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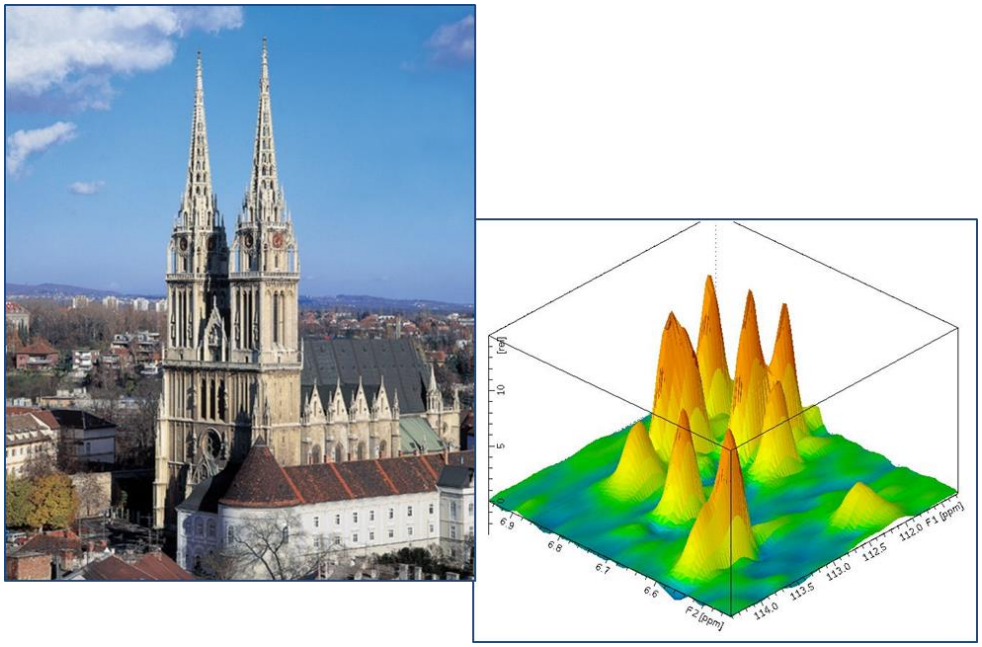

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ABSTRACTS (LECTURES)

NMR Spectroscopy in Croatia

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The first NMR spectrometer in Croatia was a home-made 29 MHz ^{1}H wide-line instrument for solid state research. This NMR device was built at Ruđer Bošković Institute from 1957 to 1959, under the supervision of dr. Siniša Maričić in a laboratory for magnetic resonances. Using this machine a number of important scientific articles have been published on structure of inorganic compounds (different sodium borates), proton conductivity and structure of noble gas molecules (xenon fluorides). Hence, one can say that NMR spectroscopy has a rather long tradition in Croatia.

In sixties, commercial instruments for high resolution NMR spectroscopy in liquids expanded on markets, especially for research related to organic chemistry. The first commercial NMR in Croatia was Varian A-60, purchased by petroleum industry firm INA-Zagreb in 1963, owing to dr. Dragutin Fleš, who in late fifties got acquainted with NMR during his stay in USA. At that time in NMR laboratory of INA have worked dr. Branko Černicki, dr. Jasna Mühl and dr. Vlasta Srića. The first commercial NMR instrument for academic community in Croatia was purchased at Faculty of natural sciences and mathematics of University of Zagreb in 1964. It was Varian A-60A, placed in organic synthesis laboratory of prof. Krešimir Balenović. At Ruđer Bošković Institute (RBI) the first commercial NMR has been installed in 1967 in Department of organic chemistry and biochemistry and the first Croatian NMR facility has been established, under the name ^{1}H NMR Service. The first head of NMR Service was professor Stanko Borčić. From that time on, with only few short interruptions in 47 years span, Ruđer Bošković Institute is giving NMR support to academic community and industry of Croatia.

In 1977 the first NMR with Fourier transformation and electromagnet was purchased by industry firm Chromos, Zagreb. It was Bruker WP-80 NMR machine. Very soon, in 1978 the PFT JEOL FX-100 and in 1980 the JEOL FQ-90 were installed both in Department of organic chemistry and biochemistry at RBI. Finally in 1990 the first superconductive NMR instrument in Croatia, Varian Gemini 300 was purchased by Ruđer Bošković Institute. With this machine NMR facility of RBI was participating in structural investigations of new azithromycin antibiotic (Zytromax, Summamed), generated by Croatian pharmaceutical industry firm Pliva.

In 2002 Bruker's AV-300 and AV-600 NMR spectrometers were installed at RBI and in July 2003 the NMR Centre of RBI was established. The first head of Centre was prof. Dražen Vikić-Topić. Centre has been performing NMR investigations in the field of organic, bioorganic, and organometallic chemistry, fluoroquinolone chemistry, supramolecular and gel chemistry, interactions of DNA, isotope effects, food and pharmaceutical chemistry, collaborating on numerous projects with researchers from RBI and University of Zagreb, Rijeka, Split and Osijek as well as with pharmaceutical companies Pliva, Belupo and JGL. NMR Centre is also involved in undergraduate and graduate studies at majority of Croatian Universities.

Beside NMR Centre at RBI few other NMR research groups work in Croatia - at University of Zagreb, Faculty of Science, group of prof. Predrag Novak and group of prof. Miroslav Požek (Bruker), at Faculty of Pharmacy, group of prof. Olga Kronja and prof. Valerije Vrček (Varian), in pharmaceutical industry firm Fidelta, group of dr. Ana Čikoš and ing. Biserka Metelko (Bruker) and petroleum industry firm INA, group of dr. Jelena Parlov Vuković (Bruker). These groups perform investigations of carbocations, isotope effects, structure of organic and biological molecules, conformation and kinetics, pharmaceutical compounds, polymers, petroleum, *etc*.

