ADRIATIC NMR

June 1–4, 2023, Mali Ston, Croatia

BOOK OF ABSTRACTS

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THE OYSTER OF NMR

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Croatian Chemical Society
Department of Chemistry, Faculty of Science, University of Zagreb, Croatia

ADRIATIC NMR

June 1–4, 2023, Mali Ston, Croatia

ADRIATIC NMR CONFERENCE

Mali Ston, Croatia, June 1–4 2023

BOOK OF ABSTRACTS

IMPRESSUM

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Department of Chemistry
Faculty of Science, University of Zagreb, Croatia

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Dear Participants,

It is our great pleasure to welcome you to the **7th Adriatic NMR Conference** which is taking place from 1 to 4 June 2023 at Vila Koruna, in Mali Ston, Croatia. The Adriatic NMR Conference is organized by the Croatian Chemical Society and Department of Chemistry, Faculty of Science, University of Zagreb, Croatia.

The wide scope of the conference includes but is not limited to topics regarding the theoretical basis of NMR, method development, small molecules, Bio-NMR, benchtop NMR, spectral data interpretation and simulation, metabolomics, NMR characterization of supramolecular systems, industrial applications of NMR spectroscopy and solid state NMR methods and applications.

We strongly believe that Adriatic NMR will foster the exchange of knowledge and experience among students and scientists and will serve as a forum for an extensive networking opportunity. With confidence that it will be a memorable event, we cordially invite you to join us in the wonderful Mali Ston in June 2023.

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ADRIATIC NMR

June 1–4, 2023, Mali Ston, Croatia

PROGRAMME

THURSDAY, JUNE 1	
13:00 – 14:00	**REGISTRATION**
CHAIR: Predrag Novak	
14:00	**OPENING**
14:00 – 14:40	**Geoffrey Bodenhausen:** *Drug Screening and Long-lived States in NMR of Small Molecules*
CHAIRS: Janez Plavec, Christina Thiele	
14:40 – 15:10	**Markus Schade:** *NMR of PROTACs*
15:10 – 15:30	**Matija Modrušan:** *Peptides As Versatile Ion-Binding Receptors*
15:30 – 16:00	**COFFEE BREAK**
16:00 – 16:30	**Goran Landek:** *NMR in Pharmaceutical Industry: from Structure Elucidations to Protein-Ligand Interactions*
16:30 – 16:50	**Kristina Smokrović:** *Optimization of Alanine EPR Dosimetry for Flash-beam Irradiations*

18:00	**WALK & TASTE STON**

FRIDAY, JUNE 2	
CHAIRS: Alexej Jerschow, Pavletta Shestakova	
9:00 – 9:40	***Christian Griesinger**: NMR Spectroscopy in Chemistry and Biology with Applications in Protein/Protein Recognition and Neuroprotection*
9:40 – 10:10	**Maja Marušič:** *When PNA Meets RNA*
10:10 – 10:30	**Gyula Batta**: *Dynamics of the Anti-Candida Proteins NFAP2 and PAFC*
10:30 – 11:00	**COFFEE BREAK**
11:00 – 11:40	**TUTORIAL – Guido Pintacuda**: *Biomolecular NMR with 1H-detection and Fast Magic-angle Spinning*
11:40 – 12:10	**Marko Trajkovski**: *Structural Characterization of DNA Relying on 4'-SCF3-modifiфaction in Role of Sensitive 19F NMR Spectroscopic Probe*
12:10 – 14:00	**LUNCH BREAK**
CHAIRS: Marko Trajkovski, Tomislav Jednačak	
14:00 – 14:30	**Ivo Piantanida**: *In a Search for the Smallest Cation-binding Peptide: Cu- and Ag- Selective Dipeptide*
14:30 – 14:50	**Marija Alešković**: *NMR Insights into Diamondoid Ammonium Salt Inclusion Complexes with Cyclodextrins*
14:50 – 15:10	**Sanel Suljić (Magritek / Alphachrom)**: *New Benchmark For Benchtop NMR Magnet Homogeneity: How Spinsolve Ultra NMRs Boost Solvent Suppression Performance For 1- And 2D NMR*
15:10 – 15:20	**Mettler Toledo d.o.o.**
15:20 – 15:50	**COFFEE BREAK + POSTER SECTION 1**
15:50 – 16:20	**Marina Šekutor**: *Design and Characterization of Diamondoid Covalent Assemblies*
16:20 – 16:40	**Nikolay Vassilev**: *In Situ Irradiation NMR Spectroscopy in the Design of New Functional Materials*
16:40 – 17:00	**Marija Cvetnić**: *Ureido-calix[4]arene Derivatives in the Role of Hosts for Anions, Ion-pairs, and/or Diacetatocalix[4]arenes*

SATURDAY, JUNE 3

CHAIRS: Klaus Zangger, Markus Schade	
9:00 – 9:40	**Janez Plavec**: *Heterocyclic Ligand Intercalation into a G-rich Quadruplex DNA Structures*
9:40 – 10:10	**Wiktor Koźmiński**: *High Dimensionality and High Resolution NMR Experiments for Biomolecules*
10:10 – 10:40	**COFFEE BREAK + POSTER SECTION 2**
10:40 – 11:20	**Christina Thiele**: *Investigating Structure, Dynamics, and Intramolecular Interactions in a Peptide Catalysed Reaction by Modern NMR Methods*
11:20 – 11:50	**Pavletta Shestakova**: *Diffusion NMR Spectroscopy – Applications for Investigation of Aggregation Phenomena and Reaction Mechanisms*
11:50 – 12:10	**Mirjana Bukvić**: *Macrozones - New Antimicrobial Thiosemicarbazone-based Azithromycin Conjugates: Design, Synthesis and Characterization*
12:10 – 12:30	**Claudia Napoli (Bruker Italia)**: *Authenticity And Quality Control Of Food By Automated 1H-NMR Spectroscopy*
12:30 – 14:00	**LUNCH BREAK**

15:00	**EXCURSION + CONFERENCE GALA DINNER (OFF SITE)**

SUNDAY, JUNE 4

CHAIRS: Wiktor Koźmiński, Nikola Bregović	
9:00 – 9:40	**TUTORIAL – Klaus Zangger**: *Pure Shift NMR*
9:40 – 10:20	**Alexej Jerschow**: *NMR Relaxation Mechanisms Studied by Experiment and Computation*
10:20 – 10:50	**COFFEE BREAK**
10:50 – 11:20	**Ernest Meštrović**: *Stabilization Mechanisms of Antibiotics in Solution: a Case Study of Ceftriaxone*
11:20 – 11:40	**Danijela Bakarić**: *Synergy Of NMR And FTIR Spectroscopy In The Characterization Of Asymmetric Liposomes*
11:40 – 12:00	**Ionel I. Mangalagiu**: *Azaheterocyles: synthesis, structure, applications*
12:00 – 12:30	**Branimir Bertoša**: *Structural And Dynamical Properties Of Selected Manganese Metallosensors*
12:30	**CLOSING**

LEGEND:

PLENARY LECTURES 40'	INVITED LECTURES 30'	SECTION LECTURES 20'	TUTORIAL 40'	SPONSOR'S PRESENTTATION

POSTER SESSION

Part 1 Friday, June 2, 15:20 – 15:50	
P01	**Iva Rezić, Maja Somogyi Škoc, Ernest Meštrović:** *Characterization of Sol Gel Precursor in Functionalization of Antibacterial Biopolymers*
P02	**Alma Ramić, Marina Poljak, Jakov Borovec, Ines Primožič, and Tomica Hrenar:** *Classification Models for Fragrant Compounds Based on NMR Spectroscopy*
P03	**Josip Požar, Andrea Usenik, Marija Alešković, Sunčica Roca, Iva Markuš, and Marina Šekutor:** *Comparative Study of Cyclodextrin Binding Affinities for Diamondoid Alcohols*
P04	**Tomislav Jednačak, Ivana Mikulandra, Kristina Smokrović, Monika Kapustić, Kristina Delaš, Ivo Piantanida, Marta Jurković, Klaus Zangger, and Predrag Novak:** *NMR and Fluorometric Studies of Bioactive Macrozone Interactions with Their Biological Targets*
P05	**Valentina Martinez, Senada Muratović, Bahar Karadeniz, Gregor Mali, Yulia Krupskaya, Vladislav Kataev, Dijana Žilić, and Krunoslav Užarević:** *Unraveling the Structural Changes of Zinc-copper MOFs by Combining ss-NMR and EPR Spectroscopy*
P06	**Markus Rotzinger, Nathalie Schuster, and Klaus Zangger:** *SelEx - a Fast and Easily Setup 1D Exchange NMR Spectroscopy Experiment*
P07	**Jasna Alić, Ivana Biljan, Zoran Štefanić, and Marina Šekutor:** *Characterization of Diamondoid Ether Self-assemblies on a HOPG Surface*
Part 2 Saturday, June 3, 10:10 – 10:40	
P08	**Karina A. Stadler, Lesly J. Ortiz-Joya, Amit Singh, Christoph Buhlheller, Karl Gruber, Tea Pavkov-Keller, Sergio Pulido, Marcel Marín-Villa, Klaus Zangger, and Nina Gubensäk:** *Structural Elucidation of the PH Domain of Akt-like Kinase in Trypanosoma cruzi: a New Target for Treatment of Chagas Disease*
P09	**Ionel I. Mangalagiu, Liliana Oniciuc, Vasilichia Antoci, and Violeta Mangalagiu:** *Heptacyclic Benzo[f]quinoline Derivatives: a NMR Study Concerning Structure Determination*
P10	**Violeta Mangalagiu, Dorina Amariucai-Mantu, and Ionel I. Mangalagiu:** *A NMR Study Concerning Conformational Equilibria of Some Pyridazinones Derivatives*
P11	**Luka Fotović, Andrea Usenik, Tea Babić, Nikola Bedeković, Katarina Pičuljan, and Josip Požar:** *Complexation Thermodynamics of Benzene Derivatives with Cucurbit[7]uril and β-cyclodextrin*
P12	**Gyula Batta, András Czajlik, Gai Jiawei, Réka Erdei, László Izsépi, Pál Herczegh, and Ilona Bakai-Bereczki:** *Vancomycin Antibiotics and Antifungal Proteins as Antiviral Agents? The use of NMR Spectroscopy and Artificial Intelligence*
P13	**Monika Galić, Leda Divjak, Kristina Smokrović, Predrag Novak:** *Synthesis and Characterization of a Macrozone 4'' Derivative of Azithromycin Derived from 4-aminobenzoic Acid and Salicylaldehyde and its Complex with Nickel(II) and Copper(II)*
P14	**Karla Kukina Gradečak, Katarina Leko, Andrea Usenik, Nikola Cindro, Vladislav Tomišić:** *Complexation of Alkali and Alkaline Earth Metal Cations by Calix[6]arene Receptors*

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PLENARY LECTURES

DRUG SCREENING AND LONG-LIVED STATES IN NMR OF SMALL MOLECULES

Geoffrey Bodenhausen

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Putative drug molecules that have an affinity for macromolecular targets such as proteins can be identified if parameters like chemical shifts, relaxation times, translational or rotational diffusion constants, intermolecular Overhauser effects, intermolecular spin-diffusion rates, etc., feature a good contrast between free and bound forms of the putative drug molecules. Long-lived states (LLSs) correspond to populations of spin states (or imbalances of their populations) that have life-times T_{LLS} that are longer than the longitudinal spin-lattice relaxation time T_1. On the other hand, long-lived coherences (LLCs) correspond to a special class of zero-quantum coherences that span pairs of states of different symmetry and that have life-times T_{LLC} that are longer than the transverse relaxation time T_2. The determination of the lifetimes T_{LLS} and T_{LLC} opens the way to greatly improved contrast between free and bound forms, and hence to improved methods for drug screening. Our recent discovery that one can easily excite and observe LLSs and LLCs involving protons of neighbouring CH_2 groups in aliphatic chains of common achiral molecules may broaden the scope of drug screening, provided the signal intensity can be boosted by hyperpolarization by suitable methods such as DNP, CIDNP or SABRE.

