ADRIATIC NMR

18-20 September 2025, Vodice, Croatia

BOOK OF ABSTRACTS

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Dear Participants,

It is our great pleasure to welcome you to the 8th Adriatic NMR Conference which will take place at Imperial Park Hotel, Vodice, Croatia, from 18 to 20 September 2025.

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 beautiful place of Vodice in September 2025.

Looking forward to seeing you!

Predrag Novak

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ADRIATIC NMR

18–20 September 2025, Vodice, Croatia

PROGRAMME

WEDNESDAY, SEPTEMBER 17	
18:00 – 19:00	**REGISTRATION**
CHAIR: Janez Plavec	
19:00 – 20:00	**Naoki Sugimoto (pre-conference lecture)**: *"To B or Not to B" in Nucleic Acids Chemistry*
20:00 – …	**CONFERENCE RECEPTION / WORKSHOP**

THURSDAY, SEPTEMBER 18	
8:30 – 9:00	**REGISTRATION**
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CHAIR: Ago Samoson	
9:05 – 9:45	**PL1 – Guido Pintacuda**, *In situ Magic-Angle Spinning NMR of Membrane Proteins: From Vision to Practice*
9:45 – 10:25	**PL2 – Mariana Sardo**, *Combining Solid-state NMR Methods & Computation to Study Confined CO_2 Chemistry in Porous Materials*
10:25 – 10:50	**COFFEE BREAK**
CHAIR: Dean Marković	
10:50 – 11:10	**IN1 – Adrijana Vinter**, *Data-driven Fragment Screening: Combining NMR Spectroscopy and Artificial Intelligence*
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11:30 – 11:50	**IN3 – Valerije Vrček**, *Environmentally Relevant Transformations of Pharmaceuticals Monitored By NMR Spectroscopy*
11:50 – 12:10	**IN4 – Nives Galić**, *Characterization of Cyclodextrins Complexes with Poorly Soluble Drugs in Solution and Solid State*
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14:50 – 15:10	**IN7 – Miroslav Požek**, *Measurements of Fast Relaxing NMR Spectra in Cuprate Superconductors*
15:10 – 15:30	**IN8 – Luís Mafra**, *Measuring Acidity or Confinement Effects in Zeolites? An Answer from Integrated SSNMR and Modeling*
15:30 – 15:50	**IN9 – Ivana Sofrenić**, *NMR Spectroscopy Revealing Challenges in Monitoring in teraction of Small Molecules with Proteins*
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16:30 – 16:50	**IN10 – Maja Marušič**, *The Imperfections Make it Better: An Increased Affinity of PNA-RNA Triplexes with Bulges*
16:50 – 17:10	**IN11 – Markus Rotzinger**, *Enhancing Sensitivity and Resolution of 19F NMR Spectra of PFAS through Band-selective Homonuclear Decoupling*
17:10 – 17:30	**IN12 – Sanja Đokić**, *New Hybrid Antitumour Agents Against Drug Resistance: Design, Synthesis, And NMR Analysis*
17:30 – 17:50	**IN13 – Nikola Cindro**, *Mechanochemical Anion-templated Synthesis of Cyclopeptides*

FRIDAY, SEPTEMBER 19

Dedicated to Dražen Vikić-Topić on the occasion of his 70th birthday	
CHAIR: Predrag Novak	
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9:15 – 9:55	**PL3 – Janez Plavec**, *NMR Reveals Dynamic G-quadruplex DNA Modulation by Methylation and Molecular Interactions* *DVT, the catalyst in search for coherence and advance of NMR (in the two countries)*
9:55 – 10:15	**IN14 – Sunčica Roca**, *Twenty Years of the Gift of Scientific Collaboration with Prof. Vikić-Topić*
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9:40 – 10:20	**PL5 – Tristan Richard**, *Tracking Wine Quality and Origin with ^{1}H-NMR: From Data to Traceability*
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P02	**HYDROLYSIS OF ENVIRONMENTALLY RELEVANT ANTICANCER DRUGS MONITORED BY NMR SPECTROSCOPY** Antonio Ljulj, Ivana Lovrić, Jerko Vujević, Edi Vuljanković, Martin Lončarić, Valerije Vrček
P03	**SYNTHESIS AND CHARACTERIZATION OF POROUS ORGANIC POLYMERS CONTAINING AZO AND AZODIOXY LINKAGES** Barbara Panić, Željka Car, Nikola Cindro, Vesna Petrović Peroković, Mladen Borovina, Ivana Biljan
P04	**RUDIMENTARY INSTRUMENTAL SETUP FOR IN SITU STUDY OF PHOTOSWITCHING BY NMR SPECTROSCOPY: CASE STUDY OF BRIDGEHEAD NITROGEN HETEROCYCLIC AZOBENZENE** Boban Anđelković, Dunja Pupavac, Andrea Nikolić, Igor Opsenica, Jovana Ljujić, Katarina Simić, Ivana Sofrenić
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P06	**RECENT ADVANCES IN TITANIUM DIOXIDE (TIO_2) NANOLAYERING FOR ENHANCED MEDICAL DEVICE PERFORMANCE IN DENTISTRY** Iva Zeneral Žuža, Dean Marković, Sandra Kraljević-Pavelić
P07	**BAYESIAN FRAMEWORK FOR OVERCOMING SCARCE NMR DATA: CASE STUDY OF THE HALDANE SYSTEM BONO** Ivan Jakovac, Mihael Srđan Grbić, Maxime Dupont, Sylvain Capponi, Nicolas Laflorencie, Yuko Hosokoshi, Masashi Takigawa, Steffen Krämer, Mladen Horvatić
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P12	**STRUCTURAL CHARACTERIZATION OF DISULFIDE-CONTAINING NITROSOARENES BY VARIABLE TEMPERATURE NMR** Laura Nuić, Ivana Biljan
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P14	**MECHANISTIC INVESTIGATIONS OF A COPPER-MEDIATED CARBOXYLATIVE C-C CROSS-COUPLING REACTION** Maria Kolympadi Markovic, Nikolina Vidović, Pegi Pavletić, Lucijana Svetić, Barbara Cetin, Valerije Vrček, Dean Marković
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P17	**^{1}H NMR AND GC–MS METABOLOMICS REVEAL HERBICIDE-INDUCED BIOMARKERS IN GRAPEVINE (VITIS VINIFERA L.)** Stefan Ivanović, Katarina Simić, Marija Gavrović-Jankulović, Jovana Ljujić, Iris Đorđević, Ivana Sofrenić, Boban Anđelković
P18	**HOST–GUEST CHEMISTRY OF DIAMANTANE AMMONIUM SALTS AND MODIFIED Β-CDS** Sunčica Roca, Nikola Bregović, Marija Alešković, Marina Šekutor
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PLENARY LECTURES