In spite of all mentioned, the Croatian academic community still suffers from the lack of modern NMR instrumentation, since the number of scientists and researchers, especially young ones, who have interest in NMR spectroscopy, rapidly increases. Therefore, the purchasing of several new and higher field NMR spectrometers for universities and institutes is a must.

Ruđer Bošković Institute applied several projects (OZIP, MediPoint, etc) to European structural funds for modernization of its research equipment, which includes purchasing of NMR spectrometers as well.

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NMR based metabolomics in disease diagnosis, plant, and food analysis

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NMR spectroscopy is a powerful non-targeted technique that has been used successfully in the analysis of body fluids especially in the field of metabolomics. It allows rapid analysis of the complex mixtures like biological fluids or plant extracts. In combination with multivariate chemometric analysis has been used successfully in the fields of drug toxicology and disease diagnosis as well as food quality control. Capabilities of NMR based metabolomics for the discovery of new biomarkers will be presented through different examples.

The first example provides the analysis of 120 newborn urine samples collected and analyzed previously by screening based mainly on MS for the diagnosis of inborn errors of metabolism. Metabolites usually not detected with other techniques (formate, dimethylamine, N-oxide trimethylamine) can be identified and monitored with NMR. Complex cases with more than one deficiencies were detected by NMR where conventional screening techniques failed. Typical examples are a) detecting increased urocanic acid in a patient with diagnosed methylmalonic aciduria b) in samples of a patient with ornithine transcarbamoylase deficiency in addition to the expected increased levels of uracil, and orotic acid increased uridine has been also determined. More importantly metabolites never been described previously in newborn errors of metabolism have been detected and identified using 2D techniques namely gluconolactone, and 4-hydroxyphenyllactate.

The second example refers to the NMR based study of the metabolome alterations during the step-wise acquisition of cancer cells resistance to doxorubicin (DXR). Metabolic profiling of osteosarcoma cell lines that were increasingly resistant DXR concentrations suggested that protein biosynthesis and glutathione metabolism, are reduced in resistant cells while other biochemical pathways like pyrimidine and purine metabolisms are also perturbed. The study depicts how cellular metabolomics may provide valuable information shedding light to complex biological mechanisms.

In a third case a pharmacometabonomic analysis will be exemplified. Pharmacometabonomics utilizes metabolic profiling to predict individual response to drugs and has important implications for personalized medicine applications. The metabolic determinants of the response of patients to clopidogrel and notably the possibility to predict development of resistance to the drug through a pharmacometabonomic analysis will be presented.

In the analysis of food and especially wine and olive oil we will describe the method for creating a database of NMR spectra for both food products. The statistical analysis of the spectroscopic data with multiparameter methods results in the creation of appropriate statistical models that allow us to analyze identification of the origin.

NMR and Olive oils: geographical origin, quality and authenticity

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The increasing ability of high field NMR spectroscopy to solve spectra of complex mixtures and to recognize and quantify each component without chemical separation, has found a constantly increasing application in metabolomics and food chemistry[1]. In this presentation I will highlight the milestones of the history between the Nuclear Magnetic Resonance spectroscopy and olive oil[2]. The quantitative analysis of major and minor compounds along with the application of a suitable statistical analysis has allowed the olive oils characterization in terms of geographical origin[3,4], variety[5] and authenticity[6]. Here, some significant applications will be reported.

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NMR Studies on Extrinsic Proteins of Photosystem II

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The Psb proteins include the extrinsic proteins found in the thylakoid membrane of higher plants and green algae [1]. PsbQ together with PsbP, PsbO and PsbR are involved in the regulation of stable oxygen production, which apparently depends on the proper assembly of these proteins in the oxygen evolving centre. While information about their structures is essential [2, 3] for the understanding of their functions, the presence of significant amounts of unstructured regions suggests an important role of dynamics in these processes. The X-ray crystallographic structure of PsbQ has been solved [4], but residues 14-33 remain unresolved, as a so-called "*missing link*". Recently, we provided initial assignment of the backbone as well as the secondary structure of PsbQ [5]. As the resonance assignment is now nearly complete including the exceptionally large number of prolines we have started solution structure and dynamics analysis. The results obtained show significant deviations compared to the available crystallographic structure. The N-terminal part of the molecule indicates a short α-helical segment embedded in a largely disordered dynamic chain at the location, where a short *β*-strand had been proposed previously.

Acknowledgments
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Simplifying proton NMR spectra by slice-selective excitation

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Protons are the most often used nuclei for NMR structure elucidation of organic and biological molecules. Compared to other NMR detectable nuclei, ^{1}H spectra typically suffer from low resolution and severe signal overlap, mainly due to extensive scalar coupling between protons. Homonuclear broadband decoupling (pure shift spectra), which leads to a collapse of ^{1}H signals into singlets vastly increases the resolution, which in some cases corresponds to a theoretical signal dispersion of NMR spectrometers at several GHz [1]. One of the most often used approaches for homonuclear broadband decoupling in the indirect dimension of two- and multidimensional NMR spectra uses frequency-selective pulses during a weak gradient field [2]. We recently reported an adaption of this method to achieve homonuclear broadband decoupling during acquisition [3]. Scalar coupling information, which is often key in analyzing chemical structures, is of course completely lost in such experiments. Two methods, which constitute a compromise between pure-shift spectra and fully coupled spectra will be presented: real-time SERF spectra [4] and real-time J-scaled proton spectra. With the first of these, 1D spectra are obtained which contain scalar coupling to one selected signal only, while J-scaling allows the recording of proton spectra with reduced coupling constants, reminiscent of off-resonance heteronuclear decoupling.

