR experiments. Long-range distance estimation (up to 5.0 Å) on the base of nuclear Overhauser enhancement approach (NOE) at conditions of extreme-narrow limits ($\omega_0\tau_c \ll 1$) was used to determine the quantitative level the internuclear distances between protons H6 and H8 situated in the rigid part of molecules and the nearest *ortho*- and *meta*-protons in mobile phenyl rings Ph⁵ and Ph², respectively. The distance difference between the calculated and experimental values in all cases was not more than 10%. These results allowed us to prove that a dominant regioisomer (**3a**) has *para*-methoxy-substituted rings at positions **9** and **12** of seven-membered ring C, and a minor regioisomer (**3d**) has these rings at positions **10** and **12**. The results of an independent approach based on the comparison of the chemical shifts of the ¹H and ¹³C nuclei of the regioisomers under study are in full agreement (or do not contradict) with the obtained conclusions based on the quantitative NOE measurements of interproton distances. The methodological approach on the basis of long-range distance estimation by NOE tested in this work can be used to establish the structure of inseparable mixtures of two or more compounds or to solve similar problems under conditions of complex mixtures of closely related organic compounds.

1 Introduction

Extremely high resolution of NMR spectroscopy and its relatively low sensitivity are the best known, respectively, positive and negative characteristics of this physical method [1]. From the moment of its appearance (in the late 1950s) to the present, all the efforts of NMR equipment manufacturers have been aimed at improving the quality of the information obtained not only by increasing the operating frequency of the spectrometer [2], but also by using special methods to simplify its spectral representations [3]. This led to the possibility of NMR analysis of the structure and dynamics of large objects (molecular systems, macromolecules ~ 100 kDa) [4], as well as mixtures of complex composition [5] of natural or artificial origin, including metabolic products [6] or nonequilibrium reaction mixtures [7].

As a result, a lot of new effective directions of NMR applications appeared in the field of conformational analysis of molecules in liquid such as chemical shift-based methods described by Williamson [8], **JBCA** method (**J**-based configuration analysis) of Matsumori et al. [9], and **computer-assisted structure elucidation (CASE)** NMR-approach which was recently analyzed by Reynolds et al. [10] and Navarro-Vázquez [11]. At the same time, there was an improvement in existing methods [12] and development of new ones [13, 14] for accurately recording these spectral characteristics: special variants of homonuclear (COSY, *J*-COSY and NOESY) and heteronuclear (HSQC, HMBC and so on) experiments [15] and absolutely new experiments, such as for example “*J*-doubling in Frequency Domain” for exact determination of very small scalar constants [16], extraction of chemical shift values from overlapped proton spectra [17] or detection of minor conformers in conformationally flexible molecules [18, 19].

The direct consequence of the existing variety of ways to solve the problem is the necessity to choose the most effective (in each specific case) approach or a set of elements of each of them, the most successful (i.e., or convenient, simple, direct, evidence-based, spectacular, unexpected) for solving the problem, which, in turn, will constitute a research methodology that is different from the others (i.e., new), which is understood to mean not only the totality of these elements, but also the sequence of their use. This provides interchangeability, mutual verifiability and, therefore, the validity of the conclusions and the reliability of the results.

We have recently discovered the possibility of forming a benzazepine moiety based on the Pd(II) catalyzed oxidative cycloaddition of quinazoline-2,4-dione and diarylacetylenes. The importance of the benzazepine backbone as a structural element may make this method attractive for synthetic or medicinal chemistry. However, while working, it was found that when using unsymmetrical diarylacetylenes, inseparable mixtures of two regioisomeric 1*H*-azepino[3,2,1-*ij*]quinazoline-1,3(2*H*)-diones were obtained as reaction products. All attempts to separate these mixtures by chromatography or fractional crystallization have failed. Therefore, to establish the structure of each regioisomer, we carried out detailed NMR analysis.

It should be noted that the methodology of directed C–H activation, aimed at the formation of the C–C and C-heteroatom bonds, has recently undergone a lot

of development, mostly due to its environmentally friendly properties [20–22]. For example, the palladium-catalyzed stepwise oxidative cycloaddition of alkynes via a C–H/N–H bond cleavage step has shown to be reliable in the formation of the corresponding nitrogen-containing heterocycles. Jiao et al. described an elegant approach to the construction of indoles from anilines and alkynes using Pd-catalyzed oxidative C–H/N–H activation, including the formation of a five-membered ring [23]. Wang’s work investigated the palladium-catalyzed oxidative cycloaddition of isatins and alkynes, resulting in benzazepine derivatives [24].

In this work, we came across some of the problems listed above when proving the structure of two regioisomers that are formed during the interaction of compounds (1) and (2) and cannot be isolated individually to be studied in the traditional way. We do not deal with the chemical mechanism of this new reaction (see the scheme in Fig. 1), which will be described in a separate publication, but describe and analyze the most significant arguments of NMR evidence for the structure of each of the two regioisomers (3) in chloroform- d_1 solution directly in their mixture.

The total number of possible regioisomers of compound (3) is four (see the table in Fig. 1) and they differ only in the position of two *para*-methoxy-substituted phenyl rings in positions 9, 10, 11 and 12 of the seven-membered ring C. Thus, in the aromatic region of the proton spectrum of such mixture, in addition to signals of the protons H6–H8 of ring A, there should be signals of 46 aromatic protons of the Ph¹–Ph⁵ rings of two regioisomers of compound (3).

2 Results and Discussion

2.1 Signal Assignments in ¹H and ¹³C Spectra of Two Regioisomers

Figure 2a shows a fragment of the low-field region of the routine ¹H NMR spectrum of compound (3), which was obtained without additional processing of the initial free induction decay (FID) signal. It is clearly seen that it contains two separate multiplet signals at 8.12 and 7.28 ppm with a relative intensity equal to 1 proton for each of them. In the spectral regions 7.46–7.37 and 6.66–6.47 ppm, overlapping multiplet signals of four protons are found, and in the most complex region, 7.17–6.72 ppm, there are overlapping signals of 16 protons. The listed values of the integral intensities are in good agreement with the structure of the compound (3).