"TO B OR NOT TO B" IN NUCLEIC ACIDS CHEMISTRY

Naoki Sugimoto

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In this lecture, I will provide an overview of the basic concepts, methods, and recent applications of predicting the stabilities of nucleic acid structures. I explain the theory of the most successful prediction method based on a nearest-neighbor (NN) model. To improve the versality of prediction, corrections for various solution conditions considered hydration have been investigated. I also describe advances in the prediction of non-canonical structures of G-quadruplexes and i-motifs. Finally, studies of intracellular analysis and stability prediction are discussed for the application of NN parameters for human health and diseases.

Acknowledgements. The author is grateful to the colleagues named in the cited papers from my laboratory, institute (FIBER), and others. This work was supported by the Ministry of Education, Culture, Sports, Science and Technology (MEXT) and the Japan Society for the Promotion of Science (JSPS) (Grant No. JP17H06351, 18KK0164, 19H00928, and 20K21258), especially for Grant-in-Aid for Scientific Research (S) (22H04975), JSPS Core-to-Core Program (JPJSCCA20220005), and The Chubei Itoh Foundation.

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IN SITU MAGIC-ANGLE SPINNING NMR OF MEMBRANE PROTEINS: FROM VISION TO PRACTICE

Guido Pintacuda

Lyon High-Field NMR Center (CNRS/ENS Lyon/UCBL), Villeurbanne, France
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Membrane proteins play essential roles in biology, but their structural study is complicated by the need for simplified mimetic systems that often fail to capture the complexity of native membranes. Solid-state NMR, with its sensitivity to local environments, is uniquely suited for in situ studies in physiologically relevant contexts. Enabled by advances in isotopic labeling, high magnetic fields, fast sample rotation and proton detection, in situ Magic-Angle Spinning (MAS) NMR now allows atomic-level insights directly in native membranes. I will review recent progress in this field and highlight our study of the E. coli outer membrane protein A (OmpA) in native outer-membrane vesicles, performed up to 100 kHz MAS and 1.2 GHz fields. These experiments revealed the first high-resolution structure of OmpA in its physiological setting, establishing a general workflow for probing membrane proteins in complex environments.

COMBINING SOLID-STATE NMR METHODS & COMPUTATION TO STUDY CONFINED CO_2 CHEMISTRY IN POROUS MATERIALS

Mariana Sardo, Daniel Pereira, Márcio Soares, Ildefonso Marin-Montesinos and Luís Mafra

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Porous CO_2-chemisorbent materials are critical for addressing rising atmospheric CO_2 levels, enabling applications such as direct air capture and industrial flue gas separation. However, understanding their reactivity and the atomic-level details of CO_2 adsorption at the gas-solid interface remains a significant challenge, hindering the design of improved materials. This talk highlights our group's recent advancements in elucidating CO_2 speciation in confined spaces under both dry and humid conditions, combining surface-enhanced solid-state NMR spectroscopy with computational modelling.