NMR SPECTROSCOPY IN CHEMISTRY AND BIOLOGY WITH APPLICATIONS IN PROTEIN/PROTEIN RECOGNITION AND NEUROPROTECTION

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NMR spectroscopy is versatile for the investigation of structure and dynamics of molecules important in chemistry and biology. After an introduction into the technique, examples of the determination of the configuration of organic molecules will be shown with quantities down to a few 10s of micrograms.[1]

Fast kinetics of protein dynamics will be discussed on examples of folded and unfolded proteins as well as for recognition processes.[2]

Interaction studies between diphenylpyrazole (DPP) compounds and α-Synuclein in membranes will be shown which indicate binding between these compounds and the backbone of α-Synuclein. These interactions are of interest since DPP compounds lead to disease modifying therapies for Parkinson's, Alzheimer's, Creutzfeldt Jacob disease and Type II diabetes mellitus.[3]

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HETEROCYCLIC LIGAND INTERCALATION INTO A G-RICH QUADRUPLEX DNA STRUCTURES

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The quadruplex DNA structure is a non-canonical DNA structure formed by stacking planar guanine bases in four-stranded structures called quartets. G-quartets are formed by the interaction of four guanine bases held together by a cyclic arrangement of hydrogen bonds. The G-rich DNA sequences that form G- and AGCGA-quadruplex structures are commonly found in telomeres, the protective caps at the ends of chromosomes, and in certain regulatory regions of the genome. Quadruplex structures play important roles in regulating gene expression, replication, and repair and are therefore of interest for both basic research and drug discovery. The structure and stability of G-quadruplexes can be studied using a variety of biophysical techniques, including Nuclear Magnetic Resonance (NMR) spectroscopy.[1-5]

Quadruplex DNA can interact with a variety of small molecules through specific recognition of the G-quartet structure. In addition, small molecules that specifically bind to quadruplex structures have been shown to be potential therapeutics for cancer and other diseases.[6] The interactions between ligand and quadruplex DNA can have significant effects on the biological function of quadruplex DNA as well as on the pharmacological properties of the ligands themselves. Binding of ligands to quadruplex DNA can stabilize or destabilize DNA structure, leading to changes in gene expression or other cellular processes.

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INVESTIGATING STRUCTURE, DYNAMICS, AND INTRAMOLECULAR INTERACTIONS IN A PEPTIDE CATALYSED REACTION BY MODERN NMR METHODS

Jens Nowag,[a] Matthias Brauser,[a] Raffael C. Wende,[b] Peter R. Schreiner,[b] Christina M. Thiele[a]

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We present our study on a peptide-based organocatalyst that enables the enantioselective monoacylation of racemic *trans*-cycloalkane-1,2-diols.[1] A dynamic binding pocket formed by the acylated catalyst intermediate was proposed by molecular mechanics as well as by DFT computations. Herein, we describe a structural study using RDCs and NOEs yielding a conformer ensemble that allows a detailed analysis of the catalyst's mode of action.[2]

Furthermore, we present an NMR titration strategy[3] of the peptide catalyst alone and in mixtures with the two diol enantiomers to quantify intramolecular interactions by several complementary NMR methods. The values obtained are well in-line with the synthetic results and the observed selectivity. The results indicate that aggregation plays a key role in this system.[4]

Acknowledgements. This work has been supported by the German Research Foundation (DFG) under grant agreement TH1115/12-1.

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NMR RELAXATION MECHANISMS STUDIED BY EXPERIMENT AND COMPUTATION

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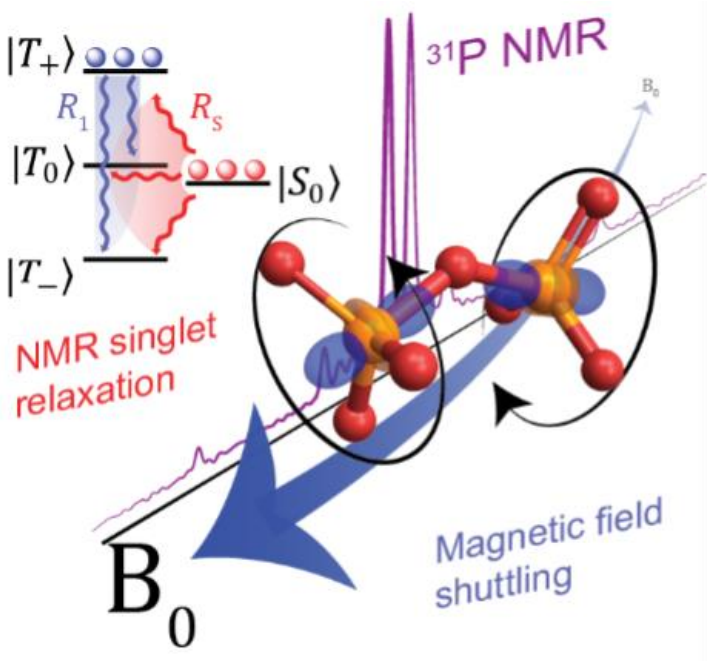
Over the years, several relaxation mechanisms have been identified, including dipolar coupling, chemical shift anisotropy, paramagnetic relaxation, spin rotation and spin-internal motion, and the scalar relaxation of the second kind. While in principle, many of the mechanisms are well understood, estimating their size can be difficult. Furthermore, multiple experimental examples have been found that decidedly defy expectations.

We present here work on directly estimating singlet relaxation mechanisms from molecular dynamics simulations. Nuclear spins states have been shown to exceed spin-lattice relaxation times several fold, with impressive demonstrations of singlet lifetimes of more than an hour in organic molecules in solution. Here we show calculations for intermolecular mechanisms and find good agreement with experiment. It is particularly surprising to see that such mechanisms as intermolecular coupling to ^{35}Cl and ^{37}Cl nuclear spins (of the chloroform solvent) could be rate limiting for singlet states.

In addition, the spin-rotation relaxation mechanism is shown to be pronounced for both singlet and Zeeman order. Good agreement between experiment and computation is achieved. Furthermore, we discuss the importance of taking into account internal motion when calculating spin-rotation tensors.

Calculations of this sort may help in the design of particularly long-lived singlet states, or could be used to identify new probes for dynamics.



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TUTORIAL LECTURES

BIOMOLECULAR NMR WITH 1H-DETECTION AND FAST MAGIC-ANGLE SPINNING

Guido Pintacuda

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Since the first pioneering studies on small deuterated peptides dating more than 20 years ago, 1H detection has evolved into the most efficient approach for investigation of biomolecular structure, dynamics, and interactions by solid-state NMR. The development of faster and faster magic-angle spinning (MAS) rates (up to 150 kHz today) at ultrahigh magnetic fields has triggered a real revolution in the field. This new spinning regime reduces the 1H–1H dipolar couplings, so that a direct detection of 1H signals, for long impossible without proton dilution, has become possible at high resolution. The switch from the traditional MAS NMR approaches with 13C and 15N detection to 1H boosts the signal by more than an order of magnitude, accelerating the site-specific analysis and opening the way to more complex immobilized biological systems of higher molecular weight and available in limited amounts. This tutorial reviews the concepts underlying this recent leap forward in sensitivity and resolution, presents a detailed description of the experimental aspects of acquisition of multidimensional correlation spectra with fast MAS, and summarises the most successful strategies for the assignment of the resonances and for the elucidation of protein structure and conformational dynamics. It finally outlines some examples where 1H-detected MAS NMR has contributed to the detailed characterisation of a variety of crystalline and noncrystalline biomolecular targets involved in biological processes ranging from catalysis through drug binding, viral infectivity, amyloid fibril formation, to transport across lipid membranes.

TUTORIAL: PURE SHIFT NMR

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NMR spectroscopy is a versatile tool to determine structural, chemical and physical properties of molecules. 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.[1-3] One of the approaches for homonuclear broadband decoupling in the direct and indirect dimension of two- and multidimensional NMR spectra uses frequency-selective pulses during a weak gradient field[4] While this slice-selective decoupling could be used both during the evolution time of multi-dimensional NMR experiments and also during detection,[5] potentially in a single scan, other pure shift techniques yield superior performance but can only be used in a pseudo-2D fashion. In this tutorial talk the methods are presented and their differences discussed.

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INVITED LECTURES

NMR OF PROTACS

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Proteolysis Targeting Chimeras (PROTACs) are progressing into the clinic, but guidelines for achieving oral bioavailability are poorly documented. Here we report solution NMR methods for measuring the 3D conformational and physicochemical properties of PROTACs and derive guidelines for improving degradation potency and oral bioavailability. We demonstrate how experimental physicochemical properties in solution differ from calculated 2D properties in silico and point out key differences from Rule-of-5 small molecule medicines, particularly an upper limit of two solvent-exposed H bond donors.

NMR IN PHARMACEUTICAL INDUSTRY: FROM STRUCTURE ELUCIDATIONS TO PROTEIN-LIGAND INTERACTIONS

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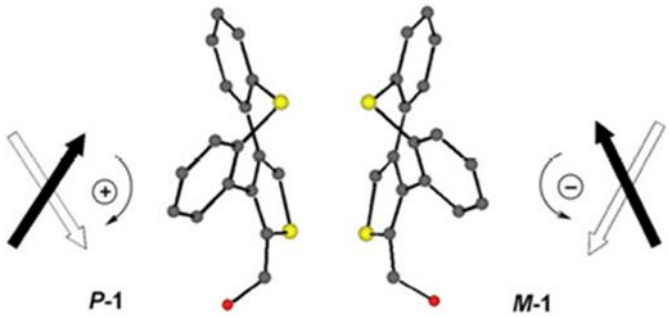
Creating a new drug is extremely lengthy and costly process. Any methodology which can assist and speed-up this process is highly appreciated. NMR is one of the many of the analytical techniques which have found its place in pharma industry. From early stages of discovery to later phases of development and manufacturing, NMR is used as “eyes” of researchers to control and direct synthetic pathways in a chosen direction.

In this talk, role of the Selvita Zagreb NMR group in supporting medicinal chemistry projects will be highlighted. One illustrative example is the combined use of dynamic NMR, line-shape analysis, molecular modelling and chiral HPLC analysis for probing conformational properties of dibenzo[b,f]thieno[3,4-d]-fused oxepines and thiepines.[1]



Alongside with many structural verifications and unknown reaction product determinations for various projects and molecule sizes, NMR was also involved in fine-tuning for lead optimization of IL-17A/IL-17RA macrocyclic disruptor through its STD and epitope mapping methods.[2]

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WHEN PNA MEETS RNA

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The ability to control the dynamic equilibria of the structured RNA is an important tool in RNA biology and has the potential for therapeutic targeting of RNAs. Triplex forming oligonucleotides that bind within duplex major groove through Hoogsteen interaction are an attractive option but are mainly used to target DNA due to the low stability of complexes with RNA and slow complex formation. Peptide nucleic acids (PNAs) increase triplex stability and binding specificity and are thus an excellent alternative for targeting RNA helices. Moreover, base modifications of PNA residues allow for triplex formation at neutral pH and salt concentrations compatible with cellular conditions. Recently, PNAs were shown to form unusually stable and sequence-specific triple helices with single-purine-nucleotide bulges.[1] We have explored structural requirements for this sequence specificity, showing that PNA efficiently shifts bulged residue conformation to adjust for uninterrupted triplex formation.

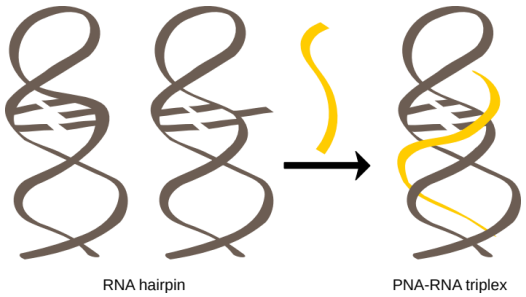

Figure 1. PNA binding to RNA helices shifts equilibria of bulged residues.

Acknowledgements. This work has been supported by Slovenian Research Agency [P1-0242].

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STRUCTURAL CHARACTERIZATION OF DNA RELYING ON 4'-SCF_3-MODIFIFACTION IN ROLE OF SENSITIVE ^{19}F NMR SPECTROSCOPIC PROBE

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Modified nucleotide analogues are often designed to broaden the structural and chemical range of synthetic oligonucleotides, thereby to support emergence of novel or improved therapeutic applications.[1] On the other hand, their use as probes for providing new insights, e.g. into molecular pathways, crucially relies on discreetness of the modifications in terms of preserving the native nucleic acid features. In this regard ^{19}F NMR studies relying on the use of fluorinated nucleotides have importantly contributed to understanding of nucleic acid structures and functions.[2,3,4]

This presentation will focus on structural characterization of oligonucleotides designed to carry 4'-SCF_3-thymidine (T4'-SCF_3) in role of NMR spectroscopic probe. Comparative analysis of the high-resolution structural details of the duplex exhibiting a single T4'-SCF_3 residue and non-modified duplex shows no major structural changes. Moreover, our results are consistent with a flexible orientation of 4'-SCF_3 group in the minor groove of DNA duplex, whereby the isolated spin system of the three magnetically equivalent fluorine atoms offers high ^{19}F NMR sensitivity. In view that the minor groove of a DNA duplex represents biologically important interface, we demonstrated amenability of using T4'-SCF_3 for ^{19}F NMR-monitoring of DNA-protein interactions. The approach also enables detection of single nucleotide polymorphism.[5] Other applications of using T4'-SCF_3 as a sensitive ^{19}F NMR spectroscopic probe will be discussed as well, including studies of the ratios of different (non-canonical) forms of DNA in equilibrium.