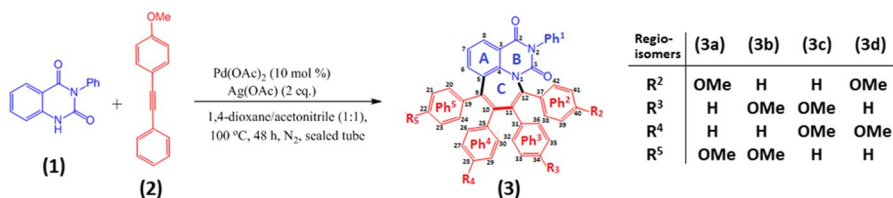


Fig. 1 Scheme of reaction between compounds (1) and (2). To the right—the table of possible regioisomers of compound (3)

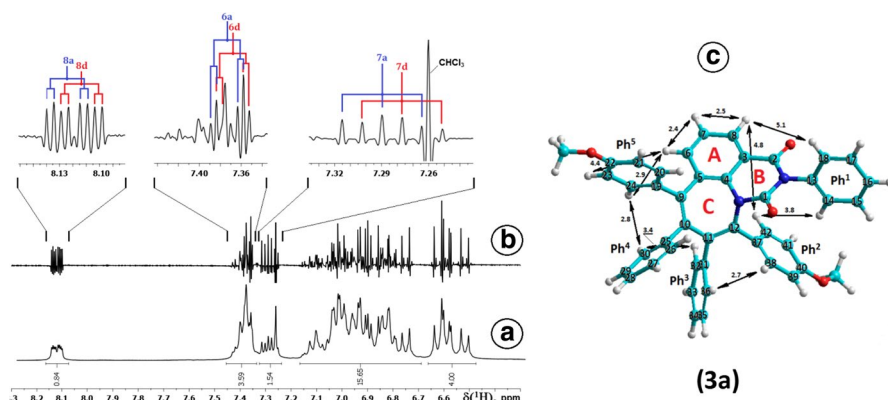


Fig. 2 Low-field region of NMR- ^1H spectra for compound (**3**) in CDCl_3 ; **a** without any additional processing; **b** after Lorentz–Gauss transformation of FID ($l_b = -3$ Hz, $g_b = 2.3$ Hz); **c** calculated (MM2) [25] spatial structure of regioisomer (**3a**), nearest interproton distances are shown by arrows and their values are given by figures (in Å)

However, only after an additional and more thorough adjustment of the constant magnetic field homogeneity and a longer accumulation of FID ($n_s = 128$, $d_1 = 10$ s), as well as the use of the Lorentz–Gauss transformation (LG: $l_b = -3.0$ Hz, $g_b = 2.3$ Hz), due to the additional narrowing of all lines, a quite obvious division of the obtained spectrum into two sets of signals almost identical in intensity was observed (Fig. 2b). The spectral separation is especially clearly seen when analyzing pairs of the multiplet signals of the protons H8 (**dd**, $J_{\text{H-H}} = 1.6, 7.5$ Hz), H7 (**dd**, $J_{\text{H-H}} = 7.5, 7.8$ Hz), and H6 (**dd**, $J_{\text{H-H}} = 1.6, 7.8$ Hz), which have only a very small difference (~ 0.006 – 0.013 ppm) in chemical shift values for the two regioisomers, indicated in Fig. 2b as (**3a**), (**3d**) and their overlapped multiplets are shown by blue and red colors, respectively. This chemical shift difference ($\Delta\delta = \delta^{(3a)} - \delta^{(3d)}$) leads to a distorted perception of the multiplicity of these signals. For example, the total signal of the H6 proton from two regioisomers looks like a doublet of triplets (**dt**, $J_{\text{H-H}} = 7.8, 1.6$ Hz), while the H8 and H7 signals can be described as **ddd** ($J_{\text{H-H}} = 1.6, 3.2, 7.6$ Hz) and **dt** ($J_{\text{H-H}} = 3.8, 7.6$ Hz), respectively. The ratio of regioisomer populations $P_{(3a)}:P_{(3d)}$ in CDCl_3 was obtained by carefully integrating the corresponding signals and turned out to be 56:44 with an accuracy within $\pm 2\%$.

Since scalar proton–proton interactions exist only within the spin systems of aromatic rings or the three-spin system H6–H7–H8, the possibility of determining the structure of the discovered regioisomers can be associated with the use of through-space dipole–dipole interactions (NOE) [26], or using such time-consuming methods such as INADEQUATE [27] and establishing, by scalar interactions between neighboring carbon atoms ($^1J_{\text{C-C}}$), the position of each of the phenyl rings Ph^2 – Ph^5 with respect to the C9–C12 atoms. In the first case, the identification of all proton signals is required, and in the second one, the signals of ^{13}C nuclei for each of the regioisomers are required.

In addition, the first of these two potential possibilities for proving the structure of regioisomers is limited by the well-known high sensitivity of the cross-relaxation

rate σ to interproton distances r : $\sigma \sim (r)^{-6}$ [26, 28]. Consequently, quantitative estimation of the distances between H6 and, especially, H8 protons with the *ortho*-protons of the Ph⁵ and Ph² rings nearest to them, respectively (2.9 Å and 4.8 Å, see Fig. 2c), may require additional costs for accumulating the corresponding weak cross peaks in NOESY spectra [29]. Rather large molecular weight of the studied regioisomers ($M=628$) and the possibility of reducing the sensitivity of NOE because of poor performance of the extremely narrowing condition ($\omega_0\tau_c < 1$) in the region where the rate of rotational diffusion (τ_c^{-1}) is commensurated with the Larmor frequency (ω_0) of precession nuclear spins [26] should also be taken into account. Therefore, all NOE measurements were carried out at a relatively low frequency of 300 MHz.

The presence of the mixture of two regioisomers in a solution of compound (3) is most easily proved using the *J*-COSY [30] spectra and/or some phase-sensitive COSY-type experiments [31]. Figure 3 shows fragments of the low-field region of these spectra, in which the signals of H6, H7 and H8 protons are located. For example, in the *J*-COSY spectrum (Fig. 3a), due to the development of spin systems due to only scalar constants, each signal structure (**dd** or **t**) belongs to a certain chemical shift, which is located in the center of multiplet signal. In the phase-sensitive COSY spectrum, which in Fig. 3b is presented as a spectrum of absolute values (modulus of the COSY spectrum), the cross peaks corresponding to each of the two regioisomers are shifted relatively to each other in two frequency coordinates by the difference in the chemical shifts of each of the interacting protons. For the dominant and minor regioisomers, these pairs for the 6/7 cross peak are shown in blue and red colors, respectively.