We demonstrate the power of NMR-assisted adsorption methods, NMR relaxation, and chemical shift anisotropy to quantitatively discriminate between six distinct CO_2 species—ranging from physisorbed CO_2 to chemisorbed carbamic acid, carbamate ion pairs, and moisture-induced bicarbonate. Unlike conventional volumetric or gravimetric techniques, NMR uniquely resolves these species, enabling the generation of individual CO_2 isotherms for each adsorbed component. This approach provides unprecedented insights into their molecular dynamics and adsorption mechanisms. A key breakthrough presented in this talk is the first successful application of Magic-Angle Spinning (MAS) – Dynamic Nuclear Polarization (DNP) NMR to study CO_2 adsorption in real-world scenarios. This innovative approach overcomes the sensitivity limitations imposed by the low natural abundance of ^{13}C in atmospheric CO_2 (~ 1 %), achieving unprecedented signal enhancement while maintaining speciation integrity. We demonstrate its success in resolving chemi- and physisorbed CO_2 species in amine-modified SBA-15 exposed to ambient air, marking a significant step towards the detection of CO_2 surface species.

The versatility of our methodology is demonstrated across diverse porous systems, including amine-modified mesoporous silicas and covalent organic frameworks (COFs). Additionally, we highlight the potential of the Calgary Framework 20 (CALF-20) MOF, which exhibits exceptional CO_2 uptake, selectivity, and durability under humid conditions. This work offers a unified framework for understanding CO_2 adsorption and guiding the design of next-generation adsorbents.

Acknowledgements. We thank CICECO-Aveiro Institute of Materials, UIDB/50011/2020, UIDP/50011/2020 & LA/P/0006/2020. The NMR spectrometers are part of the National NMR Network (PTNMR) and are partially supported by Infrastructure Project 022161 (cofinanced by FEDER through COMPETE 2020, POCI and PORL and FCT through PIDDAC). This work has received funding from the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation program (Grant Agreement 865974). We also acknowledge support from the This work was conducted within the European PANACEA from the European Union's Horizon Europe research and innovation programme under grant agreement No. 101008500 (2021-2026).

NMR REVEALS DYNAMIC G-QUADRUPLEX DNA MODULATION BY METHYLATION AND MOLECULAR INTERACTIONS

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[b] University of Ljubljana, Faculty of Chemistry and Chem. Techn., Ljubljana, Slovenia
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Noncanonical DNA structures include G-quadruplexes (G4) - four-helical structures that may form when the sequence contains more than four tracts of guanine bases. Cytosine methylation, a key epigenetic modification that regulates gene expression, has drawn interest for its potential impact on G4 structures and their stability. Using NMR spectroscopy—a powerful tool for probing structural dynamics—we examined how methylation affects the well-characterized bcl2Mid G4, which is involved in regulating BCL-2 gene expression. By combining solution-state NMR with other biophysical methods, we found that cytosine methylation induces local rearrangements within the bcl2Mid G4, leading to changes in its thermodynamic stability. Unexpectedly, methylation not only modifies stability but also alters the folding pathway by which the G4 adopts its predominant form. Specifically, methylation shifts the equilibrium between the major G4 conformation and a previously unidentified minor form. In some cases, the methylated sequence even favors the minor form more strongly than the unmethylated counterpart. Overall, our results demonstrate that cytosine methylation does more than fine-tune G4 stability—it can act as a molecular switch that redirects G4 folding pathways, with potentially significant consequences for gene regulation.[1]

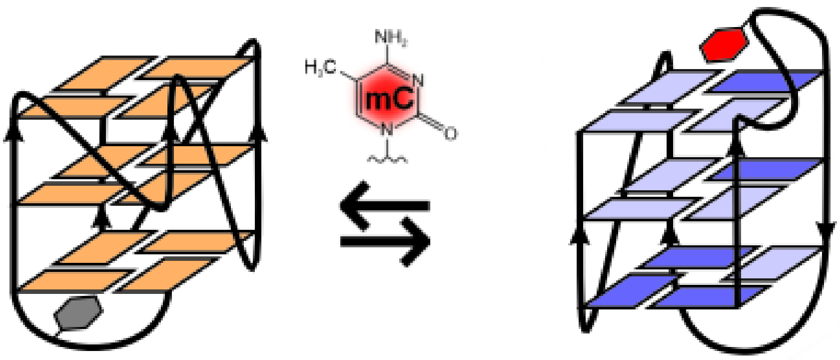

Figure 1. G4 structures adopted by the G-rich sequence originating from the BCL-2 promoter region whose equilibrium between the major (3+1) hybrid and the minor parallel topology is controlled by 5-methylcytosine substitution.

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NMR IN WINE ANALYSIS: PAST, PRESENT AND FUTURE

Pavel Solovyev

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Wine falsification has a time span as long, as probably the history of winemaking itself. However, the laws controlling wine quality, safety and especially genuineness are recently new in historical perspective, appearing only in the Industrial age.[1] With the development of analytical instrumentation such as chromatography and mass spectrometry (MS), later isotope ratio mass spectrometry (IRMS) in the second half the 20th and 21st centuries those began to be used [2]. Nuclear magnetic resonance (NMR), however, seemed to lag behind, mostly because of its lower sensitivity compared to MS, approximately 10 orders of magnitude. NMR's advent in wine analysis started in the late 1970 s first with ethanol measurement and later site-specific natural isotopic fractionation (SNIF) for geographical determination. In this century, with significant progress in computation power, metabolomics came to stage, both targeted and untargeted. [3].