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NMR Strategies for Macrolide Antibiotics Interactions

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NMR spectroscopy has experienced a rapid growth and widespread applications in drug research and development. A variety of NMR techniques has been developed for structure-based inhibitor design studies and for establishing structure activity relationships. Macrolide antibiotics are therapeutically important class of compounds that are effective inhibitors of bacterial protein biosynthesis. Macrolide antibiotics bind to the large 50S ribosomal subunit at or near the peptidyl transferase centre and block the elongation of the peptide chain. Understanding the mechanism by which macrolides exert their activity is crucial for the design of novel molecules possessing bioactivity. However, the increasing resistance to antibiotics has become a global problem and much effort is now directed toward new and more potent classes of drugs to overcome resistance mechanisms. An effective approach to solve this problem is to understand the principles of how the drugs interact with their biological targets.

In this talk an NMR strategy to study macrolide interactions will be presented and discussed. It includes NOE based experiments such as saturation transfer difference (STD) spectroscopy which provides information on the bound state macrolide conformation and their binding epitopes. Furthermore, the application of paramagnetic relaxation enhancement (PRE) induced by an inert lanthanide complex can reveal the immersion depth of macrolide antibiotics bound to their biological targets. The obtained results can help in designing novel and more potent classes of antibiotics active against the resistant bacteria.

ssNMR Study of Deuterated Paramagnetic Bis(amino acid)copper(II) Complexes. Information on Crystal Forms, Stereo-isomerism, and Molecular Mobility Available from ^{2}H Fast-MAS Spectra

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Identification of stereoisomers in paramagnetic organometallic complexes is of general interest both in liquid and solid state. In crystalline solids single-crystal diffraction is the standard method for providing molecular structure. However, solid-state NMR has much to offer the chemist because it can both supplement and complement the diffraction results. Unfortunately, NMR spectroscopy of paramagnetic compounds is often elusive since chemical shifts may change unpredictable and signals broaden beyond detection.

A recently proposed solid-state NMR methodology, coined by Ishii and co-workers as very-fast MAS method [1], turned out to be advantageous for paramagnetic compounds [2]. ^{2}H MAS spectroscopy, utilizing the quadrupolar interaction, is widely used to detect internal motions present in crystals. Concerning the resolution the ^{2}H MAS spectroscopy offers, in diamagnetic compounds ^{2}H chemical shielding effects are normally too small to be useful in stereochemical assignments, however, in paramagnetic compounds this is not always so.

In this lecture we are going to show that the enhanced ^{2}H chemical shift dispersion of deuterons touched by the paramagnetic effects is normally huge and thereby ^{2}H spectra carry molecular-level information not only on the solid-state mobility but, simultaneously, also on the stereochemistry, crystalline habit, polymorphism, and hydrogen-bonds of the molecules studied. Obviously this nucleus suffers from low sensitivity, a problem that can be overcome only by the further improvement of NMR sensitivity. At the same time deuteration of the exchangeable protons (H/D) provides a convenient, though rather limited, solution. For demonstration, we will use well known simple bis(amino acidato)copper(II) complexes (glycine, alanine, valine, *iso*-leucine, etc.) of different deuteration degrees.

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DNA G-Quadruplexes: Intermediates and Cation Interactions

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G-quadruplexes are higher order secondary structures formed by guanine-rich DNA sequences that can be found in biologically significant regions of the genome such as telomeres, immunoglobulin switch regions and promoter regions of eukaryotic cells. The main building blocks of G-quadruplex structures are stacks of square-planar arrays of G-quartets, consisting of four guanines that are linked together by eight Hoogsteen hydrogen bonds. The presence of cations such as K^+ or Na^+ seems to be essential for the formation of G-quadruplexes due to their role in reducing repulsions amongst guanine carbonyl oxygen atoms within G-quartets and additionally enhancing base-base stacking interactions.
With the use of solution-state NMR spectroscopy and other experimental techniques (PAGE, TDS, UV, CD, DSC) we have studied the behavior of guanine-rich sequences and explored their features in an environment almost completely free of G-quadruplex promoting cations.[1] Experimental data has shown the formation of a new structure, which can be considered as an intermediate on the way to folding into G-quadruplexes. It is interesting to note that the guanine bases are not held together by Hoogsteen hydrogen bonds like in G-quartets but rather by alternative base pairing. Guanine-rich DNA sequences that are pre-organized in the way shown here could fold very rapidly and not by chance into a large number of structurally diverse G-quadruplex structures with mechanisms of varying complexity. G-quadruplex formation by telomeric repeats and gene regulatory G-rich regions could simply go through populating various intermediate states, which are generally unstable and hard to detect. This kind of studies where intermediate states can be characterized structurally, thermodynamically and kinetically provide an important step in the quest to elucidate general principles by which G-quadruplexes adopt their native folds.

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Structural insights into the folding intermediates of Trp-cage miniproteins

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The 20-residue long Trp-cage is the smallest protein known and thus has been the subject of several *in vitro* and *in silico* folding studies. Here we report the multi-state folding scenario of the miniprotein in atomic detail. We detected and characterized different intermediate states *via* temperature dependent NMR measurements of the ^{15}N and $^{13}C/^{15}N$ labeled protein, both at neutral and acidic pHs. We developed a deconvolution technique to characterize the invisible – fully folded, unfolded and intermediate – states. Using non-linear fitting methods we can obtain both the thermodynamic parameters (ΔH^{F-I}, T_m^{F-I}, ΔC_p^{F-I} and ΔH^{I-U}, T_m^{I-U}, ΔC_p^{I-U}) and the NMR chemical shifts of the conformers of the multi-state unfolding process. During the unfolding of Trp-cage at least two distinct intermediates evolve: a fast-exchanging intermediate is present under neutral conditions, while a slow-exchanging intermediate emerges at acidic pH. The fast-exchanging intermediate has native-like structure with a short α-helix in the G^{11}-G^{15} segment, while the slow-exchanging intermediate has elevated dynamics, with no detectable native-like residue contacts in which the G^{11}-P^{12} peptide bond has either *cis* or *trans* conformation. Heteronuclear relaxation studies combined with MD simulations revealed the source of backbone mobility and the nature of structural rearrangements during these transitions. The ability to detect structural and dynamic information about folding intermediates *in vitro* provides an excellent opportunity to gain new insights into the energetic aspects of the energy landscape of protein folding. Our new experimental data offer exceptional testing ground for further computational simulations.