No less informative is the region 7.0–6.7 ppm of the *J*-COSY spectrum (Fig. 4), in which all eight doublet signals of the *ortho*-protons of aromatic rings Ph²–Ph⁵ of both regioisomers are clearly visible, and in the region of 6.66–6.48 ppm four doublet signals of the corresponding *meta*-protons of the *para*-methoxy-substituted rings of the regioisomers of compound (3) are observed. Separation of the signals of *ortho*-protons of *para*-substituted and unsubstituted rings due to the fact that these protons belong to different spin systems (four-spin system: AA'BB' and five-spin system: AA'BB'C, respectively) can be carried out based on the magnitude of their observed doublet splitting (shown by double arrows), which in the case of

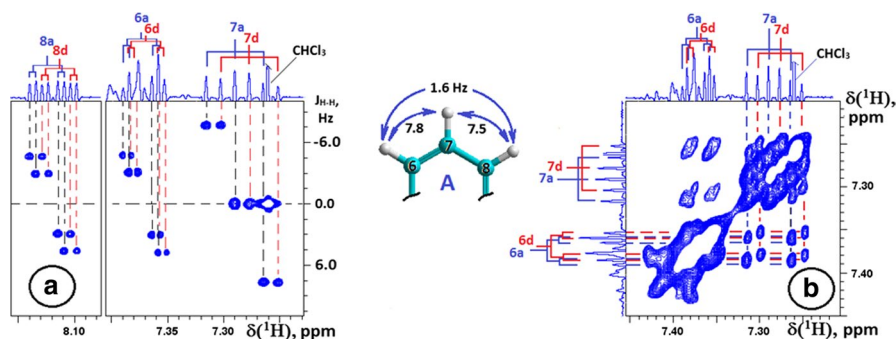


Fig. 3 Low-field fragments of *J*-COSY (a) and COSY (b) spectra of compound (3)

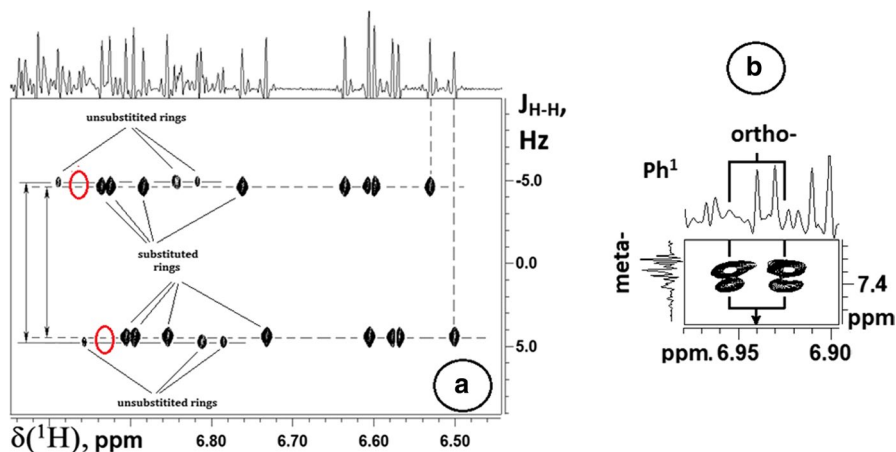


Fig. 4 Fragments of J -COSY (a) and COSY (b) of compound (3) in CDCl_3 . (a) The difference between doublet components of proton signals for substituted and unsubstituted phenyl rings is shown by double arrows and the position of *ortho*-protons in Ph^1 at 6.94 ppm is indicated by red ellipses on base COSY-data (b)

unsubstituted rings turns out to be somewhat larger (by 0.4 Hz) than in the *para*-substituted case. It should also be noted that in the ^1H NMR spectrum, the doublet signals of two (out of four) *ortho*-protons of unsubstituted rings completely overlap with each other, and this is clearly seen in the J -COSY spectrum as a doubling of the intensity of the doublet at 6.83 ppm compared to two other individual similar doublets at 6.80 and 6.97 ppm.

The procedure for detecting doublet signals of *ortho*-protons (H14 and H18) of the unsubstituted ring Ph^1 in the regioisomers of compound (3) turned out to be the most difficult. Complete absence of these signals in the J -COSY spectrum in the region of 6.96–6.93 ppm (in Fig. 4 the position of this doublet is shown with the help of red ovals) is apparently explained by the dynamic broadening of this signal and by the corresponding reduction in the lifetime (T_2) of the spin state compared to other *ortho*-protons. Therefore, to determine the position in the spectrum of the signals of these protons, the COSY experiment was used, in which the cross peak between the signals of *meta*- and *ortho*-protons 15.17/14.18 at 7.39/6.94 ppm are easily detected (see fragment of COSY spectrum in Fig. 4, right).

The position in the NMR ^1H spectrum of the remaining signals of the *meta*- and *para*-protons of unsubstituted rings was determined using the COSY and/or NOESY spectra, based, respectively, on the scalar and/or spatial interactions of these protons with the corresponding vicinal neighbors (see Fig. 5 and Table 1). In the most complex cases of the formation of strongly coupled spin systems and/or overlapping of signals, the correlation methods HSQCnd [32] and HMBC [33] were used.

For example, the above identification of the signals of the *ortho*-protons H14, H18 of the Ph^1 rings at 6.94 ppm, which have the same value for each of the regioisomers, makes it possible to detect the signals of the *meta*-protons H15 and H17 of this ring at 7.40 ppm, and the signals of the *para*-proton H16, respectively, at

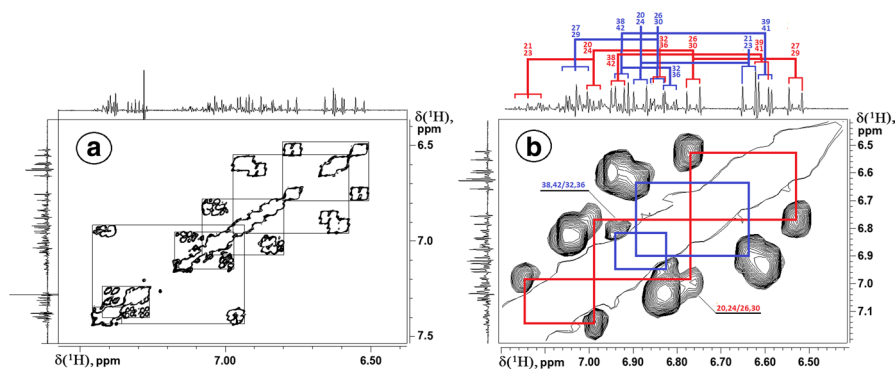


Fig. 5 Fragments of COSY (a) and NOESY (b) spectra of a mixture of two regioisomers (**3**) in CDCl_3 . Indirect (scalar) and direct (through-space) interactions between protons are shown by rectangles

