Analysis of NOESY spectra to obtain accurate information on the structure and dynamics of some 5,7-substituted pyrazolo[1,5-a]pyrimidine derivatives in solution

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Introduction

The nuclear Overhauser effect (NOE) is one of the most popular phenomena, which is currently intensively used to solve structural and dynamic problems in organic and bioorganic chemistry. This interest is due to the extremely strong sensitivity of the cross-relaxation rate σ_{ij} between the magnetic nuclei "i" and "j" on the distance between them r_{ij}, and also due to the dependence of σ_{ij} on the motion of the vector r_{ij}, which is determined by the overall rotational diffusion and intramolecular mobility of this pair of nuclei: $\sigma_{ij} \sim \tau_c^{eff}(r_{ij})^{-6}$, where $\tau_c^{eff.}$ is the effective correlation time of overall diffusional and internal motions.

A lot of experimental and methodological problems of obtaining, processing and interpreting NOE data have been discussed during the last sixty years. The accuracy of quantitative estimates of interproton distances and the need for correct averaging of the results of their measurements in the presence of intramolecular dynamic processes that are fast within the NMR time scale hold a special place among these problems [1–3]. Ignoring the established methodology leads to a violation of one of the important I. Solomon requirements on the invariance of the interproton distance r_{ij} with time in the canonical description of the NOE phenomenon [4]. Another well-known requirement is the spherical shape of the studied molecule in solution, which ensures the absence of rotational diffusion anisotropy and the fulfillment of the requirement on the same correlation times τ_c for all pairs of magnetic nuclei compared with each other in a given molecule. Thus, the selection and correct use of existing approaches to eliminate or compensate factors leading to quantitative errors in the estimation of experimental distances based on NOE are important for the practical application of this NMR tool for studying the structure and dynamics of molecules in solution.
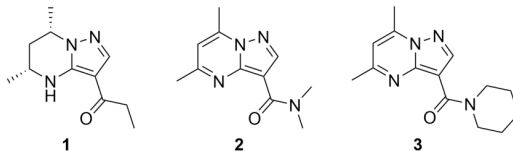

Figure 1. Structures of the studied pyrazolopyrimidine derivatives

This report presents the use of NOESY data to study in detail some 5,7-substituted pyrazolo[1,5-a]pyrimidine derivatives in solution (Fig. 1). Compound **1** was recently used to evaluate the possibility of accurately (within ±5% error) measuring the long-range distance $r_{2\text{-}6eq}$, which exceeds 6 Å [5]. It was shown that the main source of experimental errors is the

distortion of integral intensities when using the Whittaker smoother procedure [6]. So another algorithm was proposed for determining the exact values of volume integrals in NOESY spectra. In this work, these methodological features of data processing were applied in structural and dynamical studies.

Results and Discussions

Compounds **1**–**3** turned out to be extremely convenient models to analyze the problems of registration and processing of cross-peaks in NOESY spectra. Our efforts to further study structural features of compound **1** were aimed at determining the exact values of the distances between the H_{6eq} proton and protons H_{5ax} and H_{7ax} apparently symmetrically located relative to it (Fig. 2a).
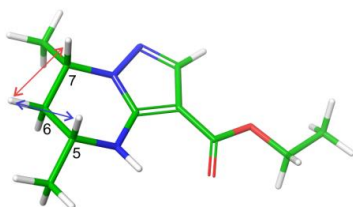

*Figure 2. Spatial structure of compound **1**, obtained by conformational search and subsequent geometry optimization, where two-ended arrows indicate considered distances*

The procedure of optimizing the geometry of the molecule **1** consistently produced a small difference between the distances $r_{6eq-5ax}$ and $r_{6eq-7ax}$ (blue and red arrows, respectively, in Fig. 2). Since this difference indicates a slight asymmetry in the location of the axial protons H_5 and H_7 with respect to the equatorial proton at position 6, we tried to use NOE to determine whether the calculated values of these distances correspond to the reality or are a consequence of optimizing method errors. To solve this problem Isolated Spin Pair Approximation (ISPA) and Peak Amplitude Normalization for Improved Cross-relaxation (PANIC) approaches were used to process the NOESY data obtained at short mixing times. These approaches are based on the use not the absolute values of the volume integrals of the corresponding cross-peaks, but their normalized values relative to the volume integrals of the diagonal cross-peaks.
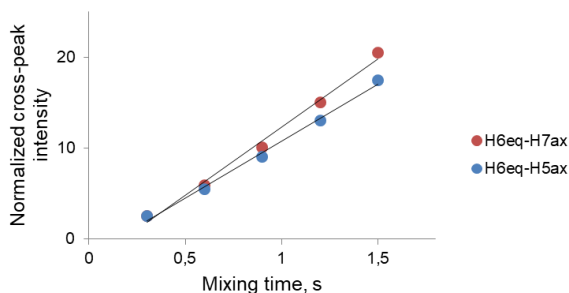
Figure 3. Graphical dependence of the normalized cross-peak intensity (S_{ij}/S_{ii}) on the mixing time τ_m for the distances $r_{6eq-7ax}$ (red points) and $r_{6eq-5ax}$ (blue points)

However, when using the minimum possible mixing time of 0.3 s for considered distances in two identical experiments, almost the same result was obtained within the measurement accuracy. Therefore, similar measurements of the normalized cross-peaks were conducted at longer mixing times. The results obtained were used to plot the dependence of normalized cross-peak intensities on mixing time (Fig. 3), which allowed estimating the corresponding cross-relaxation rates. Then we experimentally evaluated the difference in the corresponding distances using one of the calculated distances as a reference one within the considered pair. Consequently, the experimental difference in distances between considered protons turns out to be 30% higher than that obtained by the previously performed calculations [5]. Thus, it can be stated that the distance $r_{6eq-5ax}$ is really greater than the $r_{6eq-7ax}$ distance.

A similar study of the NOESY spectra of compounds **2** and **3** was carried out to determine the distances between H_2 and H_6 protons located in the rigid part of these molecules with the mobile protons of the methyl groups at positions 5 and 7, as well as with the mobile protons of the amide fragments. In this case, for quantitative estimates of cross-relaxation rates, effective values of the distances averaged by rapid exchange of mobile protons of methyl groups to the protons H_2 and H_6 were used. This report discusses various methods for obtaining and correctly processing data from NOESY spectra of such dynamic systems.

Conclusions

The presented results show the possibility of quantitative estimates of interproton distances in small molecules in solution between rigid protons, as well as between rigid and mobile protons with the high accuracy based on NOESY data using ISPA and PANIC approaches.

Acknowledgements

References

1. S. I. Selivanov, A. G. Shavva. – *Russ. J. Bioorg. Chem.*, 28(3), 194-208 (2002).
2. S. I. Selivanov, A. Y. Solov'ev, S. N. Morozkina, A. G. Shavva. – Russ. J. Bioorg. Chem., 33(3), 302-309 (2007).
3. S. I. Selivanov, S. Wang, A. S. Filatov, A. V. Stepakov. – Appl. Magn. Reson., 51, 165-182 (2020).
4. I. Solomon. – Phys. Rev., 99, 559-565 (1955).
5. D. Novikova, A. Al Mustafa, T. Grigoreva, S. Vorona, S. Selivanov, V. Tribulovich. – Molecules, 28, 6584 (2023).
6. P. H. C. Eilers. – Anal. Chem., 75, 3631-3636 (2003).