ABSTRACTS (POSTERS)

New Imidazolium Salts: NMR Study of Their Complexation with Zinc Acetate

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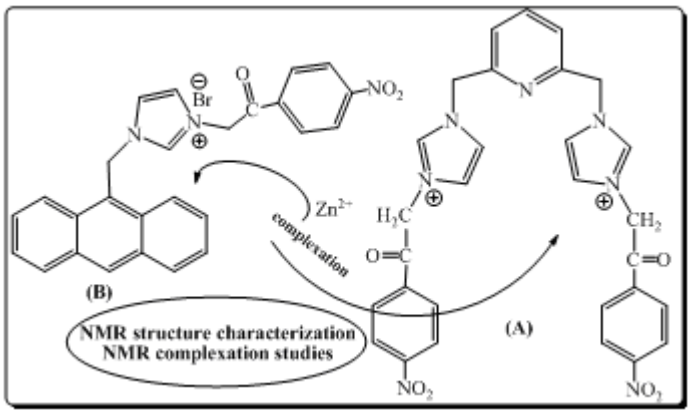
It is well known that imidazolium salts are compounds with biological properties: antibacterial, anti-inflammatory, anticancer.[1,2] Also, pyridine is intensively used as scaffold for drug development.[3] Besides, chemosensors consist imidazolium receptors and anthracene fluorophores have a great interest in host–guest chemistry, due to their biological and environmental significance.[4,5]



Having in view these considerations we synthesized two different classes of compounds having imidazol units: *bis*-imidazolium pyridine (**A**) and anthracene-imidazolium (**B**) salts. The syntheses were done in two steps: *N*-alkylation of imidazole with *bis*-bromomethyl pyridine respectively 9-chloromethyl anthracene, followed by quaternizations with bromo *p*-nitro fenacil. The structures of new compounds were proved by NMR experiments (^{1}H NMR, ^{13}C NMR, 2D-correlations). The complexation studies (^{1}H-NMR) were done using solution of salts ($2x10^{-3}$M) in deuterated acetonitrile and zinc acetate (solution in D_2O).

The NMR spectra have been recorded on a Bruker Advance III 500 spectrometer, equipped with a 5 mm PABBO detection probe, operating at 500.1 and 125.7 MHz for ^{1}H and respectively ^{13}C nuclei. In ^{1}H and ^{13}C spectra, chemical shifts are reported in δ units (ppm) relative to the residual peak of solvent (ref: DMSO, ^{1}H: 2.50 ppm; ^{13}C: 39.52 ppm).

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Spectroscopic Characterization and Biological Activity of Fatty Hydroxamic Acids Derived from Olive Oil Triacylglycerides

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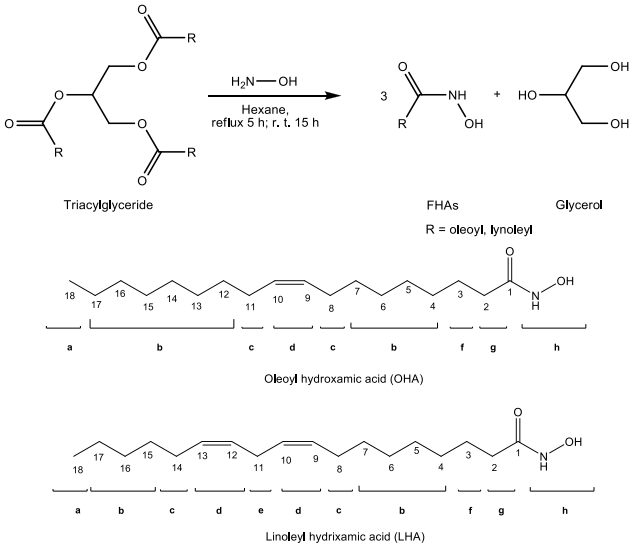
Fatty hydroxamic acid (FHAs) mixture, composed mainly of oleoyl and linoleyl hydroxamic acid (OHA and LHA respectively), was derived from olive oil triacylglycerides (Scheme). The FHAs mixture was elucidated by means of IR, Raman, MS, one- and two-dimensional homo- and heteronuclear ^{1}H and ^{13}C-NMR spectroscopy in comparison with corresponding olive oil spectra. The ratio of the OHA and LHA (4:1) was confirmed by MALDI-TOF/TOF mass spectrometry.



The results of FHAs cell toxicity on normal fibroblast (BJ) and tumour cell linee (HeLa) revealed that the normal cell line was sensitive to FHAs in whole range of applied concentrations while HeLa tumor cell line was sensitive only at higher FHAs concentrations.The radical scavenging, metal chelating and the antioxidant activity in β-carotene-linoleate assay, as well as the reducing power of FHAs, gave positive results of FHAs activity in these tests. The ADMET predictor parameters revealed that the FHAs in comparison to their corresponding carboxylic precursors (oleic and linoleic acids) are more prominent as leading compound for further research.

Keywords
Fatty hydroxamic acids, synthesis, IR, Raman, NMR and MS spectroscopy, antioxidant activity, cytotoxic activity, ADMET

Fragment Screen on BRD4 using NMR Spectroscopy

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Saturation Transfer Difference (STD) NMR[1] presents one of the most sensitive screening techniques used in fragment based lead discovery (FBLD). The STD NMR experiments are based on saturation of protein resonances through spin diffusion, subsequent magnetization transfer from protein to bound ligand and detection of the free ligand relaxation following the dissociation. NMR screen involves several fragments in a cocktail aimed at testing as many compounds as possible using minimum time and material. Individual screening can also be performed if the objective is to avoid the competition and verify the hits.