7.38 ppm. In the NMR ^1H spectrum, they overlap between themselves, forming a strongly coupled spin system ($\text{AA}'\text{BXX}'$) and with the signal of the H6 proton at 7.37 ppm (here the average value for two regioisomers is given). The positions of these aromatic protons were determined using the HSQC spectrum without decoupling from ^{13}C nuclei (HSQCnd), a fragment of which is shown in Fig. 6. In this spectrum, triplet proton signals located at the *meta*- and *para*-carbon-13 atoms (C15, C17 at 129.15 ppm and C16 at 128.55 ppm, respectively) are less sensitive to the strong scalar coupling effects of these protons, and their chemical shifts are easily determined by the central components of the triplet signals, the ratio of the intensities of which (1.8:1.0) practically corresponds to 2:1. In addition, a slight clockwise slope of the cross peak $\text{C6}^{(\beta)}/\text{H6}$ ($\text{C6}^{(\beta)}$ – here index β means quantum state of carbon-13) components gives grounds for the correct assignment of the carbon signals of the C6 carbon atom in the dominant (**3a**) and minor (**3d**) regioisomers: the low-field proton signal of the dominant regioisomer (**3a**) corresponds to the low-field signal of its carbon atom (Fig. 6b).

Similarly, the chemical shifts of the *meta*- and *para*-protons for unsubstituted aromatic rings were defined, which form a complex spectral pattern in the region of 7.17–6.98 ppm (see the full spectrum HSQCnd in Fig. S5, Suppl. Inf.). To check the signal assignments of protonated carbons and to identify the signals of quaternary ^{13}C atoms, we used the data of the HMBC method, which optimized the value of the $^{2,3}J_{\text{C-H}}$ constant equal to 8.0 Hz. The general form of this spectrum is given in Fig. S6 (Suppl. Inf.) and its most informative fragments are presented in Fig. S10.

It should be noted that when determining the proton and carbon signals in the corresponding NMR spectra, the ratio of their integral intensities, which differs only by 12%, was taken into account. This small difference in most cases turned out to be quite sufficient for determining the belonging of the signals to both protonated and quaternary carbon-13 atoms. As an example, Fig. S8 (Suppl. Inf.) shows fragments of the ^{13}C NMR spectrum of a mixture of regioisomers (**3a**) and (**3d**), which demonstrate the results of integration. In almost all cases, the integral intensity of the signal of the dominant isomer (**3a**) exceeds the integral intensity of the signal of

Table 1 Chemical shifts δ (ppm) of ^1H and ^{13}C nuclei of regioisomers (**3a**) and (**3d**) in CDCl_3

No. of atoms	^1H		^{13}C		№ of atom	^1H		^{13}C	
	3a	3d	3a	3d		3a	3d	3a	3d
1	–	–	152.53	152.53	23 (m)	6.62	7.10	113.01	127.70
2	–	–	162.45	162.45	24 (o)	6.87	6.97	131.64	130.43
3	–	–	132.82	132.48	25 (i)	–	–	139.24	131.42 ^{c)}
4	–	–	149.07	149.07	26 (o)	6.83	6.75	130.34	131.42
5	–	–	118.23 ^{b)}	118.29 ^{b)}	27 (m)	7.01	6.52	126.96	112.36
6	7.373 ^{a)}	7.367 ^{a)}	137.93	137.75	28 (p)	7.03	–	126.38	157.95
7	7.289 ^{a)}	7.276 ^{a)}	125.04	125.07	29 (m)	7.01	6.52	126.96	112.36
8	8.124 ^{a)}	8.113 ^{a)}	127.43	127.35	30 (o)	6.83	6.75	130.34	131.42
9	–	–	139.91	140.36	31 (i)	–	–	137.27 ^{c)}	137.22 ^{c)}
10	–	–	143.68 ^{b)}	143.43 ^{b)}	32 (o)	6.80	6.83	130.64	130.64
11	–	–	137.80	138.13	33 (m)	7.02	7.02	127.16 ^{c)}	127.19 ^{c)}
12	–	–	141.25	141.33	34 (p)	7.06	7.06	126.72	126.72
13	–	–	134.79	134.79	35 (m)	7.02	7.02	127.16 ^{c)}	127.19 ^{c)}
14(o)	6.94	6.94	128.16	128.16	36 (o)	6.80	6.83	130.64	130.64
15(m)	7.40	7.40	129.15	129.15	37 (i)	–	–	129.66	129.66
16 (p)	7.38	7.38	128.55	128.55	38 (o)	6.913 ^{a)}	6.922 ^{a)}	131.05	131.05
17 (m)	7.40	7.40	129.15	129.15	39 (m)	6.587 ^{a)}	6.594 ^{a)}	112.98	112.98
18 (o)	6.94	6.94	128.16	128.16	40 (p)	–	–	158.53	158.53
19 (i)	–	–	133.88	141.59	41 (m)	6.587 ^{a)}	6.594 ^{a)}	112.98	112.98
20 (o)	6.87	6.97	131.64	130.43	42 (o)	6.913 ^{a)}	6.922 ^{a)}	131.05	13,105
21 (m)	6.62	7.10	113.01	127.70	<i>p</i> -OCH ₃	–	–	–	–
(Ph ²)	3.674 ^{a)}	3.679 ^{a)}	54.94	54.94	–	–	–	–	–
22 (p)	–	7.06	158.12	126.66	<i>p</i> -OCH ₃ (Ph ^{4,5})	3.692 ^{a)}	3.674 ^{a)}	54.91	54.85

^{a)} at a chemical shift difference of less than 0.01 ppm, values with an accuracy of ± 0.001 ppm were used

^{b)} reversion of values for stereo-isomers (**3a**) and (**3d**) is possible

^{c)} established on the basis of a comparison of the integral intensities of ^{13}C signals (Fig. S8 in Suppl. Inf.)

the minor regioisomer (**3d**), although the measurement error of the integrals is comparable to the difference in the populations of these adducts ($\sim 10\%$) in the mixture under study, which was obtained by integrating the proton signals having a higher S/N ratio in the ^1H NMR spectrum than signals in the ^{13}C NMR spectrum. Moreover, based on the integration of carbon signals, it was possible to explain the absence of the signal of the quaternary *ipso*-carbon atom C25^(3d) in the ^{13}C spectrum of the minor regioisomer (**3d**), which turned out to be completely overlapped by the more intense signal of the chemically equivalent *ortho*-carbons C26^(3d) and C30^(3d) at 131.42 ppm. This overlap of three carbon signals leads to the intensity of 0.20 units of relative integral intensity being higher than the signal intensity of two equivalent *ortho*-carbons C20^(3a) and C24^(3a) at 131.64 ppm, similar to the *para*-methoxy-substituted aromatic ring Ph⁵ of the dominant regioisomer (**3a**). If we take into account