Acknowledgments. This research was funded by Slovenian Research Agency (ARRS) (grant no. P1–0242 and J1-1704)

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IN A SEARCH FOR THE SMALLEST CATION-BINDING PEPTIDE: Cu- AND Ag- SELECTIVE DIPEPTIDE

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Among many proteins and peptides, which bind metal cations, Phe-Arg-His (FRH) peptide motif (Scheme 1) attracted our attention, because it is known as an efficient ligand for Cu^{2+} in biorelevant conditions, protecting synapses from copper-related oxidative damage by resisting ROS generation.[1,2] Recently we reported several novel analogues of the Phe-Arg-His (FRH) peptide motif, prepared by replacing the histidine heterocycle with triazole and consequent triazole-fluorophore (coumarin) extension and also replacing arginine with less voluminous lysine. So constructed Phe-Lys-Ala(triazole) (FKA(triazole)) peptidoids bind Cu^{2+} cations in water with a strong, nanomolar affinity comparable to the parent FRH, demonstrating that triazole can coordinate copper similarly as histidine. Moreover, even truncated KA(triazole)coumarin dipeptide (**C**) showed submicromolar affinity to Cu^{2+}.[3]

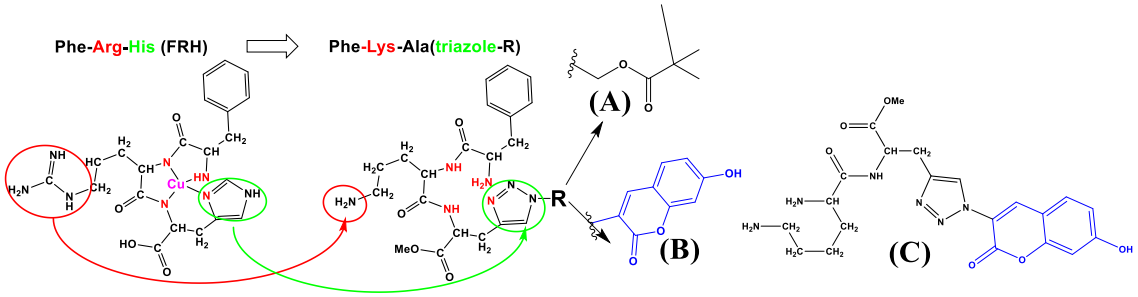

Figure 1. Comparison of naturally occurring FRH peptide (drawn as a complex with Cu^{2+}) sequence with here designed and prepared FKA(triazole) analogues (**A**), (**B**) and truncated dipeptide (**C**). Red denotes the replacement of Arg- with Lys-; green denotes the replacement of Hys-imidazole- with triazole-; blue denotes coumarin chromophore.

Such unexpectedly high affinity of dipeptide (**C**) toward Cu^{2+} prompted us to study in more detail the transition metal cation binding of novel peptidoids, whereby surprisingly selective fluorimetric response toward copper (I) and (II) ions was observed. Detailed NMR study yielded the structure of peptidoid(**C**): Cu^{2+} complex, stressing the importance of fine-tuning of orientation and distances of metal-coordinating positions, as well as the essential role of triazole in cation coordination.

Acknowledgements. This work has been supported by HrZZ IP-2018-01-5475.

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DESIGN AND CHARACTERIZATION OF DIAMONDOID COVALENT ASSEMBLIES

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Diamondoid compounds can find applications in various fields, especially as scaffolds in nanomaterial design. Diamondoid covalent assemblies are especially promising derivatives that typically consist of several diamondoid cage subunits connected with a heteroatom or a functional group. We recently prepared and fully characterized such compounds that had an ether linker in order to test their self-assembly capabilities on surfaces.[1,2] Their unambiguous characterization was achieved using NMR and was made easier due to their inherent high symmetry. On the other hand, structural assignment of their on-surface self-assemblies is challenging due to their non-planar nature so spatial identification was accomplished using a combination of microscopy and computational tools.

Spontaneous self-assembly of such systems is predominately governed by London dispersion interactions (Figure 1). In order to gain more insight into the nature of the agglomeration process, we also studied their arrangement in helium nanodroplets, which is an environment suitable for characterizing weakly-bound supramolecular clusters.[3,4] Deeper understanding of the forces governing the self-organization of diamondoid covalent assemblies opens a way to their tailored design and further application as building blocks in nanotechnology.

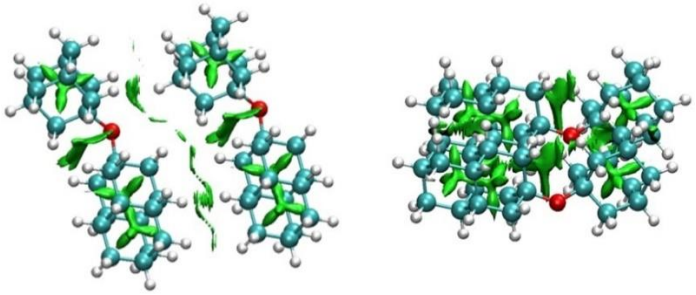

Figure 1. Self-assembled structure consisting of two 1-adamantyl-4-diamantyl ether molecules; non-covalent interactions are depicted in green.

Acknowledgements. This work has been supported by the Croatian Science Foundation (UIP-2017-05-9653).

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HIGH DIMENSIONALITY AND HIGH RESOLUTION NMR EXPERIMENTS FOR BIOMOLECULES

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Studies of biomolecular structure and dynamics by NMR spectroscopy at atomic resolution require acquisition of multidimensional spectra. However, the recording time of sufficiently resolved multidimensional spectra is often very long due to the sampling limitations. A variety of different methods, mostly based on non-uniform sampling, were proposed to overcome this limitation in multidimensional NMR spectroscopy. They could be utilized in two different ways, either to shorten the experiment duration without loss of resolution, or to perform experiments that are not obtainable conventionally, i.e. with significantly improved resolution and/or of high dimensionality. Most often first of these two, so called “Fast NMR” approach, is shown as the example of the utility of these methods, as it saves expensive spectrometer time. However, in many cases spectra which are not possible to record conventionally, featuring extraordinary resolution and high number of dimensions may be more interesting from scientific point of view as they reveal effects that are hidden, when spectral lines are broad, or enable resolving spectral ambiguities when peaks are overlapped. This second approach we refer to as “Accurate NMR”. Its full potential is manifested when the overall experiment time is less important than a new information available from spectra of high dimensionality (4-6D) or of high resolution approaching natural line-width. The new methods were applied for NMR studies of intrinsically disordered proteins, where the structural disorder in combination with highly repetitive amino-acid sequences causes severe peak overlap in the spectra. Several novel 4-7D pulse sequences are proposed. The new experiments employ non-uniform sampling that enables achieving high resolution in indirectly detected dimensions.

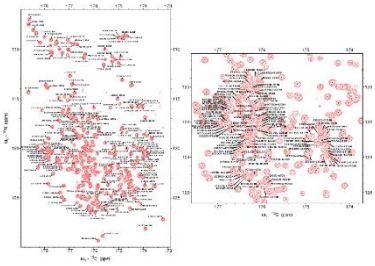

Figure 1 Resonance assignment of Tau3x (354 aa) shown on CON projection from 3D HNCO[1]

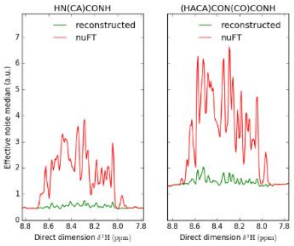

Figure 2 Noise median for 5D HN(CA)CONH (*left*) and 5D (HACA)CON(CO)CONH (*right*) SSA-reconstructed and nuFT spectra with respect to direct dimension δ ^{1}H chemical shift[2]

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DIFFUSION NMR SPCTROSCOPY – APPLICATIONS FOR INVESTIGATION OF AGGREGATION PHENOMENA AND REACTION MECHANISMS

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Within the wide toolbox of NMR techniques diffusion NMR spectroscopy has proven its power as a useful method for the investigation of complex mixtures, intermolecular interactions and aggregation phenomena in solution.[1] The method has well established methodology and has been successfully applied for the investigation of non-covalent and covalent type of interactions. In combination with other advanced NMR techniques diffusion NMR has great potentials as a valuable research strategy for characterization of complex fluids and reaction mechanisms.

Within this context examples from our research on the application of integrated diffusion NMR based approach for investigation of non-covalent and covalent intermolecular interactions will be presented.[2-5]

We applied ^{1}H diffusion NMR, in combination with 2D NMR and molecular dynamics simulations for the investigation of aggregation phenomena and for determination of particles size, shape, structure and polymer partitioning in complex multicomponent surfactant-polymer mixtures (mixed micelles and sterically stabilized liposomes).

Diffusion NMR based approach has been also successfully applied by our group for identification and characterization of *in situ* generated catalytic intermediates allowing deeper insight into the mechanism of specific intermolecular interactions and better understanding of the mechanism of chemical reactions. Examples include polyoxometalate promoted hydrolysis of biologically relevant substrates and asymmetric borane reduction of ketones in the presence of chiral ligands.

Acknowledgements. This work has been supported by the Bulgarian Ministry of Education and Science under the project INFRAMAT, part of the Bulgarian National Roadmap for Research Infrastructures.

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STABILIZATION MECHANISMS OF ANTIBIOTICS IN SOLUTION: A CASE STUDY OF CEFTRIAXONE

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Antimicrobial resistance poses a significant challenge for the future of healthcare. As bacteria become increasingly resistant to antibiotics, it becomes more difficult to treat infections and prevent the spread of disease. This can lead to longer hospital stays, increased healthcare costs, and higher rates of morbidity and mortality. Therefore, continuous efforts are being made to discover new antibiotics and to increase the stability and efficacy of existing molecules.

There is also an approach known as antimicrobial stewardship, with the overall aim of balancing the need for effective treatment of infections with the need to minimize the emergence of antimicrobial resistance.

By embracing antimicrobial stewardship principles, we can further drive the need to utilize current molecules and improve their stability in solution. A prime example of this approach in action is the development of a ready-to-use formulation of vancomycin, which has been made available to healthcare providers. Meanwhile, b-lactam antibiotics remain one of the most important classes of antibiotics, and are still widely used in the fight against bacterial infections.

In this study, Ceftriaxone was used as the model compound to investigate the stabilization mechanism based on degradation kinetics, using various techniques. The results show that the stabilization mechanism is specific to the molecule, but understanding the possible non-covalent interactions is still of great benefit for its utilization.[1]

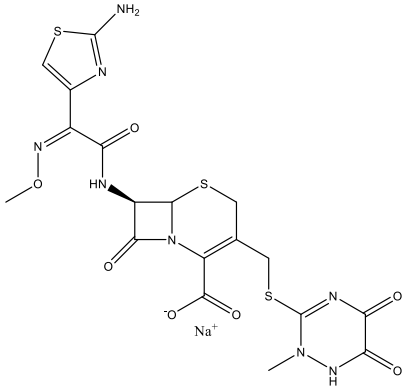

Figure 1. Molecular structure of Ceftriaxone sodium salt

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STRUCTURAL AND DYNAMICAL PROPERTIES OF SELECTED MANGANESE METALLOSENSORS

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Homeostasis of manganese ions is essential for bacteria survival. Manganese ions (Mn^{2+}) are cofactors for some of the key enzymes of basic cellular processes, such as DNA replication and resistance to oxidative stress, as such, are also essential for bacterium.[1,2] At the same time, too high cellular concentration of Mn^{2+} ions is toxic for the bacterium cell. Therefore, bacteria have developed sensitive cellular mechanisms for regulation of manganese homeostasis based on transcriptional factors that regulate transcription of proteins involved in transport and storage of Mn^{2+} ions in cell. [1,2] Since such mechanisms are different between bacteria and eukaryotes, mentioned transcriptional factors in pathogenic bacteria present potential targets for the development of novel antibiotics. In the presented research, structural and dynamical properties of selected manganese metallosensors regarding the presence or absence of Mn^{2+} ions in the binding sites, were studied computationally and experimentally. Computational methods consisted primarily of molecular dynamics (MD) simulations using explicit solvent model. Starting from available crystal structures, different systems with and without Mn^{2+} ions in the binding sites were generated *in silico* and prepared for all-atom MD simulations. Parametrisation of interactions between Mn^{2+} ions and amino acid residues of the binding sites were achieved using quantum mechanics (QM) calculations. Validation of computational results was achieved through several different experimental methods, such as microcalorimetry (ICT and DSC) and circular dichroism (CD) spectroscopy. Since NMR spectroscopy is the most suitable experimental method for investigation of proteins' structural and dynamical properties in solution, NMR measurements are planned as well. For the purpose of NMR experiments, bacteria with plasmids harbouring genes of interest was grown in ^{13}C and ^{15}N-labelled medium.