Acetylation of histone lysines has a key role in regulating chromatin architecture and transcription. BRD4[2] "reads" lysine acetylation state through binding to it. It is a member of BET family of bromodomains and a known target for various forms of carcinoma. The goal of studies on BRD4 is to find a small molecule which will selectively prevent the interaction of BRD4 with acetylated lysine without affecting the function of other bromodomain-containing proteins.

STD NMR was tested as fragment screening method against BRD4. A known binder I-BET762 was used to adjust the experimental conditions of the screen. This resulted in the identification of five unambiguous binders (excluding the known binder I-BET762). Comparison with Surface Plasmon Resonance (SPR)[3,5] data revealed a good correlation between the techniques identifying three fragments which qualified as orthogonalized binders. Experimentally determined protein-ligand crystal data[4,5] was obtained for three out of five NMR hits. Furthermore, STD NMR screen revealed two additional weak binders undetected by other techniques that warrant further investigation in fragment-to-lead chemistry.

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NMR STUDY of SOLUTION DYNAMICS of Pd AND Pd NHC COMPLEXES with ALLYL LIGANDS

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Preliminary results in our research group led to design and synthesis of fused imidazolidene N-heterocyclic carbene (NHC) complexes. Allyl palladium complexes of type **1** were obtained via base-induced *in situ* carbene generation and consequent carbene capture with relevant (η^3-allyl)palladium(II) complex. Such one-pot synthesis has the advantage of avoiding multi-step reactions.

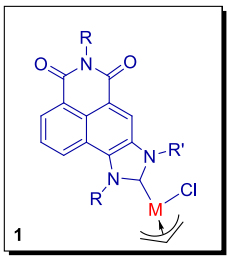

Fig. 1. Complexes of type **1**

The mechanisms of η^3-η^1-η^3-isomerization [1] and apparent allyl-palladium rotation [2] cause the chemical exchange of organometallic allyl complexes in solution in the NMR time scale. However unsymmetrical NHC allyl palladium complexes are rarely described in literature. Elucidation of structure and dynamic behaviour of complexes of type **1** (with various allyl substituents) in solution using 1D and 2D variable temperatures NMR techniques, are used not only for complete characterization of complexes, but the results may improve the fine tuning of palladium-catalyzed reactions which involves participation of allylic system. DFT calculations of ground state and transition state structures of complexes of type **1** help to prove the mechanism and origin of chemical exchange.

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Antibody-mediated ^{15}N-GS saturation transfer difference

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Fragment-based drug design has been successfully applied to challenging targets. The detection of the weak protein-ligand interactions is a key element in this approach. Pure, homogeneous protein is needed during the detection of the protein-ligand interactions by ^{1}H saturation transfer difference (STD) NMR. Recently, ^{15}N-GS STD was introduced for heterogeneous ligand library.[2] Testing this technique, ^{15}N-labelled Gal-1 protein-lactose interaction was studied. The measurements revealed that the ^{15}N-filetered excitation of the protein resonances resulted in an effective homogenous saturation, yielding signal rich STD spectra with higher intensity peaks compared with the direct ^{1}H saturation.
In this work, we present an antibody-mediated ^{15}N-GS-STD technique, which is suitable for impure proteins (Figure 1). The saturation is carried out on a ^{15}N-labelled antibody, which recognizes the target protein and the magnetization is relayed to the ligand. This way, the protein-ligand interaction can be identified in a multicomponent system such as impure protein samples. The proposed technique was tested and demonstrated on the anti-Galectin-1 antibody - Galectin-1 - lactose system.

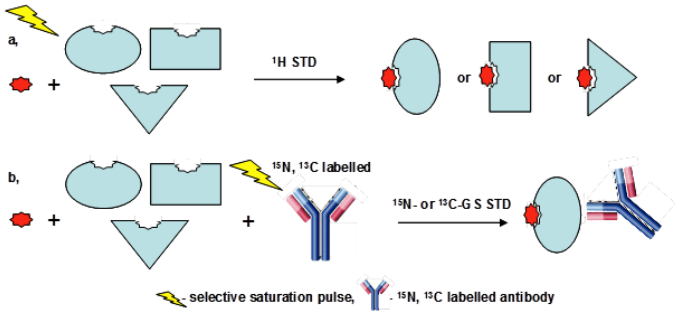

Figure 1. Antibody mediated 15N-STD technique.

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NMR Spectroscopy of Cataract

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Nowadays cataract is the most common cause of blindness. Cataract is a consequence of aging in most cases, but it can have many other causes. To understand the physiological state of crystallin proteins in the lens and how they are changed in the cataractous condition, it is important to inspect the state of water in the crystalline lens. NMR spectroscopy, and especially pulsed-gradient NMR allows us to measure diffusion rates in the water-protein solution. It is essential to use the temperature as an independent variable, to reveal phase transitions and other changes in the states of the crystallins, respectively. We had to develop a customized NMR-probe, because there was no commercial probe, which satisfied all the requirements. We have succeeded in having built the probe able to perform with the required stability, the frequency is adjustable between 98MHz and 329MHz, its coil has the proper volume and in the wide temperature range of 200 to 400 K.

NMR and Quantum Chemical Studies of Hydrogen Bonding in Acetylacetone and Benzoylacetone Derived Enaminones

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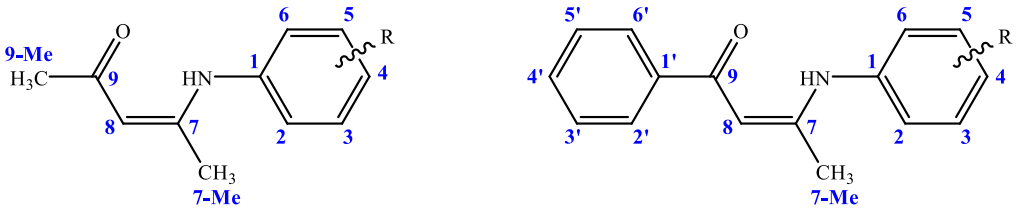
Enaminones represent an important class of organic molecules with interesting biological properties, such as anticonvulsant, antimicrobial, antioxidant, antitumor and cytogenetic activity.[1,2] They might exist in two tautomeric forms: keto-amine and enol-imine.