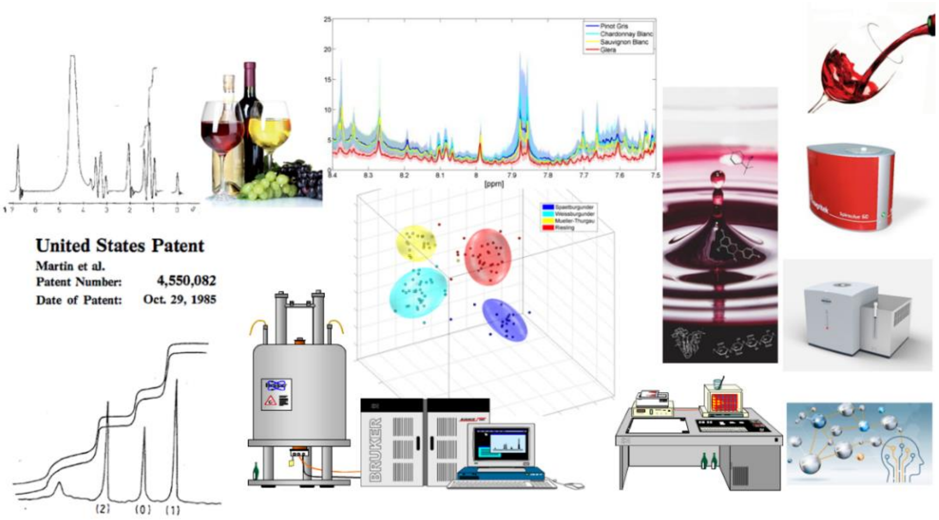

Figure 1. A collage depicting NMR use for wine analysis in the past present and possible future.

The latest steps in evolution of this method for wine analysis show a comeback of low-field or so-called benchtop NMR devices. In the future, artificial intelligence (AI) models will most probably enter the stage of this technology (see Figure 1). This lecture will cover all this in detail.

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TRACKING WINE QUALITY AND ORIGIN WITH ^{1}H-NMR: FROM DATA TO TRACEABILITY

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Gregory Da Costa, Tristan Richard

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Wine is a premium agri-food product and, as such, is particularly susceptible to fraud. Efforts to combat counterfeiting primarily focus on verifying the consistency between the product and its labeled attributes — such as grape variety, vintage, and geographical origin. Achieving this level of verification requires robust analytical techniques combined with advanced data processing methods. Among these, quantitative ^{1}H-NMR spectroscopy has proven to be a powerful approach, enabling rapid, non-destructive analysis of a wide range of compounds with minimal sample preparation [1,2,3].

In this study, we present the development and validation of an open metabolomic strategy based on ^{1}H-NMR spectroscopy to assess wine traceability. The proposed approach relies on standardized, flexible, and accessible protocols for both analytical procedures and data processing. In addition, several application examples are provided to illustrate the method's relevance and versatility in real-world scenarios.

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NEW PERSPECTIVES IN CROATIAN WINE PRODUCTION, ANALYTICS AND EDUCATION

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The Department of Viticulture and Enology, University of Zagreb Faculty of Agriculture, has played a pivotal role in shaping Croatian wine production for over a century through scientific research, education, and innovation. Building upon its experimental station and advanced analytical and sensory laboratories, the Department contributes to the preservation of native grape varieties and the development of modern enological technologies. Current projects such as *CroVitiRestart* focus on revitalizing indigenous cultivars through tailored winemaking technologies [1], while *VitiResist* applies metabolomics and molecular tools for breeding disease-resistant varieties. In education, the *MERGO* Erasmus+ project introduces game-based olfactory learning and tangible user interfaces to modernize wine sensory training. The most recent project applies DOSY-NMR spectroscopy and Deep Reinforcement Learning [2], to create reliable database for wine authenticity, varietal classification, and origin verification. By merging tradition with innovation, Croatian enology strives to enhance wine quality, authenticity, and market value while ensuring sustainability and global learning opportunities.

Acknowledgements. This work has been supported by NPOO.C3.2.R3-I1.06.0211

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INVITED LECTURES

DATA-DRIVEN FRAGMENT SCREENING: COMBINING NMR SPECTROSCOPY AND ARTIFICIAL INTELLIGENCE

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Fragment-based drug discovery (FBDD) has become a cornerstone for identifying novel chemical starting points, with NMR spectroscopy serving as a highly sensitive tool for detecting weak fragment–protein interactions and guiding optimization. Recent advances have expanded the scope of NMR-based screening and improved throughput and sensitivity, reinforcing its role as a key technique in early drug discovery.

At the same time, artificial intelligence and machine learning are emerging as powerful complements to these experimental approaches. From automating spectrum interpretation to supporting fragment prioritization and optimization, data-driven methods are helping overcome long-standing bottlenecks in fragment screening.

This talk will explore how the integration of NMR spectroscopy with AI-driven analytics is reshaping fragment-based drug discovery, offering new opportunities to accelerate workflows, increase efficiency, and enhance the success rate of fragment-to-lead development.