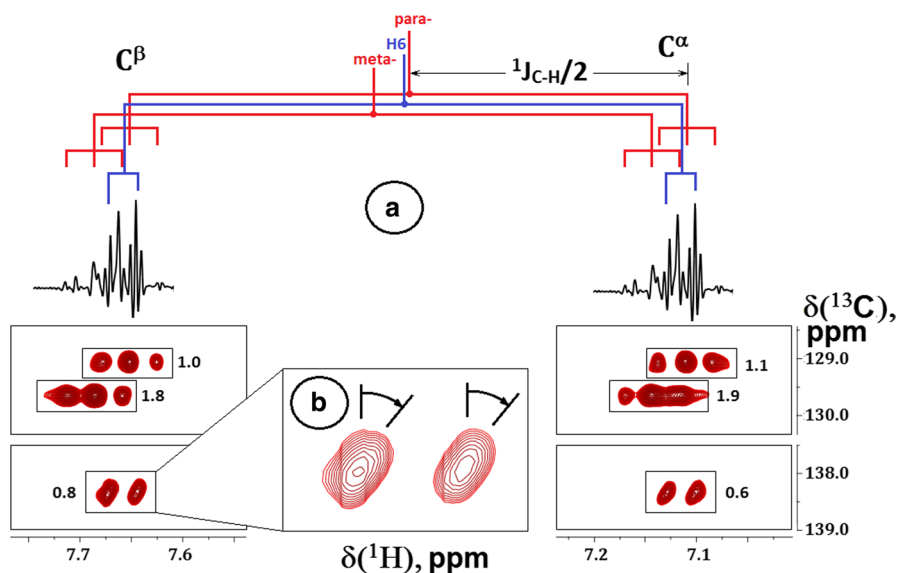


Fig. 6 Fragments of the HSQCnd spectrum of a mixture of two regioisomers of the compound (**3**): **a**) low-field (left) and high-field (right) components of doublet cross peaks for ^{13}C -H pairs of signals from the *ortho*-, *meta*- and *para*-protons of the Ph^1 ring and the H6 proton; **b**) increased cross peak C6^(B)/H6, which has a clockwise slope due to unequal chemical shifts of the ^1H and ^{13}C nuclei in the dominant and minor isomers. Relative integral values of cross-peak intensities are shown by figures

that the total intensity of the signals of two *ipso*-carbon atoms C37^(3a) and C37^(3d) at 129.66 is 0.62 units, then the fraction of the minor regioisomer is about 0.27 units of the relative integrated intensity. Assuming the same rate of longitudinal relaxation of the same type carbon signals C25^(3d) and C37^(3d), we can conclude that the integral intensity of the signal of two equivalent *para*-carbons C26^(3d) and C30^(3d) is about 1.91 units, which turns out to be less than the value of the integral for a similar signal of *ortho*-carbons C20^(3a) and C24^(3a) at 131.64 ppm in the ^{13}C spectrum of the dominant regioisomer (**3a**). Therefore, the assumption of random overlap of the C25^(3d) signal at 131.42 ppm by the total signal of *ortho*-carbons C26^(3d) and C30^(3d) is quite justified and confirmed on a quantitative level.

2.2 Structural Analysis of Regioisomers (**3a**) and (**3b**) on Base Long-Range NOEs

The most interesting for establishing the structure of regioisomers (**3a**) and (**3d**) is the determination of the position of the protons of the Ph^5 and Ph^2 rings, the *ortho*-protons of which are located most closely to the H6 and H8 protons, respectively. This information, after identifying all proton signals for each of the regioisomers (see Table 1), was obtained by analyzing the NOESY spectrum (Fig. 7). Along with intense cross peaks between the signals of vicinal *para* aromatic protons, the distance

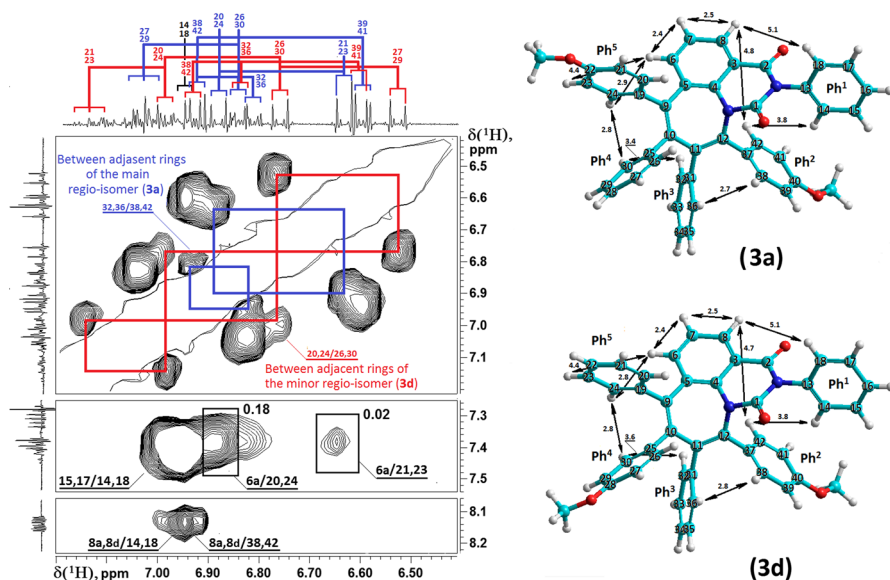


Fig. 7 Fragments of NOESY spectrum of compound (**3**) at mixing time 0.5 s in CDCl_3 (left) and spatial structures of dominant (**3a**) and minor (**3d**) regioisomers (right). The nearest proton–proton distances are shown by two arrow lines and the calculated (MM2) distance values depicted by figures (in Å). The most important through-space interactions in isomers (**3a**) and (**3d**) are presented, respectively, by blue and red lines in NOESY and ^1H spectra. Some values of cross-peak volume integrals (in relative units) are presented using rectangles with figures

between which is only 2.5 Å, weaker cross peaks are observed, which are of greatest interest.