The structure and hydrogen bonding of acetylacetone **1a**–**1d** and benzoylacetone **2a**–**2d** derived enaminones (Figure 1) in DMSO and $CDCl_3$ solutions have been studied by a combination of NMR spectroscopy and quantum chemical (PM6 and DFT) methods. It has been demonstrated that the investigated compounds mainly existed in the localised keto-amine tautomeric form. Down-field chemical shifts of NH and OH protons and significant line-broadening have clearly indicated that both groups formed hydrogen bonds, which has further been supported by quantum chemical calculations. Concentration and temperature dependent NMR measurements have shown that amine protons are involved in strong intramolecular hydrogen bonding of the NH···O=C type in both solvents. On the other hand, hydroxyl protons are engaged in weaker intermolecular hydrogen bonds with solvent molecules in DMSO, while in chloroform intermolecular interactions between two molecules are dominant. The presented results can further be used for better understanding and exploiting properties these compounds possess, especially their bioactivity.



1a: R = 2-Me, 4-OH
1b: R = 2-OH, 5-Me
1c: R = 2-OH, 4-Me
1d: R = 2-OH, 5-NO_2

2a: R = 2-Me, 4-OH
2b: R = 2-OH, 5-Me
2c: R = 2-OH, 4-Me
2d: R = 2-OH, 5-NO_2

Figure 1. The structures and atom numbering of **1a**–**1d** and **2a**–**2d**.

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Amorphous versus nano-structured hydroxyapatites: cut-off averaging of CP MAS kinetics

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High data point density $^{1}H–^{31}P$ cross-polarization kinetics measurements have been carried out for amorphous and nano-structured calcium hydroxyapatites (CaHA). Bruker AVANCE III HD NMR spectrometer operating at resonance frequencies of 400 and 162 MHz for ^{1}H and ^{31}P respectively was applied. The chosen setting of the sampling frequency of $5{\cdot}10^{4}$ s^{-1} allowed to reveal all spin interactions having the dipolar splitting $b \leq 25$ kHz. In the case of $^{1}H–^{31}P$ interaction this means that the structures with the internuclear distances $r \geq 0.45$ nm could be resolved. The novel processing of CP MAS data has been developed introducing the variable cut-off Gaussian distribution of the dipolar coupling. It was used in the averaging of CP kinetic curves and the fitting to the experimental results. This procedure allowed to describe the oscillatory kinetics and CP curves in nano-structured materials over a wide range of contact time and to determine the characteristic size profile and composition of the spin clusters (fig. 1).

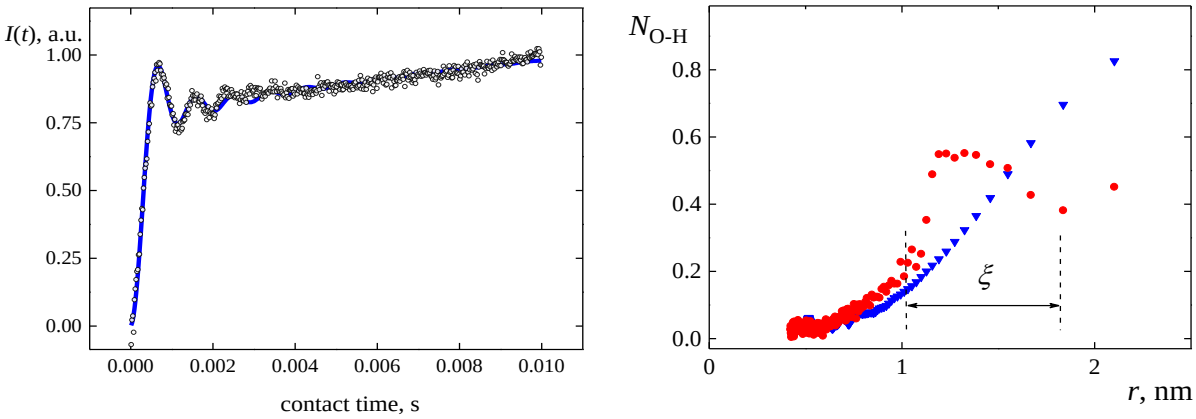

Figure 1. (Left) Oscillatory CP MAS kinetic curve, circles – experimental data points, blue line – fitting curve. (Right) Characteristic size of spin clusters, red dots – nano-structured CaHA, blue triangles – amorphous CaHA.

The characteristic size of $^{31}P–(^{1}H)_n$ spin nano-cluster being within 1.2 - 1.8 nm has been determined for nano-structured CaHA (fig. 1). Supposing that mobile H_2O molecules from adsorbed water are concentrated on the surfaces of the crystallites mainly the spatial distribution of ^{1}H spins can be considered as the approximate size distribution of nano-crystallites in CaHA.

The research was partly funded by the European Community's social foundation under Grant Agreement No VP1-3.1-ŠMM-08-K-01-004/KS-120000-1756.