HYBRID AND CHEMERIC AZAHETEROCYCLES: SYNTHESIS, APPLICATIONS AND NMR CONSIDERATIONS

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Azaheterocycles derivatives are invaluable scaffolds in modern chemistry, mostly because of their invaluable applications in agriculture (mostly as grow up factors, insecticides and herbicides), [1] medicine and pharmacy (possessing a large variety of biological activities such as antibacterial, antifungal, anti-inflammatory, anticancer, antihypertensive, anticoagulants, antidepressant, etc.),[2] opto-electronics (as electroluminescent and fluorescent materials, semiconductor devices, sensors, etc.) [3], etc. Polyphenols are natural and synthetic compounds of great interest in medicine, especially because of their antioxidant, antimicrobial and anticancer activity.[4]

In continuation of our continuous efforts in the field of azaheterocyclic derivatives, we present herein a thoroughly study concerning the synthesis, biological activity and some NMR and X-ray considerations of some hybrid and chimeric azaheterocycles derivatives

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ENVIRONMENTALLY RELEVANT TRANSFORMATIONS OF PHARMACEUTICALS MONITORED BY NMR SPECTROSCOPY

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Reaction mechanism details of chlorination and hydrolysis of anticancer agents cyclophosph-amide (**CPA**) and ifosfamide (**IF**) have been zoomed in by the use of 1D- (^{1}H, ^{13}C, and ^{31}P) and 2D-NMR techniques. During acid catalyzed hydrolysis of **IF**, the stable intermediate was observed and its structure was resolved by HMBC and HSQC spectra (see Figure). In contrast, no intermediate was detected in the course of the hydrolysis of **CPA**, suggesting different reaction mechanisms for the two pharmaceutical isomers.

Both **CPA** and **IF**, in the absence of light (in the darkness of the magnet), undergo *N*-chlorination reaction, resulting in *N*3- and *N*7-chlorinated products, respectively. However, when exposed to UV light, the reaction rate is decreased and the chlorination inhibited. This may be due to light sensitivity of the product.

To perform chlorination in the presence of UV-light (380 nm) and/or white light (400-750 nm), the optical fiber, connected to the respective light source, was inserted in NMR tube containing reaction mixture (**CPA**/**IF** and HOCl). A coaxial NMR tubes system was used in which the fiber was not immersed directly in solution. To monitor reactions with increased rate, a two-chamber borosilicate glass NMR tube, which allows for in-tube mixing, was used.

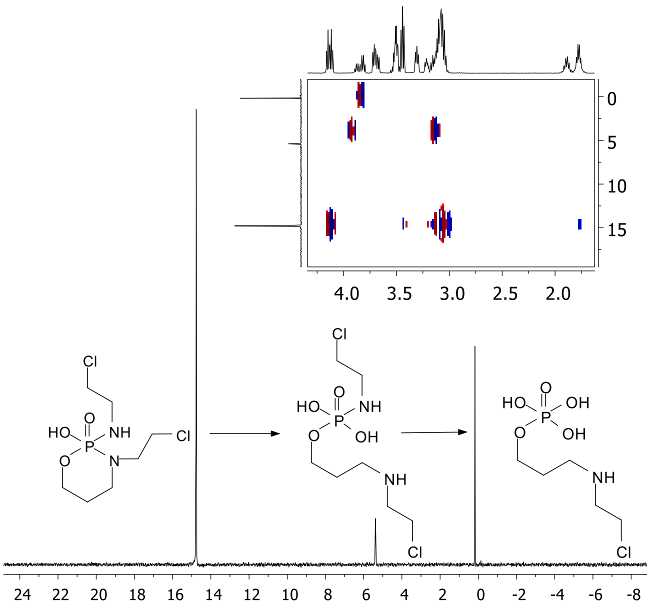

Figure. ^{31}P{^{1}H} and gHMBC (^{1}H-^{31}P) NMR spectra of the reaction mixture (ifosfamide/HOCl/D_2O)

Acknowledgements. This work has been supported by Croatian Science Foundation (projects IP-2022-10-2634 (“Pharma-Eco”) and DOK-NPOO-2023-10-7238).