For example, the cross peak between the doublet signal at 6.87 ppm of *ortho*-protons in the *para*-methoxy-substituted ring of the dominant regioisomer (**3a**) is direct evidence that in this isomer, one of the two substituted phenyl rings is located at the C9 atom. The presence of an intense cross peak between the signals of *ortho*- and *meta*-protons (20,24/21,23), as well as an extremely weak cross peak between the same *meta*-protons (H21, H23) and the proton H6, makes it possible to be not only qualitative, but also quantitative estimation of the ratio of interproton distances r_{24-23} , r_{6-24} and r_{6-23} . The calculated values of these distances were obtained using the method of molecular mechanics MM2 [25] and are equal to 2.5, 2.9 and 4.4 Å, respectively. The experimental values of the integral intensities of the corresponding cross peaks are: 1.0:0.18:0.02. Since the first of these through-space interactions corresponds to two pairs of *ortho*- and *meta*-protons, the observed value is at least two times greater than the pair interactions between the H6 proton and the H24 and H23 protons. In the last cases, we neglect the contributions into the observed NOE values from the H20 and H21 protons, since the distances $r_{6-20}=4.11$ Å and $r_{6-21}=5.26$ Å are 1.2 Å and 0.86 Å higher than the distance values which were obtained for distances between H6 and the nearest protons H24 and H23, respectively. This means that the contribution to the NOE cross peak from the interaction of the H6 proton with H₂O cannot exceed 12% of the main contribution of H6/

H24, and in the case of the second cross peak H6/H23, the contribution from the interaction H6/21 is not more than 30%. Thus, the calculated ratio of the cross-peak intensities is 1.0:0.41:0.033, and the experimental ratio, taking into account the contributions from equivalent protons, is 0.5:0.16:0.013 (or 1.0:0.32:0.026), which is in good agreement with ratio of the expected NOE values. Using the value of 2.5 Å as a reference distance, the following values can be obtained for estimating the distance between the H6 proton and the nearest Ph⁵ ring: $r_{6-24}=3.02$ Å and $r_{6-23}=4.6$ Å.

Thus, the obtained experimental distances for dominant regioisomer (**3a**) are within the same absolute error of ± 0.2 Å from the calculated values. Consequently, the relative deviations from the calculated distances are +7.6 and +4.5%, respectively, i.e., they do not exceed 10%. The obtained distance values indicate the possibility of their quantitative measurements using NOE up to 5.0 Å with an accuracy of about $\pm 10\%$. This conclusion has been much discussed recently [34] and as a result the term eNOE (exact NOE) completely corresponds to our NOE data.

For the minor regioisomer (**3d**) in the NOESY spectrum, a spatial interaction was found between the *ortho*-protons of neighboring phenyl rings Ph⁴ and Ph⁵ (see cross peaks 20,24/26,30 in Fig. 7). At the same time, the Ph⁴ ring is *para*-methoxy-substituted and has a characteristic doublet signal at 6.75 ppm in the proton spectrum. However, the NOESY spectrum does not contain its cross peak with the H6 proton, which indicates that this ring is located at the C10 atom, and not at C9. This means that in position 9 of regioisomer (**3d**) there is an unsubstituted phenyl ring Ph^{5(d)}, with *ortho*-proton signals are at 6.97 ppm next to signals at 6.94 ppm of the *ortho*-protons H14, H18 of the Ph¹ ring. The spectral closeness of these signals does not allow us to detect a cross peak in the NOESY spectrum between the *ortho*-protons H24, H20 and the H6 proton, since the signals of the *meta*-protons H15 and H17 of the Ph¹ ring at 7.40 ppm overlap with the signal of the H6 proton and have a strong spatial interaction with the *ortho*-protons H14 and H18 (see intensive cross peak 15, 17/14, and 18 in the NOESY spectrum; Fig. 7, left).

Thus, the obtained NOE information about the structural features of regioisomers (**3a**) and (**3d**) indicates a different structure of the phenyl rings in positions **9** and **10** in the dominant and minor isomers (see Fig. 7, right). This structural difference is fully confirmed by the difference $\Delta\delta$ ($\Delta\delta=\delta^{(A)}-\delta^{(B)}$) in the chemical shifts of the ¹H and ¹³C nuclei (see Table S1 in the Suppl. Inf.). For example, when passing from regioisomer (**3a**) to (**3d**), the chemical shift of *meta*-protons H21 and H23 ($\Delta\delta_{(H21,H23)}=-0.48$ ppm) and *meta*-carbons C21 and C23 ($\Delta\delta_{(C21,C23)}=-14.69$ ppm) of the Ph³ ring increases and, simultaneously, there is a decrease by the same amount of the chemical shift of the *meta*-protons H27 and H29 ($\Delta\delta_{(H27,H29)}=+0.49$ ppm) and *meta*-carbons C27 and C29 ($\Delta\delta_{(C27,C29)}=+14.60$ ppm) in the Ph⁴ ring. It corresponds to the well-known increments of the methoxy substituent in the aromatic ring for the *ortho*-position in the ¹H and ¹³C NMR spectra, respectively.

A completely opposite situation for the considered regioisomers (**3a**) and (**3d**) arises when comparing the chemical shifts of proton and carbon signals of phenyl rings in positions 11 and 12. Their chemical shift differences $\Delta\delta(^1\text{H})$ and $\Delta\delta(^{13}\text{C})$ for most of the compared signals are small (see Table S1 in Suppl. Inf.). This indicates that the *para*-methoxy-substituted and unsubstituted phenyl groups retain their position in these regioisomers. Therefore, the main issue in this case is to obtain the

evidence of the position (at C11 or at C12) of at least one of the phenyl rings in any of the two isomers.

First of all, it should be noted that the cross peak 32,36/38,42, which is well observed in the NOESY spectrum (Fig. 7, left), is common for both regioisomers, which indicates the proximity of the phenyl rings Ph² and Ph³. Unfortunately, the quantitative estimation of the corresponding distances in this case is difficult not only because of the partial or complete overlap of the doublet signals of *ortho*-protons belonging to the dominant (**3a**) and minor (**3d**) regioisomers, but also because of the proximity of this cross peak to the diagonal of the two-dimensional spectrum NOESY and impossibility of its exact integration. Therefore, another less intense cross peak with coordinates 8.12/7.93 ppm was analyzed in detail, which is a superposition of two cross peaks (8a,8d/14,18 and 8a,8d/38,42), shifted relatively to each other horizontally (i.e., on the F2 scale) by a relatively small amount (about 0.01–0.02 ppm).