Acknowledgements. This work was supported by the Croatian Science Foundation project "Manganese metallosensors" IP-2020-02- 3446.

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SECTION LECTURES

PEPTIDES AS VERSATILE ION-BINDING RECEPTORS

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During the last few decades cyclic peptides have attracted large interest due to their bioavailability and biological activity, whereby they can serve as both host and guest molecules in the corresponding binding processes.[1,2] Their enhanced binding affinity, metabolic stability and selectivity towards substrates, compared to the linear analogs, make them perfect candidates for antibiotics and membrane transport usage. Cyclic peptides are usually synthesized from the linear precursors. The main factor that affects the success of a ring-closure reaction in the synthesis of cyclic peptides, regardless of the macrocyclization strategy, is the conformational preorganization, that is the ability of a linear precursor to bring its reactive termini in the close spatial proximity. This reaction step requires template species amongst which the simplest ones used are cations and anions. Our previous work showed improved macrocyclization yields when chloride was used as a template agent[3] compared to the recommended procedure, as well as relatively high affinity of the prepared cyclic peptides towards monovalent anions.[4,5] Here we present the investigations of the binding of several monovalent (oxo)anions, as well as bivalent cations, to the linear and cyclic peptides monitored by NMR and fluorescence spectroscopy. To get an insight into the structural characteristics of the receptors and complex species and conformational changes arising from the complexation process, we have also performed detailed molecular dynamics simulations.

Acknowledgements. This work was supported by the Croatian Science Foundation under project IP-2019-04-9560 (Macrosol) and European Regional Development Fund (project CluK, KK.01.1.1.02.0016).

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OPTIMIZATION OF ALANINE EPR DOSIMETRY FOR FLASH-BEAM IRRADIATIONS

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Alanine EPR (electron paramagnetic resonance) dosimetry is an industry standard for the determination of high radiation doses (between 10 Gy and 150 kGy).[1] It relies on the formation of the stable alanine radical (SAR) that is formed by deamination of the alanine zwitter-ion. This radical species comprises 60% of the radicals formed upon irradiation of L-alanine crystals. Besides the SAR, nearly 40% of the radicals formed are the second alanine radical, R2. This radical is formed by the abstraction of the H_α atom. A third radical species is also formed, although it is present only in a minor amount. The structure of the third alanine radical has not been confirmed experimentally. [2,3]

In the advent of development of new devices for radiotherapy that can deliver exceptionally high doses of radiation in short pulses, it is necessary to establish whether the established calibration curves still valid.

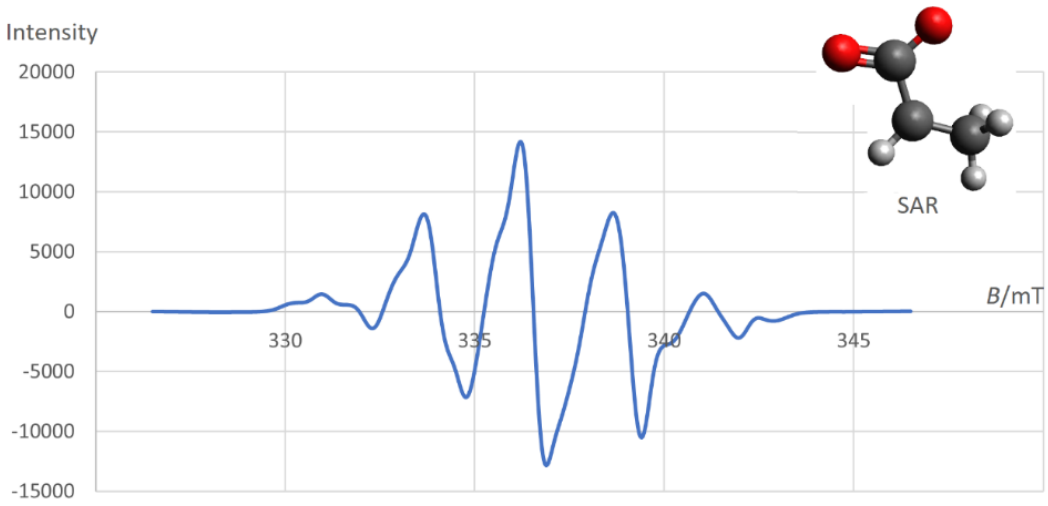

Figure 1. EPR spectra of an irradiated L-alanine pellet and the structure of the stable alanine radical.

Acknowledgements. This work has been supported by British Embassy Zagreb Impact Fund 2022-2023; project entitled "Uncertainty Improvement of Dose Estimation by Using Alanine EPR Dosimetry for Flash Radiotherapy".

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DYNAMICS OF THE ANTI-CANDIDA PROTEINS NFAP2 AND PAFC

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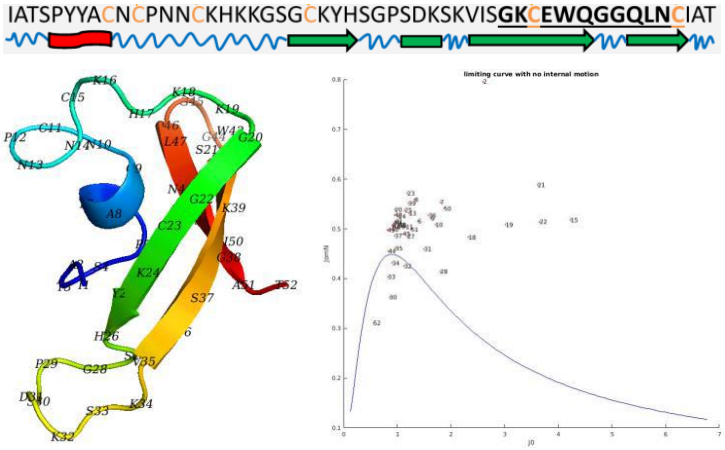
Small, cysteine-rich proteins (50-60 aa) like PAF or AFP are efficient antifungals and some have anti-*Candida* (PAFC, NFAP2) while others show anti-corona virus activity (PAF, PAFB).[1] However, their mode of action is not yet fully understood.[2] Their β-barrel tertiary structures are stabilised by 3-4 disulfide bridges lending apparent rigidity to the structures. Still, intrinsic dynamics persists as shown[3,4] by NMR ^{15}N-relaxation, ^{15}N-CEST and MD calculations that are supported by stress induced unfolding and natural abundance 13C relaxation studies. In addition to thermal unfolding we show DMSO induced transitions[5] in PAF and PAF variants as detected by NMR and DSC microcalorimetry. Partially unfolded reversible states can be biologically relevant, e.g. connected to disulfide shuffling or other thiol related transitions, while dynamic intermediates can be preferred for conformational-selection mode of molecular recognition. Practical consequences may have impact on the validation of MD simulations or protein concentration measurements.



A **B**

Figure 1. Structure of NFAP2 as predicted by AlphaFold2 artificial intelligence program (**A**). Reduced spectral density mapping of NFAP2 as derived from ^{15}N NMR relaxation data (**B**).

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NMR INSIGHTS INTO DIAMONDOID AMMONIUM SALT INCLUSION COMPLEXES WITH CYCLODEXTRINS

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Diamondoid ammonium salts (DAS) are known guest molecules capable of realizing extremely tight binding within cucurbituril (CB[n]) hosts in aqueous environment, with binding constants reaching up to 10^{15} mol^{-1} dm^3.[1] Inspired by these properties, we broadened the scope of our research concerning the binding between DAS and other hydrophobic cavities, *i.e.*, cyclodextrins (CDs).[2]

Herein we present binding capabilities of various DAS regioisomers and evaluate the effect of different nitrogen-based functionalities on complex stabilities with CDs. Stability constants were determined by ITC and ^{1}H NMR titrations in aqueous solutions. Additionally, we used a diffusion NMR ^{1}H DOSY method to estimate the percentage of complexation based on the calculated diffusion coefficients. The structural features of the complexes were assessed by the ^{1}H- ^{1}H NOESY NMR experiments, and computational studies revealed key interactions acting within the inclusion complexes.

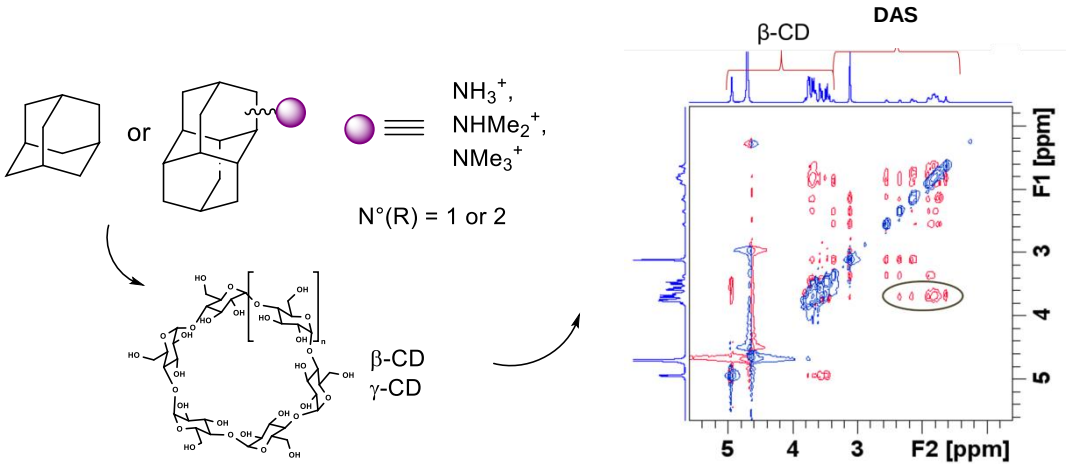

Figure 1. DAS and CDs and ^{1}H-^{1}H NOESY NMR spectra of the shown complex

Acknowledgements. This work has been supported by the Croatian Academy of Science and Arts and the Croatian Science Foundation (UIP-2017-05-9653 (DiamMat), IP-2019-04-9560 (MacroSol)).

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IN SITU IRRADIATION NMR SPECTROSCOPY IN THE DESIGN OF NEW FUNCTIONAL MATERIALS

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In situ irradiation NMR spectroscopy includes illumination of the samples inside the NMR spectrometer. The setup, which includes LEDs as light sources, allows application of whole variety of NMR methods to photochemical reactions (Fig. 1). The capabilities of this illumination NMR spectroscopic technique to perform an initiator-free thiol–ene "click" coupling reaction of an allyl-functionalized poly(allyl glycidyl ether) (PAGE) prepolymer with a number of mono- and di-oligo polyethylene glycol (PEG) thiols was demonstrated. [1]

Within the same project, the structure and conformational mobility of OLED relevant 1,3,5-triazine derivatives were recently studied by dynamic NMR and DFT calculation. [2]

Recently, the structure elucidation of eleven derivatives of 4,6-dihalogeno-3-isopropyl substituted synthetic analogues of the natural compound carvacrol was carried out by means of NMR experiments (HOBS-EXSY) and DFT calculations. Unusual $^{3}J_{CH}(180°) < {}^{3}J_{CH}(0°)$ relationship is observed. [3]

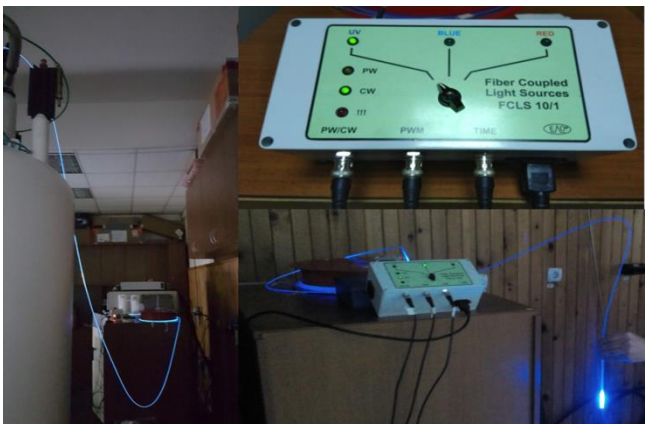

Figure 1. The setup for *in situ* irradiation NMR spectroscopy.