Diagnosing and Monitoring Methylmalonic Aciduria by NMR Spectroscopy

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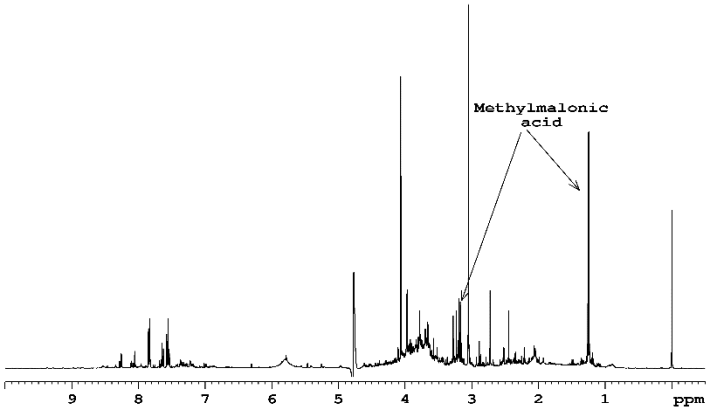
NMR spectroscopy has the potential for both targeted and untargeted screening of metabolites in biological fluids, such as urine. This technique is useful for medical research, but it may also contribute to saving lives, particularly in places where classical screening tests are not available. The NMR tests are non invasive, fast and reliable.

Methylmalonic aciduria is an inborn error of branched-chain amino acid metabolism. It is a relatively rare disorder, occurring in 1:50,000 to 1:100,000 newborns. The patients with this disorder can present high concentrations of methylmalonic acid, 3-hydroxypropionic acid, 3-hydroxyisovaleric acid and methylcitric acid in certain biological fluids.

We describe the NMR detection, quantification and follow-up of methylmalonic acid in urine of two children affected by methylmalonic aciduria. This metabolite was found to vary from 0.7 to 47 mol/mol creatinine, one child responding better to the treatment than the other (normal values 0.1-79 μmol/mmol creatinine, ref. Human Metabolom Database). In both cases, the presence of methylmalonic acid was first established by NMR analysis, the diagnostic being subsequently confirmed by classical methods.



The NMR spectrum of urine sample from one of the patients with methylmalonic aciduria.

Solid and Solution State Structural Characterization of Thiosemicarbazone Derivatives: A Combined Experimental and Theoretical Study

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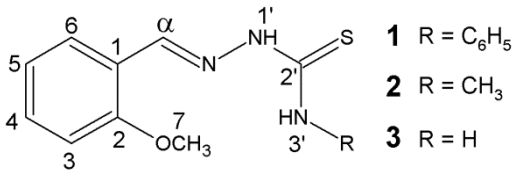
Thiosemicarbazones can exist in several tautomeric forms and conformations, with the possibility of intra- and intermolecular hydrogen bonding. Detailed structural characterization of thiosemicarbazones, as compounds that are associated with a wide spectrum of biological activities, is a prerequisite for better understanding of their bioactivity and for obtaining molecules with improved pharmacological properties.



The aim of our research is to investigate the influence of substituents, the nature of the solvent (different polarity and different proton-donor and proton-acceptor abilities) and temperature on molecular conformation, tautomerism and nature of hydrogen bonding in salicylaldehyde and 2-methoxybenzaldehyde thiosemicarbazone derivatives. We present here a part of our study regarding the effect of substituting OH with OMe group in salycilaldehyde residue on the overall structure and thione-thiol tautomerism. Solid state structures of **1** (single polymorph) and **2** (two polymorphs) were characterized by single-crystal X-ray diffraction and high resolution ^{13}C and ^{15}N solid-state NMR spectroscopy. Since the crystal structure of **3** was reported previously, we employed ^{13}C and ^{15}N solid-state NMR experiments which provided structural data complementary to those obtained by X-ray diffraction. Solid state NMR parameters were used as a reference for solution study results. Possible solvent induced conformational and tautomeric equilibrium changes were probed by multinuclear (^{1}H, ^{13}C and ^{15}N) temperature dependent NMR experiments in $CDCl_3$ and DMSO solutions. Experimental results were complemented with computational research of conformational space in vacuum and organic solvents, by combination of molecular- and quantum-mechanical methods.

Can Spin-Noise Form an Echo?

Victor RODIN[1], Norbert MÜLLER[1]

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Nuclear magnetic spin noise experiments (spectra without rf-pulses) can easily be carried out with modern high-resolution NMR spectrometers, especially when using cryogenically cooled probes. In previous work different applications (e.g. spin-noise imaging and 2D spin-noise spectroscopy) have been introduced [1–4].

We report a surprising new phenomenon, which indicated that the magnitude of spin noise depends on the gradient history.

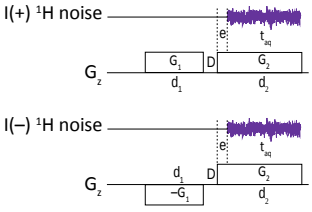

Fig.1. With this basic two gradient sequence (no rf-pulses), sets of difference experiments between accumulated noise power spectra using same sign and opposite sign gradients were performed. The integrals I(+) and I(-) are compared. Typical parameters: δ_1=2ms, δ_2=4.8ms, ε=1ms, Δ=0.15ms, G_1=G_2=(0.4–2)% G_{max}. (G_{max}= 65 G/cm).

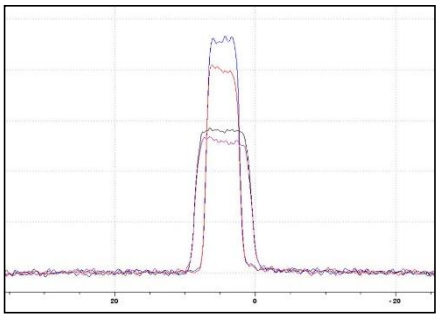

Fig.2. Spin noise power spectra acquired in 90% H_2O/D_2O (SNTO conditions [3]) with the two gradient sequences (Fig.1) using different ±G_1=G_2 strengths (% G_{max}).
G_{max} = 65 G/cm. G_1,G_2 increase from top to bottom: -0.6, 0.6; -1.0, 1.0; We attribute the amplitude increase in each pair to "refocused noise". (Bruker Avance III, 700 MHz, TCI-cryoprobe).