IN-4

CHARACTERIZATION OF CYCLODEXTRINS COMPLEXES WITH POORLY SOLUBLE DRUGS IN SOLUTION AND SOLID STATE

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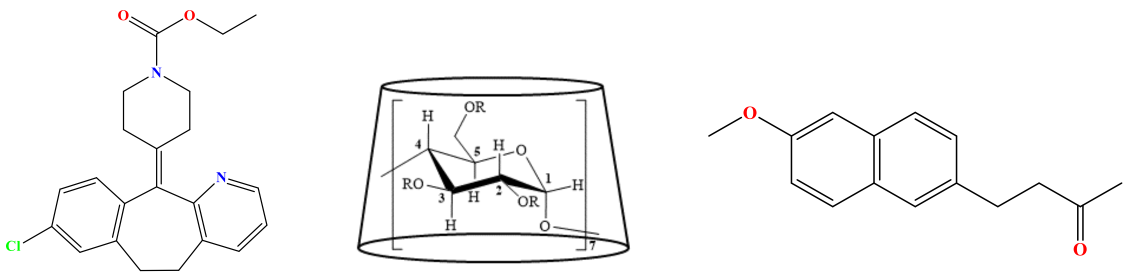
The solubility of poorly soluble drugs can be improved by complexation with β-cyclodextrin and its derivatives. The drug-cyclodextrin complexes in the solution and in the solid state are usually characterized by different methods, including UV-Vis, fluorescence, MS, NMR, DSC, IR etc.[1,2] The complexation of loratadine (LOR) and nabumetone (NAB) complexes with β-cyclodextrins (β-CD) and its derivatives (hydroxypropyl β-CD (HP-β-CD), randomly methylated β-CD (RM-β-CD) and sulfobutylether β-CD sodium salt (SBE-β-CD)) was studied by means of an integrated approach comprising ITC, MS, NMR spectroscopy, DSC, PXRD, ATR-FTIR, and computational methods.[3-5] The formation of inclusion complexes was confirmed both in solid state and in the solution, resulting with improved solubility of abovementioned poorly soluble drugs. The stoichiometry, mode of binding, and thermodynamic parameters for complexes were determined.



Loratadine β-cyclodextrins Nabumetone

Figure 1. Structures of loratadine, nabumetone and β-cyclodextrins

Acknowledgements. This work has been supported by Croatian Science Foundation under the project number HRZZ-IP-2022-10-6033.

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IDENTIFICATION AND QUANTIFICATION OF MOLECULES IN DIFFERENT MIXTURES BY NMR

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The search for molecules in simple and complex mixtures represents a crucial challenge in the field of analytical chemistry. This process involves the identification and characterization of chemical components within matrices that can range from pure solutions to complex biological samples. Nuclear Magnetic Resonance (NMR) spectroscopy, plays a fundamental role in this research, enabling the identification and quantification of molecules.

In particular, with regard to the quantification of molecules,NMR allows for the quantitative analysis of mixtures without the need for extensive sample preparation or calibration curves. The technique can provide accurate concentration measurements of individual components within a mixture.

The development of advanced software for NMR spectroscopy has revolutionized the identification and quantification of molecules in complex mixtures. Bruker has recently developed a dedicated software – Advanced Chemical Profiler (ACP) - including sophisticated algorithms and machine learning techniques to analyze NMR spectra, providing accurate and reliable results.

By automating the interpretation of NMR data, ACP significantly reduces the time and expertise required for manual analysis. It can identify molecular species, even in highly complex mixtures, and quantify their concentrations with high precision. The integration of user-friendly interfaces and robust data processing capabilities ensures that researchers can efficiently manage and analyze their samples.

In this presentation, we will explore practical applications.

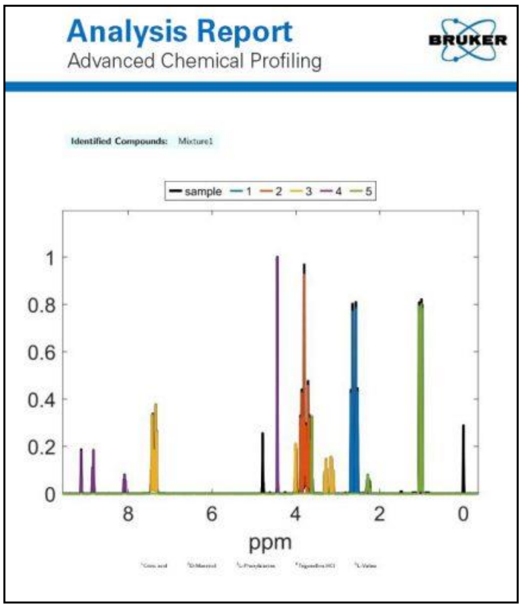

Figure 1. Advanced Chemical Profiling report one page example

IN-6

SOME MAS AND AI PERSPECTIVES

Ats Kaldma, Mai-Liis Org, Kalju Vanatalu, Meelis Rohtmäe, Liisi Karlep,
Neeme Danziger, Raiker Witter, Ago Samoson

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A lab overwiev of NMR and induced activities will be given. This entails principles of spinning beyond 250 kHz, AI application in metabolomics and negative-ion battery development. Perhaps also something else, like study of wood and ayurvedic plants.

MEASUREMENTS OF FAST RELAXING NMR SPECTRA IN CUPRATE SUPERCONDUCTORS

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NMR relaxation times in solids are much shorter than in liquids. This is even more pronounced in strongly correlated systems, such as superconductors or magnetic materials, which typically exhibit a very rich phase diagram. Relaxations in such systems are further enhanced by strong charge or spin fluctuations. In some cases, the NMR signal is completely wiped out due to fast relaxations. Such wipeout effect is often taken as a sign of a phase transition.

The NMR signal is lost if the relaxation occurs within the detector dead time (typically 10 μs). Therefore, by shortening the deadtime, properties of previously hidden parts of the phase diagram can be revealed. We have recently developed an experimental procedure to shorten the detector dead time (to 2 μs).[1] Here we show two examples of how such detection provides insight into spin and charge dynamics in previously unexplored parts of the phase diagram.