Acknowledgements. This research was funded by the Bulgarian Science Fund, grant number KP-06-H29/6. Research equipment of Distributed Research Infrastructure INFRAMAT, part of Bulgarian National Roadmap for Research Infrastructures, supported by Bulgarian Ministry of Education and Science was used in this investigation.

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UREIDO-CALIX[4]ARENE DERIVATIVES IN THE ROLE OF HOSTS FOR ANIONS, ION-PAIRS, AND/OR DIACETATOCALIX[4]ARENES

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The ultimate target of the ongoing development of anion and ion-pair receptors is their application in some real-world functions such as sensing and transport in biological systems, extraction in industrial processes, and new water purification technologies.[1–3] Among classical anion and ion-pair receptors containing hydrogen-bond donating groups,[4,5] a significant place belongs to calixarenes containing (thio)urea moieties. Despite being known for a few decades, the fundamental and the application-oriented work with the latter ion-receptors is still in progress.[6,7]

In this talk, fundamental research of binding of anions on two new simple urea-derivatives of *p*-tert-butylcalix[4]arenes (**1**, **2**; Figure 1) in acetonitrile will be presented. Thermodynamics of complexation of the selected anions (Cl^-, HSO_4^-, $H_2PO_4^-$, hydrogen pyrophosphate, acetate, benzoate) was investigated using UV/Vis, ITC, and ^{1}H NMR titrations. Macrocycle with four urea-moieties (**2**) proved to be a better anion receptor in comparison to diureido-calix[4]arene derivative (**1**) which is in accordance with the pK_a values of these calixarenes (experimentally determined by NMR). Furthermore, the cooperative effect in binding of Na^+ and several anions (Cl^-, HSO_4^-, $H_2PO_4^-$) with receptor **1** (designed also as ion-pair receptor) was evaluated. The other part of this talk will demonstrate the realization of the idea of reversible formation of supramolecular dimeric calix[4]arene capsules (**1** or **2** & **3** or **4**) based on urea – carboxylate interactions which can be governed by changing the solution acidity.

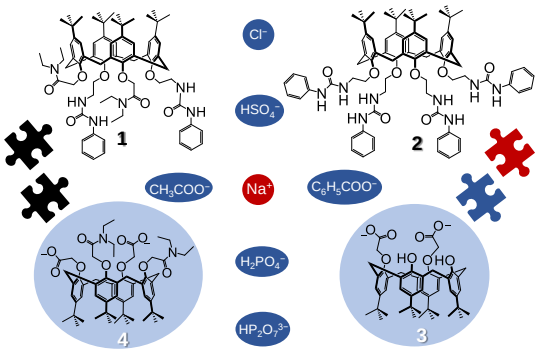

Figure 1. Structures of the investigated calixarene derivatives.

Acknowledgements. This work has been supported by Croatian Science Foundation under the project IP-2019-04-9560 (MacroSol).

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MACROZONES - NEW ANTIMICROBIAL THIOSEMICARBAZONE-BASED AZITHROMYCIN CONJUGATES: DESIGN, SYNTHESIS AND CHARACTERIZATION

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Bacterial resistance to marketed antibiotics is growing rapidly and represents one of the major global hazards to human health. Today, there is a high need for discovery of new anti-infective agents to combat resistance. A well-known class of antibacterials, 14- and 15-membered macrolides are widely prescribed to treat upper and lower respiratory tract infections. Azithromycin is a 15-membered macrolide antibiotic possessing a broad spectrum of antibacterial potency and favorable pharmacokinetics. Unfortunately, the number of newly marketed antibiotics has decreased dramatically in recent years. Withdrawal of the macrolide antibiotic telithromycin and inability of solithromycin to get marketing approval have prompted our efforts to search for new anti-infective macrolide compounds. Here we present design, synthesis and biological evaluation of a novel hybrid class of azithromycin conjugates, the macrozones. Evaluation of prepared compounds against a panel of pathogenic bacteria revealed that these molecules showed excellent activities against susceptible *S. pneumoniae*, *S. pyogenes* and *E. faecalis strains*, comparable to or better than azithromycin. Furthermore, prepared macrozones exhibited excellent activity against efflux resistant *S. pneumoniae*, 32 times better than that azithromycin, and very good activity against efflux resistant *S. aureus* strain against which azithromycin is inactive. The results described here can serve as a good basis to guide further activities directed toward the discovery of more potent macrolide anti-infectives.

SYNERGY OF NMR AND FTIR SPECTROSCOPY IN THE CHARACTERIZATION OF ASYMMETRIC LIPOSOMES

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Although asymmetric liposomes are nowadays considered the most suitable representatives of plasma membranes, their challenging preparation, which requires the use of limited lipid combinations, is further complicated by their far from straightforward characterization.[1] One of the indispensable techniques to confirm the asymmetric distribution of lipids composed exclusively of phosphatidylcholine (PC) is ^{1}H NMR spectroscopy, which in the presence of lanthanide ions such as Pr^{3+} can distinguish the protons of the choline group ($-N(CH_3)_3^+$) in the inner or outer membrane leaflet (Figure 1).[2] With the aim of identifying PC lipid molecules in a particular leaflet of asymmetric liposomes, we prepared them from 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) and 1,2-dipalmitoyl-*sn*-glycero-3-phosphoserine (DPPS) and characterized their distribution by observing the response of the $-N(CH_3)_3^+$ group by ^{1}H NMR and, for the first time, by FTIR spectroscopy.

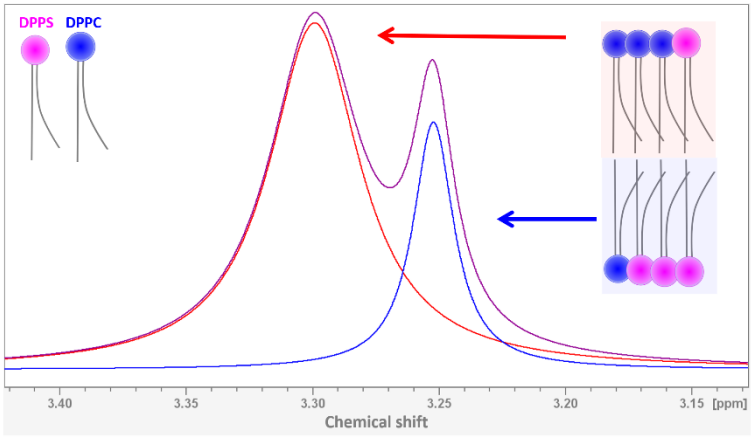

Figure 1. The deconvolution of $-N(CH_3)_3^+$ signal originated from choline moiety in asymmetric liposomes constituted from DPPC and DPPS lipids (1:1 ratio) in the presence of Pr^{3+}

Acknowledgements. This work has been supported by Croatian Science Foundation for the financial support within the project „Model of demyelination on a molecular scale at physiological and pathological conditions" (HrZZ UIP-2020-02-7669).

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AZAHETEROCYLES: SYNTHESIS, STRUCTURE, APPLICATIONS

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During the last decades azaheterocyclic derivatives has been reported as highly valuable scaffolds in medicine, optoelectronic, agriculture, etc. Thus, azaheterocycles and especially five and six member ring derivatives have a large variety of biological activities such as antimicrobial, anticancer, analgesic, antidepressant, antihypertensive, diuretics, etc. In optoelectronic azaheterocyclic derivatives have been reported as highly fluorescent materials, organic semiconductors, materials with nonlinear optical properties, etc.

As part of our ongoing research in the field of azaheterocycles with biological active and optoelectronic properties, we report herein some core results obtained by our group concerning the synthesis, structure and applications of azaheterocyles. The methods of synthesis are straight and efficient, the structure was proven unambiguously (by mono- and bi- dimensional NMR, X-ray, FT-IT, MS, etc) and the antimicrobial (antibacterial, antifungal, anti-TB) and fluorescent properties of azaheterocycles was determined.

Acknowledgements. This work was supported by a grant of the Romanian Ministry of Education and Research, project number CNFIS-FDI-2021-0546.

SPONSORS' PRESENTATIONS

NEW BENCHMARK FOR BENCHTOP NMR MAGNET HOMOGENEITY: HOW SPINSOLVE ULTRA NMRs BOOST SOLVENT SUPPRESSION PERFORMANCE FOR 1- AND 2D NMR

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Typically, NMR samples are dissolved in deuterated solvents to avoid the overlapping of the large solvent peaks with the signal of the analytes. However, if samples need to be analyzed while they are being synthesized or if the components of a liquid formulation need to be quantified the use of deuterated solvents is not an option. In these situations, the alternative known from high-field NMR is to implement solvent suppression methods that are used to drastically attenuate the solvent signals. On benchtop NMR systems, however, it is challenging to obtain efficient suppression as these methods require a highly homogeneous magnetic field, like the ones generated by superconducting magnets. Within this presentation, we demonstrate the performance of the WET solvent suppression method implemented on Spinsolve™ ULTRA NMRs to attenuate the signals of some of the most common organic solvents. The ultra-high homogeneity of the Spinsolve™ ULTRA models makes it possible to significantly attenuate the solvent peaks by two to three orders of magnitude. In this way, the overlapping of the analyte and solvent signals is reduced to the point where the analytes can be detected baseline-separated after applying the WET sequence. Due to the external hardware lock of the Spinsolve™ NMR systems, protonated solvents can be directly employed without requiring tedious sample workups to replace the regular solvents used in the reactor for their deuterated counterparts. Finally, a Spinsolve™ protocol including a CPMG filter will be shown which allows the access of analytes being completely overlapped by broad matrix signals of ^{1}H NMR samples. Within this protocol, significant T_2 time differences are utilized to filter off unwanted peaks.

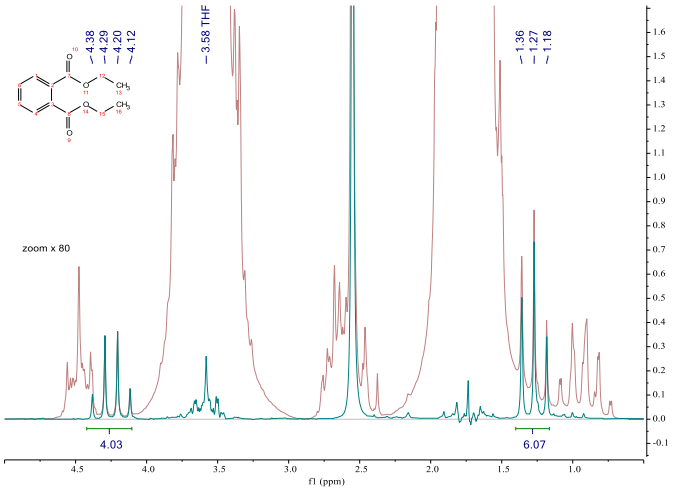

Figure 1. Zoomed comparison of a regular 1D ^{1}H NMR of diethyl phthalate in THF without ^{13}C decoupling and solvent suppression (red) and an applied 1D ^{1}H WET NMR protocol with ^{13}C decoupling and solvent suppression (cyan).

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Nuclear Magnetic Resonance Spectroscopy is a powerful and well-established technology for detailed investigations of qualitative and quantitative characteristics of complex chemical and biological samples, like food, body fluids, or plant extracts.

Yielding targeted quantification of single compounds as well as untargeted whole-matrix fingerprinting in a single run, NMR is specific and holistic likewise. Its supreme reproducibility enables worldwide lab-to-lab spectra comparison and collective database build-up. Unlimited data re-processing is given and allows to apply future statistical algorithms, re-modelling of more or different parameters, or retrospective quantification of mixture components not in the focus of interest at present.

When coupled with uni- and multivariate statistical methods, a wealth of information can be extracted from NMR data in automated processes to generate classification, discrimination, and regression models, e.g. for authenticity and quality control, or component quantification by regression.

In addition, authenticity of food products is becoming an important popular topic and consumers are increasingly demanding more information about the safety but also the origin of food. As certain fraudulent food manipulations elude detection by classical analytics, the goal is to develop methodologies able to filter out minor non-natural variations in complex matrices, and to identify and quantify single components within the matrices alike.