The results obtained support concepts of "refocused spin noise". The "spin noise echo" experiment can be applied as rf-pulse free diffusion measurement. Thus spin-noise experiments with pulse field gradient pulses could result in new approaches in studying dynamic characteristics (relaxation, exchange and diffusion) in bulk liquids and interactions in liquid mixtures.

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Acknowledgement

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Sugar Profiles of Bulgarian Oak Honeydew Honey by NMR

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In the last decade, there is a growing market in Europe for honeydew honey. In Eastern Europe, as well as in Germany, Switzerland, and Austria; the price of honeydew honeys is in general higher than the one of floral honeys, because they are regarded as more beneficial for human health[1]. A number of studies have demonstrated that honeydew honey possesses higher antibacterial and antioxidant activity compared to floral honeys[2]. For this reason, it is important to develop reliable procedures to discriminate honeydew honey from blossom honeys. Honeydew honeys contain specific microscopic elements (fungi and algae) but there are no quantitative quality criteria described so far[3]. The situation is further complicated by the fact that there are many different types of honeydew honeys[4]. Secondary metabolites and chemometric approaches, mostly PCA have also been suggested as potential solution for discrimination of honeydew honey[5]. In general, "there are no internationally accepted quality criteria for the different types of honeydew honeys"[1]

Based on the NMR carbohydrate profiles of the animeric region of 24 Bulgarian honeys we derived some specific features which set oak honeydew apart form floral honeys. The oak honeydew honeys are characterized by lower F/G ratio. Another important difference is the presence of kestose in all studied oak honeydew honeys and its absence in all studied floral honeys. Together with the presence of quercitol[6], detected in the course of the same measurement, NMR sugar profiling is a useful tool for discrimination of oak honeydew honey from floral honeys.

Acknowledgments: The financial support by the Bulgarian Science Fund (DFNI-B01-31, UNA-17, DRNF-02-13) is gratefully acknowledged.

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Structure Elucidation of Pyrroloquinoxalines and Pyrrolobezimidazoles from a One-Pot Multicomponent Synthesis

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[1] *"Petru Poni" Institute of Macromolecular Chemistry, Iasi, Romania.*

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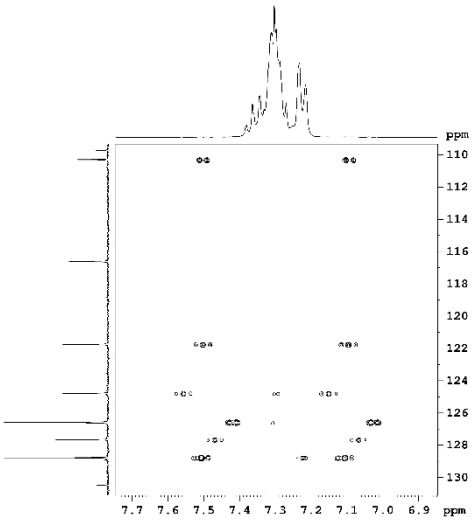
Pyrrolobenzimidazole and pyrroloquinoxaline derivatives present various biological and pharmacological properties, like anticancer, anti-tuberculosis or anti-parasitic activities. Due to these properties, there is a continuous interest in developing efficient synthetic methods and fully characterizing these compounds.

We describe the multinuclear NMR characterization of two representative compounds from these classes. The ^{1}H-, ^{13}C- and ^{15}N- NMR chemical shifts have been unambiguously assigned based on 2D NMR experiments like: H,H-COSY, H,C-HSQC, H,C-HMBC, H,N-HMBC, H,H-NOESY. In ^{1}H NMR spectra of these derivatives, the protons from the phenyl ring and the annelated benzo ring are overlapping in the region 7-8 ppm. Based on a less used undecoupled H,C-HSQC type of spectrum we assigned for the first time the individual aromatic signals, the multiplicity and the order of magnitude of the coupling constants for these classes of compounds.



Undecoupled H,C-HSQC spectrum.

NMR Spectroscopic And Theoretical Investigations Of Fluoroquinolone Norfloxacin

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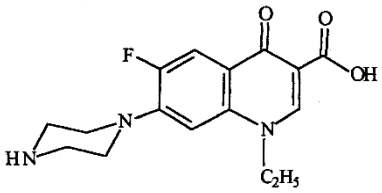
The fluoroquinolones are characterized by a broad antibacterial spectrum, good oral absorption and tissue distribution and they are widely used for treatments of different urinary, respiratory and bones infections. Although fluoroquinolones show low frequency of adverse effects, a number of them have been observed, *e.g.*inhibition of metabolism of methylxanthines-theophylline, coffeine), complexation with metal cations such as calcium, magnesium, bismuth and aluminium ions, commonly present in multivitamin and antacid preparations, *etc.* Therefore, the structure of fluoroquinolones at different pH values and the pattern of their complexation is important in studying of drug interactions.

We investigated here norfloxacin (Figure), orally active fluoroquinolone antimicrobial structurally related to nalidixic acid, by one- and two-dimensional homo- and heteronuclear ^{1}H and ^{13}C NMR spectroscopy and by semi-empirical and DFT theoretical calculations.



The two-dimensional NMR techniques: COSY, COSYLR, NOESY, ROESY, HMQC and HMBC were used for structural and conformational determination. The nuclear shieldings (chemical shifts) were calculated with GIAO/DFT theoretical method. Complexes with the following metal ions were prepared: Ca^{2+}, Mg^{2+}, Fe^{2+}, Fe^{3+}, Cu^{2+}, Zn^{2+}, Al^{3+} and the complexation was studied by, UV, IR and NMR spectroscopy, as well.

The study has revealed the fold-in orientation of the side-chain CH_3 group and pyperazine ring and the complexation with metal ions at the oxygen atoms of carboxyl and carbonyl groups of norfloxacin.