In the cuprate superconductor $La_{1.875}Ba_{0.125}CuO_4$ we show that the of relaxation rate increase is caused by magnetic rather than charge fluctuations, which finally confirms the long-suspected assumption that spin fluctuations are responsible for the wipeout effect.[1]

In lightly doped cuprate $La_{2-x}Sr_xCuO_4$ we show that qualitative changes occur when the Sr concentration increases through x = 0.05, which we interpret as a transition from an insulating state with unconnected metallic islands to a granular metal state with tunneling between the grains.[2]

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MEASURING ACIDITY OR CONFINEMENT EFFECTS IN ZEOLITES? AN ANSWER FROM INTEGRATED SSNMR AND MODELING

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The nature, strength, and spatial distribution of acid sites in zeolites are central to their catalytic performance, yet their characterization remains a formidable challenge. Using trimethyl-phosphine oxide (TMPO) as a probe for acidity, we combined solid-state NMR and computational modeling to redefine the understanding of zeolite acidity. 2D ^{1}H-^{31}P HETCOR combined with ab initio molecular dynamics revealed that confinement effects and protonated TMPO dimers – not distinct Brønsted acid strengths – explain ^{31}P NMR resonances in HZSM-5.[1,2] An atomistic view of host–guest dynamics is provided by examining aluminum siting and guest–guest interactions. Extending this approach to external zeolite surfaces, we are able to identify unique SiOH species, pore-mouth Brønsted sites, and tricoordinate Al-Lewis sites, previously undetected.[3] This talk also demonstrates the importance of optimizing TMPO adsorption methods, showing how solvent choice and gas-phase loading influence dimer formation and acid site quantification.[4] Altogether, these insights bridge internal confinement effects and external surface chemistry, offering a unified framework for a better atomic-level understanding of acid site structures in zeolite catalysts.

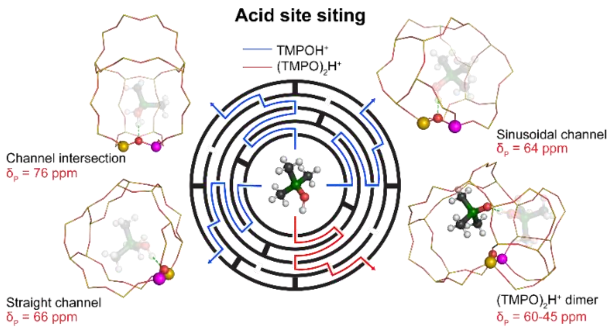

Figure 1. Structure and calculated δ ^{31}P of TMPO interacting with distinct Brønsted acid sites.

Acknowledgements. We thank CICECO – Aveiro Institute of Materials and the University of Aveiro. We also acknowledge the Infrastructure Project 022161 (FEDER-COMPETE2020-POCI/PORL). the ERC CoG-GA-865974 and FCT (10.54499/2020.00056.CEE-CIND/CP1589/CT0005 and 2020.08520.BD).

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IN-9

NMR SPECTROSCOPY REVEALING CHALLENGES IN MONITORING INTERACTION OF SMALL MOLECULES WITH PROTEINS

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Comprehending the interactions between small molecules and proteins is crutial for numerous domains of biomedical science. Nuclear magnetic resonance spectroscopy (NMR) is highly effective tool for examining intermolecular interactions, providing apparent advantages over alternative analytical methods. The observation of small molecule interactions with proteins by ligand-based NMR spectroscopy entails monitoring alterations in the NMR signals of the ligand (small molecule) when it binds transiently to a protein. [1] This technique is valuable because it primarily focuses on the ligand's resonances, facilitating detection of binding events without requiring isotopic labeling of the protein, and it is suitable for large protein targets. The technique can also facilitate structure-based drug design by providing atomic-level insights into interactions without requiring full protein structure or resonance assignments.

The *in situ* kinetics NMR experiment constitutes a pseudo 2D NMR technique that enables real-time observation of reaction rates and processes directly within the reaction environment, without disturbing or isolating reaction components. This method allows detailed observation of the temporal evolution of substrate, intermediate, and product concentration changes with great chemical specificity, eliminating the necessity for sample extraction or quenching. Additionally, provide the simultaneous tracking of numerous species by their unique NMR signals, including transient intermediates that may be challenging to detect using alternative approaches. [2] The *In situ kinetics* study using NMR spectroscopy is a potent method for elucidating reaction pathways and rates with chemical accuracy, providing substantial benefits in understanding the dynamic of molecular processes at the atomic level.

Our study demonstrates that the newly developed ^{1}H NOE pumping experiment enables the observation of interactions within a single experiment. This ^{1}H *in situ* NMR experiment has proven to be an excellent method for monitoring both enzyme inhibition and reactivation. Both experimental methodologies constitute a valuable approach and are suitable for the comprehensive evaluation of a wide array of cholinesterase inhibitors. This is especially important in the examination of neurodegenerative diseases, such as Alzheimer’s disease.