The value $r_{8-7} = 2.50 \text{ \AA}$ was used as a reference distance. The relative intensity of cross peak 8/7 with respect to the diagonal signal of the H8 proton is 9.56% (Fig. 8). In this case, the intensity of the discussed total cross peak is about 0.5%. The error of its measurement because of the uncompensated base plane in the vicinity of the coordinate 8.12/7.93 ppm (see open rectangles in Fig. 8) is about 0.1%, so the real value of this cross peak is somewhat lower, i.e., about 0.4%. Taking into account the commensurability of the distances $r_{8-18} = 5.1 \text{ \AA}$ and $r_{8-42} = 4.73 \text{ \AA}$ and neglecting the contributions to the cross peaks of spatial interactions 8/14 ($r_{8-14} = 6.05 \text{ \AA}$) and 8/38 ($r_{8-38} = 7.7 \text{ \AA}$), we obtained cross-peak intensity 8/42 equal to half of the measured value of the total cross peak,

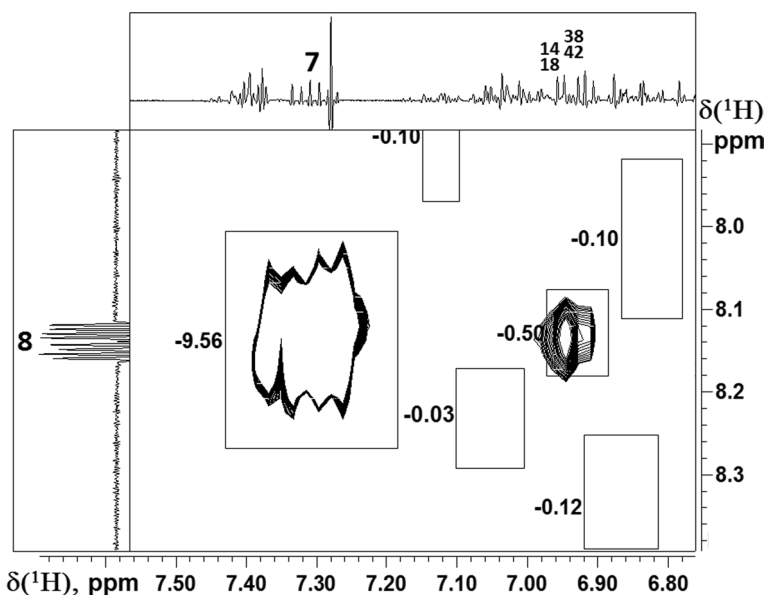


Fig. 8 Fragment of NOESY spectrum of compound (**3**) and volume integrals of cross peaks and noise inputs (open rectangles) are shown by figures

i.e., about 0.2%. Based on the comparison of these data, the experimental value of the distance r_{8-42} is 4.76 Å ($2.5 \text{ \AA} \cdot (9.56/0.2)^{1/6} = 4.76 \text{ \AA}$). This value practically coincides with the calculated distances for regioisomers (**3a**) and (**3d**): $r_{8-42}^{(3a)} = 4.74 \text{ \AA}$, $r_{8-42}^{(3d)} = 4.73 \text{ \AA}$.

We only need to add that the calculated distance $r_{8-32} = 6.7 \text{ \AA}$ turns out to be 2.0 Å longer than r_{8-42} . Therefore, the corresponding cross peak 8/32 should be 8 times smaller than the observed cross peak 8/42 (i.e., be about 0.025%), which is beyond the limits of measurement accuracy.

Thus, the involvement of quantitative estimation of interproton distances based on NOE to solve the problem of establishing the structure of two regioisomers in a mixture made it possible to obtain an unambiguous answer about the spatial structure of these compounds using NMR spectroscopy methods without resorting to other physical methods, including X-ray diffraction analysis.

3 Conclusion

High-resolution NMR spectroscopy methods at 300 MHz allowed us to solve very difficult problem to prove the structures of two similar regioisomers of 2,5,6,7,8-pentaaryl-1*H*-azepino[3,2,1-*ij*]quinazoline-1,3(2*H*)-dione containing various aryl substituents in the azepine ring. At first, complete signal assignment in the ^1H and ^{13}C spectra of each compound was made by using homo- and heteronuclear NMR methods in the condition of their almost equal (56:44) population in CDCl_3 . Then long-range distance estimation (up to 5.0 Å) by the NOE approach was used to determine (on the quantitative level) the internuclear distances between protons H6 and H8 situated in the rigid part of molecules with the nearest *ortho*- and *meta*-protons in mobile phenyl rings Ph^5 and Ph^2 , respectively. This experimental NOE data were compared with their calculated values obtained by the method of molecular mechanic (MM2) in *isolated spin-pair approximation* (ISPA) [26] at condition of extreme-narrow limits ($\omega_0\tau_c \ll 1$) for NOE registration. The comparison of calculated and experimental distances on the quantitative level was almost absolutely exact and the relative distance difference between them in any case was not more than 10%. These results allowed us to prove that the dominant regioisomer (**3a**) has *para*-methoxy-substituted rings at positions **9** and **12** of seven-membered ring **C**, and the minor regioisomer (**3d**) has these rings at positions **10** and **12**.

It should be noted that the results of an independent approach based on the comparison of the chemical shifts of the ^1H and ^{13}C nuclei of the regioisomers under study are in full agreement (or do not contradict) with the obtained conclusions based on the quantitative NOE measurements of interproton distances. The methodological approach tested in this work can be used to establish the structure of structurally related compounds or to solve similar problems under conditions of complex mixtures of closely related organic compounds.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s00723-022-01496-6>.

Acknowledgements Spectral NMR investigations were performed in the Resource Centers “Magnetic Resonance Research Center” of the Saint-Petersburg State University. This work was supported by the Ministry of Science and Higher Education of the Russian Federation (0785.00.X6019). V.M.B. is grateful to the Ministry of Education and Science of the Russian Federation (0791-2020-0006) for financial support. S.I.S. acknowledges Saint-Petersburg State University for a research grant 92425251.

Author Contributions JAP—participated in the synthesis of the studied compounds, prepared Figs. 1 and 2, compiled Table 1 based on the data obtained, and also actively participated in the discussion of the results and made an oral report on this topic at the Spinus-2022 conference. DDK—participated in the synthesis of the studied compounds and preliminary analysis of NMR spectra, and in the discussion of the text of the manuscript. VMB—participated in the synthesis of the studied compounds and an attempt to isolate them from the reaction mixture, and actively participated in the discussion of the results and the text of the manuscript. AVS—engaged in the substantiation, preparation and implementation of the synthesis of the studied compounds, took an active part in the planning of all work and discussion of the results, and also described the synthesis of the studied regioisomers and the prospects for their practical use. SIS—performed all NMR experiments on the DPX-300 spectrometer (300 MHz) and processed the obtained spectra, wrote the main text of the manuscript, including the design of spectral drawings and quantitative analysis of data on measuring interproton distances using NOEs. All authors have read and agreed to the published version of the manuscript.