Bruker NMR based solution, already commercially applied and ISO-17025 accredited for fruit juice, wine and honey profiling on the high-resolution 400 MHz FoodScreener platform, is now also available for olive oils, at 400 MHz and on the bench-top, cryogen-free, Fourier 80 system operating at 80 MHz.

Some applications and results will be presented.

POSTERS

CHARACTERIZATION OF SOL GEL PRECURSOR IN FUNCTIONALIZATION OF ANTIBACTERIAL BIOPOLYMERS

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Biodegradable polymers that have been functionalized using the sol-gel process can be used for medical devices applied locally, particularly against drug-resistant bacteria [1]. By monitoring the process variables and optimizing the chemical composition of reagents, new medical devices are produced that contain active surface coatings efficient against microorganism strains resistant to antibiotics [2]. It should be noted that the variation in the quality of the precursor materials can have a significant impact on the performance of the final product. The antimicrobial coatings are applied on the surface of materials by sol-gel process and characterized by different spectroscopic, microscopic and chromatographic methods. During the sol gel coating, acidic conditions enable the oxygen atom of the Si–OH or Si–OR group to be protonated in a rapid first step. By this the electron density is withdrawn from the central silicon atom, making it more electrophilic and thus more susceptible to be attacked by water (Figure 1, A) or by silanol groups (Figure 1, B). In this work the nature of the interface in structured organic−inorganic composites prepared from precursor (3-glycidyloxypropyl) trimethoxysilane, was characterized by NMR, SEM-EDX and FTIR techniques. The combination of this methodologies enabled useful insight into the local chemical environments and occurring dynamic heterogeneities within the deep coating reaction.

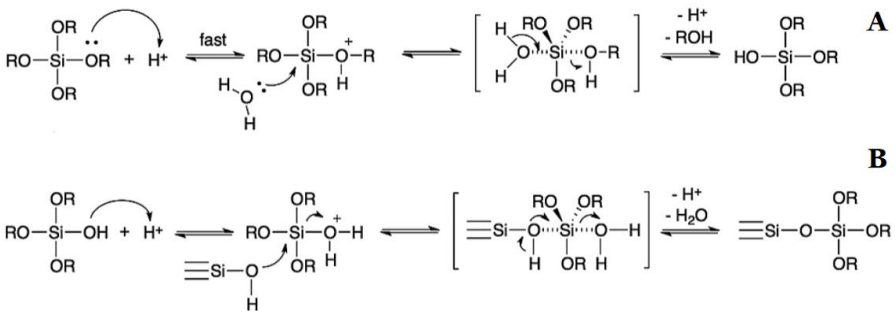

Figure 1. Hydrolysis (A) and condensation (B) in acidic catalysis with five – fold coordinated Si intermediate formation

Performing in-depth characterization of the precursor materials can lead to a better correlation between the properties of the precursor, the preparation process, and the performance of the final product, ultimately resulting in more effective medical devices with surface coatings that can combat antibiotic-resistant microorganisms.

Acknowledgements. This work was financially supported by the Croatian Science Foundation, project IP-2019-04-1381 entitled „Antibacterial coating for biodegradable medicine materials ABBAMEDICA“.

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CLASSIFICATION MODELS FOR FRAGRANT COMPOUNDS BASED ON NMR SPECTROSCOPY

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Spectroscopic measurements of 82 selected odorants were performed using ^{1}H NMR spectroscopy. This set includes 6 main types of perfumery odor notes[1] and the NMR spectral data will subsequently be used to build an accurate classification model. 2nd-order tensor decomposition tool principal component analysis (PCA) was applied to a set of obtained NMR spectra, as well as on their first and second derivatives. The quality of PCA models was evaluated by determining the optimal number of principal components for the representation in the reduced space.[2] In each case, the first principal component accounted for most of the total variance among the samples. The results were additionally improved using spectral derivatives.

Classification of these odorants was established and underlying hidden spectral differences among compounds were determined by investigating the principal component loadings.[3] These differences are directly caused by changes in the chemical composition. It was found that NMR spectroscopy coupled with PCA can distinguish between various fragrant compounds. Odorants subjected to the chemometric analyses can be divided into several major groups (clusters). Investigation of the principal component loadings determined the major differences among the NMR spectra regarding structural patterns present in the chemical structures. These differences are associated with the total number of aromatic and/or aliphatic functional groups and their structure, reflecting variations in the composition of different odor notes.

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COMPARATIVE STUDY OF CYCLODEXTRIN BINDING AFFINITIES FOR DIAMONDOID ALCOHOLS

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The temperature and solvent effect on the complexation of adamantane and diamantane alcohols by cyclodextrins (**β-CD** and **γ-CD**) in water, formamide, and ethylene glycol was investigated by means of ITC and NMR spectroscopy. The predominantly exothermic binding of all guests by **β-CD** in water was observed at 298 K, whereas the inclusion within **γ-CD** was entirely entropy-driven, with the exception of diamantan-1-ol. **β-CD** was a better receptor for explored guests, especially for adamantan-1-ol and apical diamantane alcohols. In the case of both receptors, the complex stability increased with the size of included hydrophobic surface.

A pronounced decrease of $\Delta_r H°$ and $\Delta_r S°$ with temperature, resulting in weak $\Delta_r G°(T)$ dependence, was observed for all studied systems. The effect is predominantly due to the disordering of the guest-hydrating water, which gradually shifts from classical to non-classical as the temperature increases. Unlike in water, the binding of alcohols with **β-CD** in organic solvents was exothermic and accompanied with negative entropy changes over the explored temperature range.

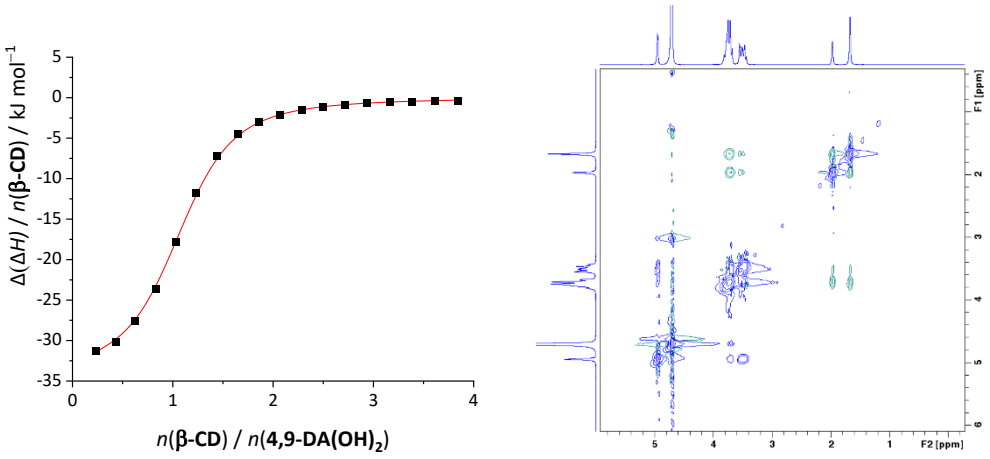

Figure 1. a) Microcalorimetric titration of **4,9-DA(OH)$_2$** with **β-CD** in H_2O at 298 K, **b)** Partial contour plot of the NOESY ^{1}H NMR spectra of the reaction mixture containing **4,9-DA(OH)$_2$** and **β-CD** in D_2O at 298 K.

Acknowledgements: This research was fully supported by the Croatian Science Foundation (projects **MacroSol**, IP-2019-04-9560 and **DiamMat**, UIP-2017-05-9653).

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NMR AND FLUOROMETRIC STUDIES OF BIOACTIVE MACROZONE INTERACTIONS WITH THEIR BIOLOGICAL TARGETS

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Macrozones are new conjugates of azithromycin and thiosemicarbazones, which exhibit very good *in vitro* antibacterial activities against susceptible and some resistant bacterial strains thus showing a potential for further development.[1,2]

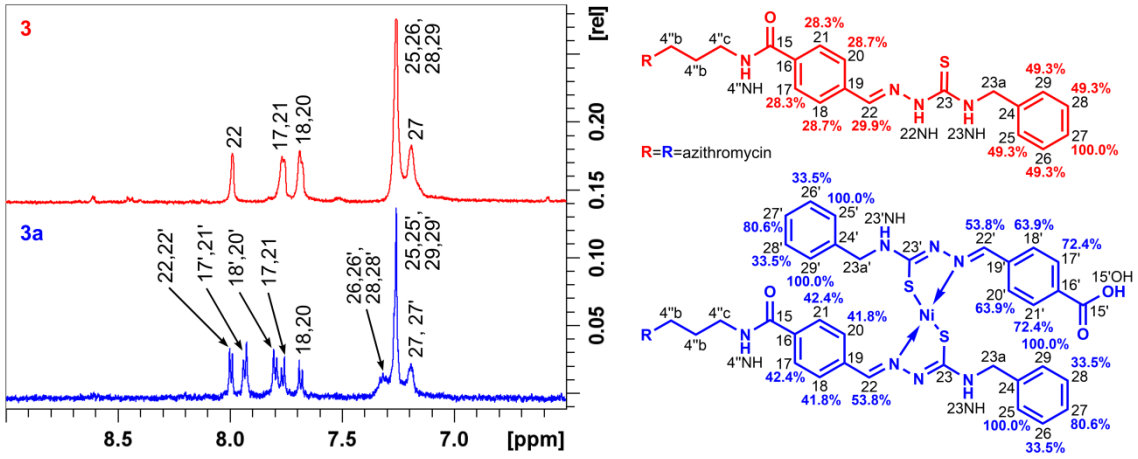

Figure 1. STD difference peaks for the protons of **3** and **3a** with the highest STD enhancements.

In this work, we applied a combination of NMR and fluorometric methods to study interactions of bioactive macrozones with their biological targets: bovine serum albumin and ribosome isolated from *Escherichia coli*. Saturation transfer difference (STD) and water-ligand observed *via* gradient spectroscopy (WaterLOGSY) spectra provided valuable data on ligand orientation, solvent exposure, binding epitopes and specificity. The binding stoichiometry and association constants were calculated from fluorometric data. These results can serve as a good starting point for the design of new macrozones and their metal complexes with improved bioactivity.

Acknowledgements. This research was funded by HRZZ, grant number IP-2018-01-8098 "Macrozones".

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UNRAVELING THE STRUCTURAL CHANGES OF ZINC-COPPER MOFS BY COMBINING SS-NMR AND EPR SPECTROSCOPY

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Metal-organic frameworks (MOFs) are nanoporous molecular networks with high structural and chemical diversity. MOFs show enormous potential due to the high tunability of their physico-chemical properties through structural changes. Solid-state nuclear magnetic resonance (ssNMR) and electron paramagnetic resonance (EPR) spectroscopy provide information on the structure and dynamics of MOFs. They complement other spectroscopic and diffraction analyses in the search for an understanding of the relationship between structure and function in MOFs. Ball milling has been used for the stoichiometrically controlled formation of bimetallic MOF-74[1] and amorphization of MOF-74 materials.[2,3] Here, bimetallic MOF-74 materials consisting of diamagnetic zinc (II) and paramagnetic copper (II) metal nodes in a 1:1 ratio, were prepared by different mechanochemical approaches and from different polymeric precursors. To find out their differences and how the introduction of defects and the collapse of the porous structure of MOF by mechanochemical amorphization affects their properties, we studied them by magnetic resonance spectroscopy (ssNMR and EPR). Synthesized crystalline materials are indistinguishable by powder X-ray diffraction, while ssNMR and EPR spectroscopy along with other analyses revealed different distributions of metals in the MOFs.[4]

Acknowledgements. This work has been supported through EU ESF grant PZS-2019-02-4129, HRZZ projects IP-2018-01-3168 and UIP-2014-09-9775, German (DAAD)-Croatian (MZO) bilateral project 910-08/18-01/00324 and ARRS (research core funding no. P1-0021 and project no. N1-0079)

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SELEX - A FAST AND EASILY SETUP 1D EXCHANGE NMR SPECTROSCOPY EXPERIMENT

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Observing chemical exchange in a variety of media is a challenge occasionally associated with labeling or derivatization to monitor dynamic processes. NMR provides powerful tools to monitor dynamic processes without sample modifications and within a reasonable timeframe. One such tool is EXchange SpectroscopY (EXSY) which offers information about chemical exchange processes on a variety of timescales.

EXSY requires acquisition of time-consuming two-dimensional spectra. This limitation was alleviated by the introduction of ultrafast NMR, an approach, which significantly decreases acquisition times by spatially encoding the incremented delay in several slices of the detection volume.[1] However, UF EXSY experiments require an intricate setup and are therefore not as readily applied. In this work we provide a just as fast alternative, via an easily setup experiment which uses spatial encoding to extract similar information in a 1D experiment. Therein, all protons are observed at once, but in different slices of the detection volume. The experiment can be carried out in a single scan to identify exchanging sites in a 1D spectrum.

Acknowledgements. This work has been supported by the Austrian Science Foundation (FWF) under the project number P30230 and through the DocFunds project BioMolStruct.

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CHARACTERIZATION OF DIAMONDOID ETHER SELF-ASSEMBLIES ON A HOPG SURFACE

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Diamondoids are a group of diamond-like hydrocarbon cage molecules that have emerged as promising candidates in a wide range of applications. For example, the inherent properties of diamondoids allow for their application as scaffolds in nanotechnology. Following that rationale, we recently prepared diamondoid ether molecules with a goal to study their self-organization on a highly oriented pyrolytic graphite (HOPG) surface.[1,2] The deposited diamondoid ethers formed ordered monolayers which were structurally characterized using scanning tunneling microscopy. We also conducted a detailed computational analysis that revealed the most favorable on-surface orientations of these rigid molecules (Figure 1) and confirmed that their spontaneous self-assembly was governed by London dispersion interactions acting between cage subunits. We also found that the oxygen atom played an important role in directing the molecules towards the graphite surface, whereas the abundant side C–H contacts between the cages were crucial for the formation of an ordered 2D lattice.

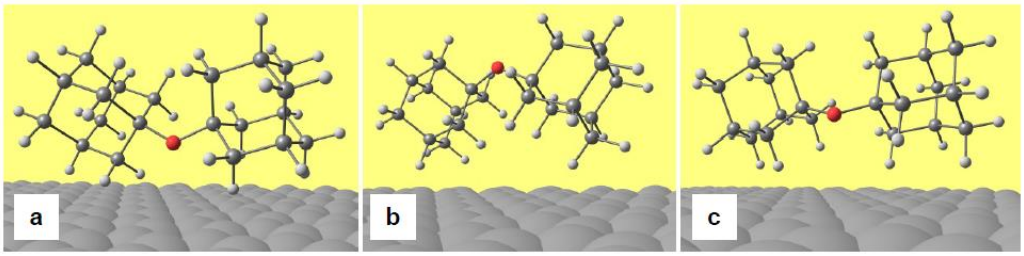

Figure 1. Feasible on-surface orientations of 1,1′-diadamantyl ether on graphite. Functional group directed, (a) towards the surface, (b) away from the surface, and (c) parallel to the surface.

Acknowledgements. This work has been supported by the Croatian Science Foundation (UIP-2017-05-9653 "Diamondoid scaffolds containing heteroatoms – preparation and application in development of advanced materials"). The support of infrastructural project CIuK co-financed by the Croatian Government and the European Union through the European Regional Development Fund - Competitiveness and Cohesion Operational Programme (grant number KK.01.1.1.02.0016) is also acknowledged.

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STRUCTURAL ELUCIDATION OF THE PH DOMAIN OF AKT-*LIKE* KINASE IN *TRYPANOSOMA CRUZI*: A NEW TARGET FOR TREATMENT OF CHAGAS DISEASE

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Chagas disease is caused by infection with the protozoan parasite *Trypanosoma cruzi*. The disease is endemic to Latin America with over 6 million people infected worldwide. Current treatment options are inefficient and cause severe side effects.[1,2] Although Akt-*like* kinase (*Tc*Akt) represents a promising drug target, its structure and mechanism of action are still not resolved.[3,4] In human Akt, the pleckstrin homology (PH) domain is binding phosphoinositides (PIP), leading to the disruption of the autoinhibitory interface between the kinase and PH domains, thus enabling activation by phosphorylation[5] (see Figure 1A). The presented 3D-structure and binding studies of the N-terminal *Tc*Akt PH domain reveal a positively charged binding pocket and PIP-induced structural changes in variable loop regions, highly associated with kinase activation (see Figure 1B). Our findings reveal unique insights into the structure and functionality of the so far scarcely understood *T. cruzi* Akt-*like* kinase, thereby forming the basis for the development of efficient drugs against Chagas disease.

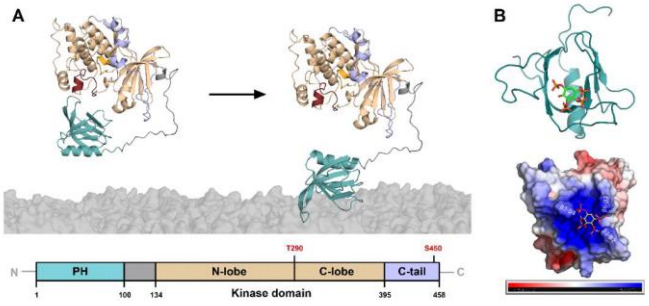

Figure 1. (A) Alphafold model of *Tc*Akt in closed formation and open conformation with the PH domain bound to PIP_3 on the surface of the membrane. (B) PH domain bound to PIP_4 shown with secondary structure elements and with electrostatic surface (red: negatively charged, blue: positively charged)

Acknowledgements. This work was supported by doc.fund Molecular Metabolism (DOC 50) funded by the Austrian Science Fund (FWF), the Land Steiermark and the City of Graz. Financial support by the Excellence Initiative BioHealth of the University of Graz is gratefully acknowledged. We are also grateful to the Land Steiermark for the Zukunftsfonds project Frontier NMR.

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HEPTACYCLIC BENZO[F]QUINOLINE DERIVATIVES: A NMR STUDY CONCERNING STRUCTURE DETERMINATION

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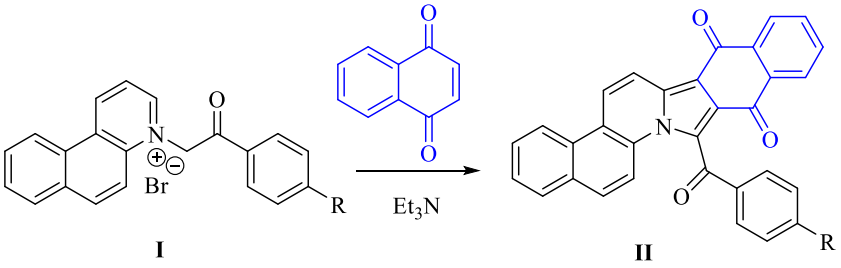
Quinoline and its fused benzo derivatives benzo[f]quinoline, are valuable motifs in medicinal chemistry, having a large variety of biological activities such as antiplasmodial and antimalarial, antitubercular, antibacterial, antifungal, antivirus, anticancer, antinociceptive and anti-inflammatory, etc.[1-4] As a result, structure determination of such compounds is an important clue for many researchers. In continuation of our continuous efforts in the field of quinoline and its derivatives,[3,4] we present herein a detailed study concerning synthesis and NMR structure determination of some heptacyclic benzo[f]quinoline derivatives. The synthesis is direct and efficient, and involves a Huisgen 3+2 dipolar cycloaddition reaction of benzo[f]quinoline salts **I** to benzoquinone, when the desired heptacyclic benzo[f]quinoline derivatives **II** are obtained.



I **II**

The stereochemistry of the heptacyclic benzo[f]quinoline derivatives **II** was studied using the NMR experiments: ^{1}H-NMR, ^{13}C-NMR, NOEDIFF and two-dimensional experiments 2D-COSY, HMQC, HMBC.

KEYWORDS: heptacyclic benzo[f]quinoline; stereochemistry; NMR experiments

ACKNOWLEDGMENTS. The authors are thankful for financial support to Romanian Ministry of Research, Innovation and Digitization, within Program 1—Development of the national RD system, Subprogram 1.2—Institutional Performance—RDI excellence funding projects, Contract no.11PFE/30.12.2021, and to CNCS - UEFISCDI, project number PN-III-P4-ID-PCE-2020-0371.

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A NMR STUDY CONCERNING CONFORMATIONAL EQUILIBRIA OF SOME PYRIDAZINONES DERIVATIVES

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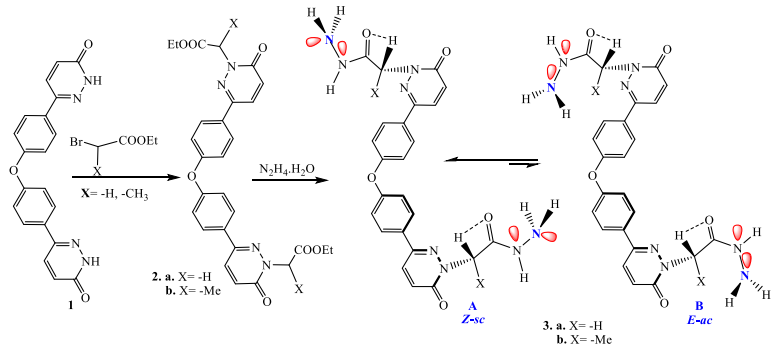
Pyridazinones heterocycles are “privileged structures” in medicinal chemistry, possesing a large variety of biological activities such as antimicrobial, cardiovascular and antihypertensive, diuretic, anti-inflammatory, analgesic, antinociceptive, etc [1-3]. In contination of our efforts in the field of azaheterocyclic derivatives, we present herein some results obtained by our group in the field of bis-pyridazinones derivatives with acetylhydrazine skeleton. The synthesis is straight and efficient, in two steps. The first step involve an initially N-alkylation of bis-pyridazinones **1** with 2–bromoalkyl esters, when the corresponding bis-pyridazinones eters **2a,b** are obtained. In the second step the bis-pyridazinones eters **2a,b** are treated with hydrazine, when the desired acetylhydrazines bis-pyridazinones **3a,b** are obtained [4].



The stereochemistry of the acetylhydrazines bis-pyridazinones **3a,b** was studied using the NMR experiments (1H, 13C, 2D HMBC) at variable temperatures. At room temperature, the NMR experiments reveal a conformational equilibrium of stereoisomers **3a,b**, the *Z-sc* conformer (A structure) being in majority (around 90%) while the conformer *E-ac* is in minor proportion, around10%. The NoeDiff 1D experiments prove unambiguously the above considerations, only the major isomer *Z-sc* showing a strong NOE between the hydrazidic NH and the –CH-R group. A thoroughly 1H NMR study related to dependence of conformational equilibrium with the temperature have been performed, indicating the presence of a single stereoisomer at temperatures higher to 80 °C, the *Z-ac* conformer.

KEYWORDS: acetylhydrazines bis-pyridazinones; conformational equilibrium; *Z-sc* and *Z-sc* conformers; NoeDiff 1D experiments

ACKNOWLEDGMENTS. The authors are thankful for financial support to Romanian Ministry of Research, Innovation and Digitization, within Program 1—Development of the national RD system, Subprogram 1.2—Institutional Performance—RDI excellence funding projects, Contract no.11PFE/30.12.2021, and to CNCS - UEFISCDI, project number PN-III-P4-ID-PCE-2020-0371.

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COMPLEXATION THERMODYNAMICS OF BENZENE DERIVATIVES WITH CUCURBIT[7]URIL AND β-CYCLODEXTRIN

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The binding of mono- and 1,4-disupstituted benzene derivatives with cucurbit[7]uril (**CB7**) and β-cyclodextrin (**β-CD**) was explored in water by means of titration microcalorimetry and NMR spectroscopy. Determined complex stability constants with **CB7** were notably higher compared to analogous complexes with **β-CD** due to more exothermic binding with the glycouril-based receptor. The differences in complexation affinities for explored guests could be rationalized by considering their hydration, electron-donor (-acceptor) properties, and the position of the introduced functionalities. Investigations in a wider temperature range (278–338 K) revealed a significant $\Delta_r H°(T)$ and $\Delta_r S°(T)$ dependence which resulted in almost complete enthalpy-entropy compensation. This fact, and the entropically favorable hosting of guests at lower temperatures, are in accord with the classic hydration of benzene.