Declarations

Conflict of Interest The authors declare no conflict of interest.

References

1. N.S. Bhacca, D.H. Williams, *Application of NMR Spectroscopy in Organic Chemistry. Illustrations from the steroid field*, (Holden-Day, San Francisco, 1964), 198 P
2. J.W. Emsley, J. Feeney, *Progr. NMR. Spectrosc.* **50**, 179–198 (2007)
3. R. R. Ernst, G. Bodenhausen, A. Wokaun, *Principles of Nuclear Magnetic Resonance in One and Two Dimensions*, (Oxford University Press, Oxford 1987), 711 P
4. K. Pervushin, R. Riek, G. Wider, K. Wüthrich, *Proc. Natl. Acad. Sci. USA* **94**(23), 12366–12371 (1997)
5. J. Wist, *Magn. Reson. Chem.* **55**(1), 22–28 (2017)
6. O. Beckonert, H.C. Keun, T.M.D. Ebbels, J. Bundy, E. Holmes, J.C. Lindon, J.K. Nicholson, *Nat. Protoc.* **2**(11), 2692–2703 (2007)
7. P.A. Keifer, *Ann. Report NMR Spectroscopy* **62**(1), 1–47 (2007)
8. M.P. Williamson, *Progr. NMR. Spectrosc.* **73**, 1–16 (2013)
9. N. Matsumori, D. Kaneno, M. Murata, H. Nakamura, K.J. Tachibana, *J. Org. Chem.* **64**(3), 866–876 (1999)
10. D.C. Burns, E.P. Mazzola, W.F. Reynolds, *Nat. Prod. Rep.* **36**(6), 919–933 (2019)
11. A. Navarro-Vázquez, *Magn. Reson. Chem.* **55**(1), 29–32 (2017)
12. W.F. Reynolds, R.G. Enriquez, *J. Nat. Prod.* **65**(2), 221–244 (2002)
13. B.L. Marquez, W.H. Gerwick, R.T. Williamson, *Magn. Reson. Chem.* **39**(9), 499–530 (2001)
14. K. Kobzar, B. Luy, *J. Magn. Reson.* **186**(1), 131–141 (2007)
15. S. Braun, H.-O. Kalinowski, S. Berger, *150 and More Basic NMR Experiments. A practical Course* (Wiley-VCH, 2nd expanded ed. 1998), 596 P
16. A. Garza-Garcia, G. Ponzanelli-Velazques, Federico del Rio-Portilla, *J. Magn. Reson.* **148**(2), 214–219 (2002)
17. D.P. Frueh, *Progr. NMR. Spectrosc.* **78**(2), 47–75 (2014)
18. S.I. Selivanov, Solov'ev A.Yu., S.N. Morozkina, A.G. Shavva, *Rus. J. Bioorg. Chem.* **33**(3), 302–309 (2007)
19. C.P. Butts, C.R. Jones, E.C. Towers, J.L. Flynn, L. Appleby, N.J. Barron, *Org. Biomol. Chem.* **9**(1), 177–184 (2011)

20. C. Zhu, R. Wang, J.R. Falck, *Chem. Asian J.* **7**, 1502–1514 (2012)
21. G. Song, F. Wang, X. Li, *Chem. Soc. Rev.* **41**, 3651–3678 (2012)
22. J.F. Hartwig, *Chem. Soc. Rev.* **40**, 1992–2002 (2011)
23. Z.-Z. Shi, C. Zhang, S. Li, D.-L. Pan, S.-T. Ding, Y.-X. Cui, N. Jiao, *Angew. Chem. Int. Ed.* **48**, 4572–4576 (2009)
24. L. Wang, J. Huang, S. Peng, H. Liu, X. Jiang, J. Wang, *Angew. Chem. Int. Ed.* **52**, 1768–1772 (2013)
25. N.L. Allinger, *J. Am. Chem. Soc.* **99**(25), 8127–8134 (1977)
26. D. Neuhaus, M.P. Williamson, *The Nuclear Overhauser Effect in Structural and Conformational Analysis* (2nd ed.), (Wiley-VCH, New York, 2000), 619 P
27. A. Bax, R. Freeman, T.A. Frenkiel, *J. Am. Chem. Soc.* **103**, 2102–2104 (1980)
28. R.A. Bell, J.K. Saunders, *Canad. J. Chem.* **48**(7), 1114–1122 (1970)
29. S.I. Selivanov, S. Wang, A.S. Filatov, A.V. Stepanov, *Applied Magn. Reson.* **51**(2), 165–182 (2020)
30. W.P. Aue, J. Karhan, R.R. Ernst, *J. Chem. Phys.* **64**, 4226–4227 (1976)
31. D.J. States, R.A. Haberkorn, D.J. Ruben, *J. Magn. Reson.* **48**(2), 286–292 (1982)
32. G. Bodenhausen, D.J. Ruben, *Chem. Phys. Lett.* **69**(1), 185–189 (1980)
33. A. Bax, M.F. Sammers, *J. Am. Chem. Soc.* **108**, 2093–2094 (1986)
34. B. Vögeli, *Progr. NMR. Spectrosc.* **78**, 1–46 (2014)

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Springer Nature or its licensor holds exclusive rights to this article under a publishing agreement with the author(s) or other rightsholder(s); author self-archiving of the accepted manuscript version of this article is solely governed by the terms of such publishing agreement and applicable law.

Authors and Affiliations

Julia A. Pronina¹ · Darya D. Komolova² · Vitali M. Boitsov³ ·
Alexander V. Stepanov^{1,2} · Stanislav I. Selivanov^{1,2,4}

✉ Stanislav I. Selivanov
s.selivanov@spbu.ru; nmr.group.spbu@gmail.com

Julia A. Pronina
pronina.iuli05@yandex.ru

¹ Saint-Petersburg State Institute of Technology (Technical University), Moskovsky prospect 26, 190013 Saint-Petersburg, Russia

² Department of Chemistry, St. Petersburg State University, 199034 St. Petersburg, Russia

³ Saint Petersburg National Research Academic University of the Russian Academy of Sciences, ul. Khlopina 8/3, 194021 Saint-Petersburg, Russia

⁴ Laboratory of Biomolecular NMR, St. Petersburg State University, 199034 St. Petersburg, Russia