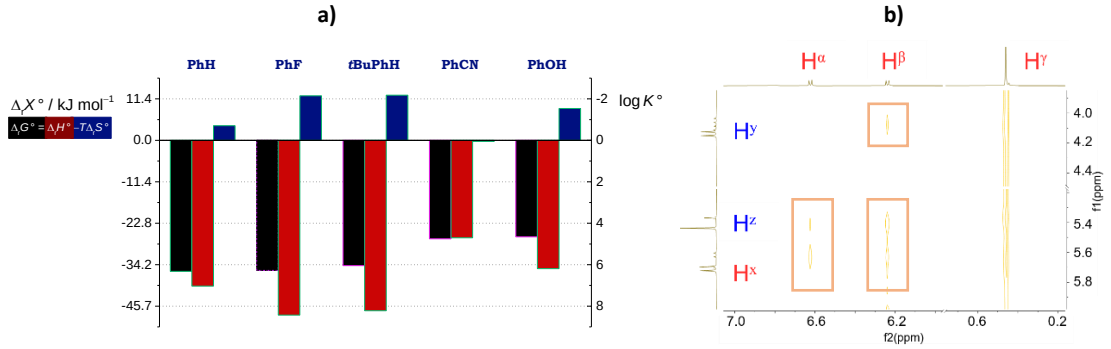

Figure 1. a) Standard thermodynamic parameters for complexation of benzene and corresponding monosubstituted derivatives with **CB7** in H_2O at 298 K; **b)** Partial contour plot of the ROESY ^{1}H NMR spectrum of the reaction mixture containing **4*t*BuPhOH** and **CB7** in D_2O at 298 K.

Acknowledgements: This research was fully supported by the Croatian Science Foundation (project **MacroSol**, IP-2019-04-9560).

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VANCOMYCIN ANTIBIOTICS AND ANTIFUNGAL PROTEINS AS ANTIVIRAL AGENTS? THE USE OF NMR SPECTROSCOPY AND ARTIFICIAL INTELLIGENCE.

Gyula Batta,[a] András Czajlik,[a] Gai Jiawei,[a] Réka Erdei,[a] László Izsépi,[a] Pál Herczegh,[b] Ilona Bakai-Bereczki[b]

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The COVID-19 pandemic and SARS-CoV-2 caused more than 5 million victims worldwide from the 2019 outbreak. New influenza viruses also mean global threat, that may cause 1 billion/year infection. Therefore, drug repurposing is an urgent research area. FDA has recently approved new glycopeptides efficient against various microbes. Some of our novel glycopeptide derivatives show promising antiviral and/or antibacterial activities.[1,2] We have recently applied the STD-NMR method proving the binding site of SARS-COV-2 spike protein to anti-cancer drug Rucaparib.[3] We continue intensive NMR and in-silico structure, dynamics, function studies of our antifungal disulfide proteins.[4] Some of them showed anti-corona virus activities[5] and their in-vitro and biological tests are in progress. Now, we started application of AI methods, e.g. AlphaFold2[6] to speed up protein structure determination in line with experimental NMR data.

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SYNTHESIS AND CHARACTERIZATION OF A MACROZONE 4'' DERIVATIVE OF AZITHROMYCIN DERIVED FROM 4-AMINOBENZOIC ACID AND SALICYLALDEHYDE AND ITS COMPLEX WITH NICKEL(II) AND COPPER(II)

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The increase in bacterial resistance to currently marketed antibiotics has prompted the need to discover new ones. The most well-known macrolide antibiotic is azithromycin, which is used to treat diseases caused by Gram-positive and Gram-negative bacteria.[1] Macrozones are conjugates of azithromycin and thiosemicarbazones. They are potential candidates for a new class of antibiotics for treatment of infections caused by resistant bacteria, because they manage to bypass the developed resistance mechanism. In order to further increase the biological activity, thiosemicarbazone complexes with metal ions are made, which might have a positive effect on their pharmacological properties.[2] The goal of this work is the preparation and characterization of the macrozone shown in Scheme 1. This molecule can bind divalent metal ions in a stechiometric ratio of 1:1. A thiosemicarbazone derived from 4-aminobenzoic acid and salicylaldehyde was prepared. The resulting thiosemicarbazone is then bound to the 4''-aminopropyl derivative of azithromycin. The possibility of complexation with copper (II) and nickel (II) ions was explored. One- and two- dimensional NMR spectroscopy was used to characterize compounds in each reaction step.

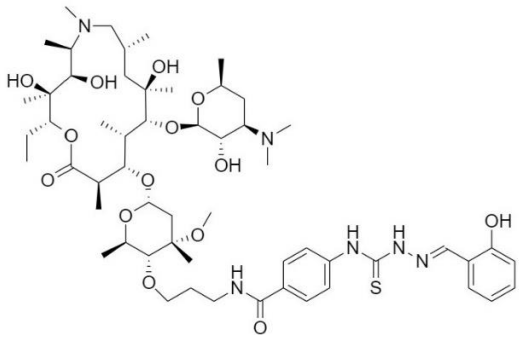

Figure 1. Structural formula of the 4''-derivative of azithromycin derived from 4-aminobenzoic acid and salicylaldehyde.

Acknowledgements. Macrozones, New Conjugates of Macrolide Antibiotics: Design, Synthesis and Interactions

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COMPLEXATION OF ALKALI AND ALKALINE EARTH METAL CATIONS BY CALIX[6]ARENE RECEPTORS

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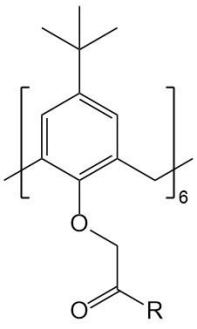
Calix[*n*]arenes are macrocyclic oligomers consisted of phenolic residues ($n \geq 4$) linked by methylene bridges in the ortho position.[1] During the past few decades, these compounds have been shown to be very efficient receptors for various chemical species depending on their size and functionalization on the upper and/or lower rim.[1–3] Numerous calixarene-based receptors for cations have been developed, and their cation-binding equilibria investigated both thermodynamically and structurally in various solvents. However, the investigations were mostly oriented towards smaller, calix[4]arene-based receptors, leaving the complexation processes involving calix[6]arenes rather unexplored.

In the scope of this work, calix[6]arene derivatives **L1**–**L3** (Figure 1) were synthesized and their binding affinities towards alkali and alkaline earth metal cations in acetonitrile were thoroughly investigated by means of spectrophotometry, NMR spectroscopy, and isothermal titration calorimetry. Thus obtained results were discussed regarding the structural properties of receptors **L1**–**L3** and their complexes, as well as the charge density of the cations.



L1 R = OEt
L2 R = NEt_2
L3 R = $N(CH_2N_3)_2$

Figure 1. Structures of studied calix[6]arene receptors.

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Visit Ston region - We live it, you will love it!

If you are a lover of historical tradition, pristine nature and superb cuisine, Pelješac peninsula is the ideal place for you!

The very beginning of the peninsula is the location of a little, magical place, Mali Ston, which is connected to Ston by the longest preserved stone wall in Europe (5.5 km). Bay of Mali Ston, known for its cultivation of shellfish, especially oysters and mussels, is an indispensable destination for true lovers of seafood delicacies. A boat ride to the oyster farms and tasting of this natural aphrodisiac straight from the sea, paired with a glass of fine wine from Pelješac, make this place a real gourmet jewel. For fans of high-quality and fresh fish dishes, our widely-known restaurants have their door always open. Only a kilometer away by car or walking along the walls (40 min), at the foot of hill Podzvizd, lies the medieval town of Ston, whose history dates as far back as the Dubrovnik Republic. Ston is rich with historical and cultural tradition, and the former value of Ston as the city of salt has been confirmed today in the plants of the oldest active saltworks in the world. The saltworks remained true to the tradition and a natural production method, which hasn't change since the era of antiquity. Only two kilometers away from Ston, surrounded by a thick pine forest and accessible beach for yachts and boats, is situated little village of Broce. One ride away down a winding road, only few kilometers long with a beautiful view of the sea, is situated one of the most stunning coves in the southern Adriatic Sea, the idyllic town of Kobaš. It is a true paradise for boaters and sailors, and its gastro-enological offer can be experienced in one of the three restaurants, located right by the sea.

By driving down the main road along the steep slopes of Pelješac, you can see kilometers of grapevines and olive trees. Their cultivation and a rich tradition are a testament to a century-old love of Pelješac inhabitants towards grapes, vine and olive oil. For an intense experience of the winery tradition, throughout the entire year, you can visit the wine cellars situated in small villages of Ponikve and Putnikovići, as well as at the newly established "House of Wine Tradition". On the coast of bay of Mali Ston, from Brijesta to Luka Dubrava, are ideal places for a family vacation in private accommodation and camps. These villages are known for shellfish cultivation, and the little islands surrounding it are ideal for one-day excursions and sports fishing.

A place you simply must visit is also the beautiful cove Žuljana. Its unforgettable sunsets, natural beauty and the most exquisite sandy and gravel beaches will leave you breathless. Also, the crystal clear sea in this area is ideal for exploring the undersea, diving and fishing.

Wherever for its natural beauty, cultural heritage, a crystal clear sea or superb wine and cuisine, Ston region has a varietal offer which can satisfy the needs of even the most demanding of guests. All these places offer an unforgettable experience of a rich historical and cultural tradition and hospitable local population. Their tales of pride and love for their homeland wait to be discovered, and once you visit this place, you will definitely want to come back again.

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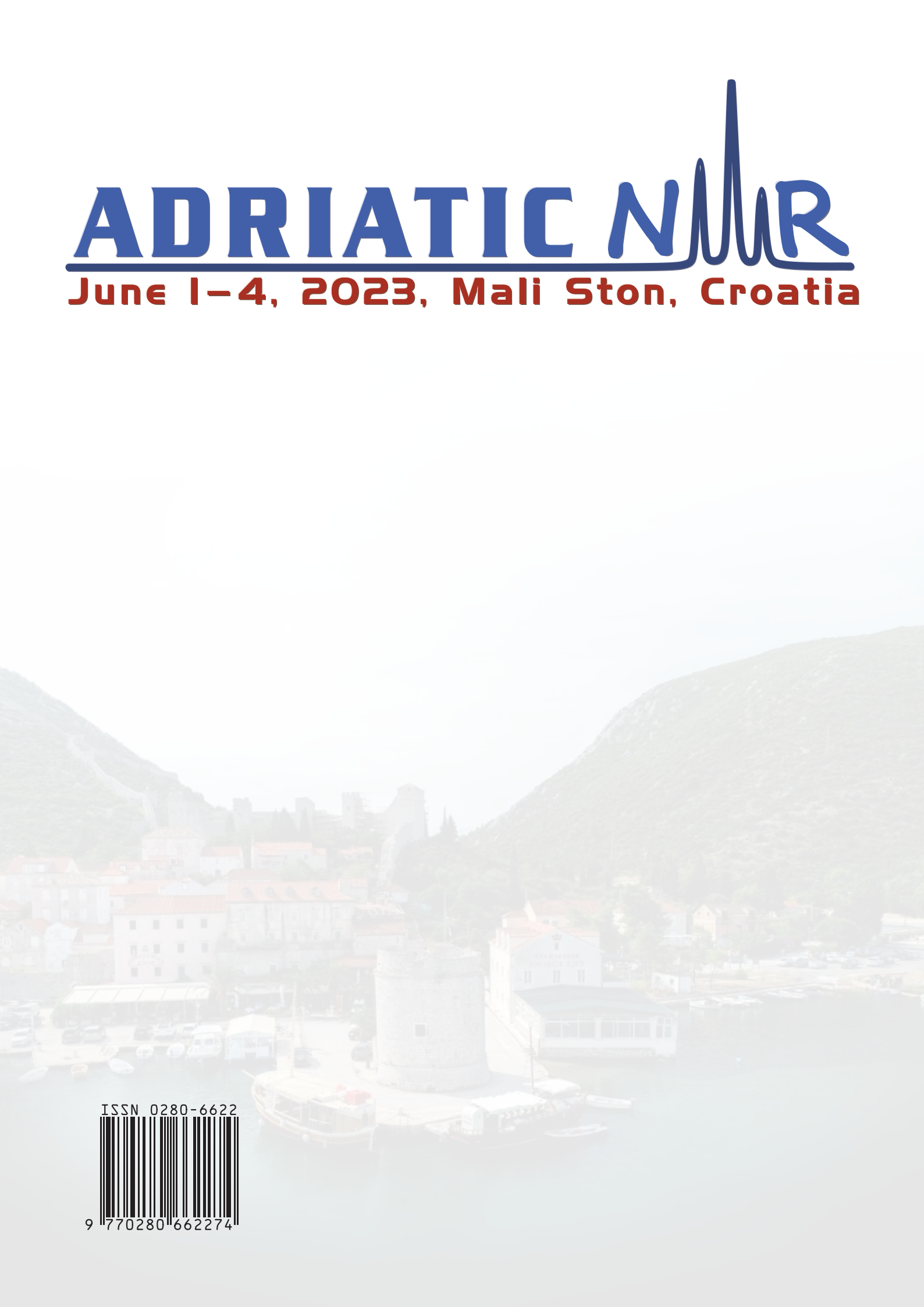

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