Acknowledgements. This work has been supported by Organisation for the Prohibition of Chemical Weapons (OPCW) Internship program, Project Nos. L/ICA/ICB-46/21 and L/ICA/ICB-307/23 and the Ministry of Science, Technological Development and Innovation of the Republic of Serbia, contract number 451-03-136/2025-03/200168.

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THE IMPERFECTIONS MAKE IT BETTER: AN INCREASED AFFINITY OF PNA-RNA TRIPLEXES WITH BULGES

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Controlling RNA structural dynamics is key in RNA biology and therapeutic targeting. Peptide nucleic acids (PNAs) enable stable, sequence-specific triplex formation with structured RNA, even under physiological conditions. They can recognize bulged purine residues and induce conformational changes that support continuous triplex formation. Using NMR spectroscopy, we found that PNA-induced remodeling is independent of the initial dsRNA conformation and is instead driven by a delicate balance between the type and number of bulge conformations within the triplex, with bulge composition further enhancing PNA affinity by up to tenfold — highlighting a tunable strategy for achieving high-affinity and sequence-specific RNA targeting.

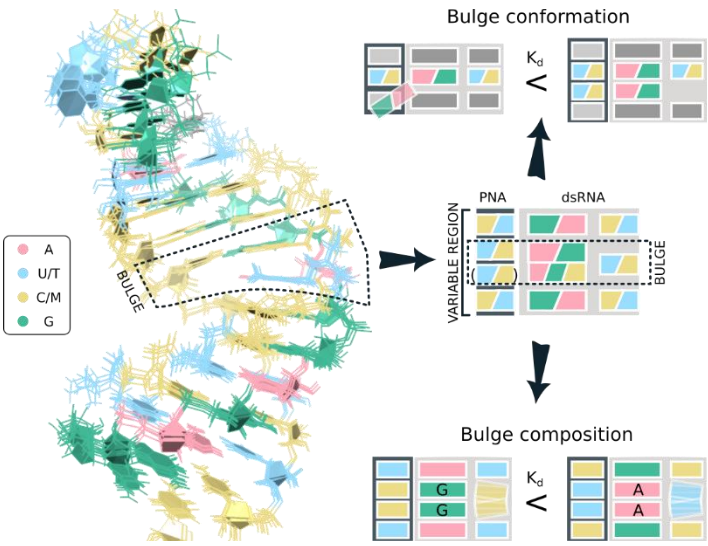

Figure 1. Mutations in the variable region of PNA-dsRNA triplexes affect the conformation and composition of the bulge, resulting in a markedly increased binding affinity.

Acknowledgements. This work has been supported by Slovenian Research Agency [P1-0242] and CERIC-ERIC consortium (projects no. 20232149 and 20242193).

ENHANCING SENSITIVITY AND RESOLUTION OF ^{19}F NMR SPECTRA OF PFAS THROUGH BAND-SELECTIVE HOMONUCLEAR DECOUPLING

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Per- and polyfluoroalkyl substances (PFAS) are a diverse class of substances defined by the presence of perfluorinated carbon atoms.[1] PFAS, which were developed more than 70 years ago, exhibit several favorable properties which made them indispensable in the manufacturing of various items such as non-stick coatings of pans, food-packaging and firefighting foams.[2] However, several of the compounds have been identified to bioaccumulate at alarming levels and are potentially toxic.[3–5] To better establish NMR in the analysis of these compounds, we developed a method which maximizes resolution as well as sensitivity for acquiring decoupled ^{19}F NMR spectra of PFAS. The experiment uses band-selective homonuclear decoupling[6-9] of the CF_3 spectral region (around -82.4 ppm) and results in spectra showing both enhanced resolution and enhanced signal intensities for perfluorinated compounds. PFAS mixtures, which show highly overlapped multiplett signals in conventional ^{19}F NMR spectra are reduced to individual separated singlets enabling not only their identification, but also quantification at concentrations of less than 10 mg/L.

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NEW HYBRID ANTITUMOUR AGENTS AGAINST DRUG RESISTANCE: DESIGN, SYNTHESIS, AND NMR ANALYSIS

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Protulactone A and asperilactone C are natural compounds isolated from a marine-derived Aspergillus species, has demonstrated moderate cytotoxic activity.[1-2] Goniofufurone and its structural analogues, have shown potent antiproliferative effects against various cancer cell lines.[3] In this study, guided by a molecular hybridization approach, we designed and synthesized a novel hybrid analogues integrating a bicyclic lactone core−characteristic of protulactone A−and a phenyl moiety commonly found in styryl lactones. Comprehensive structural elucidation of synthesized molecules was performed using 1D and 2D NMR spectroscopy. The resulting compounds (**1**-**4**) were evaluated for their cytotoxic potential across a panel of human cancer cell lines, including drug-resistant variants. Tested compounds reduced MRP1 levels and moderately increased Caspase-3 levels in K562 cells, indicating anti-MDR and pro-apoptotic activity. No Caspase-3 activation was detected in MRC-5 cells, confirming the selective cytotoxicity of these compounds toward cancer cells.

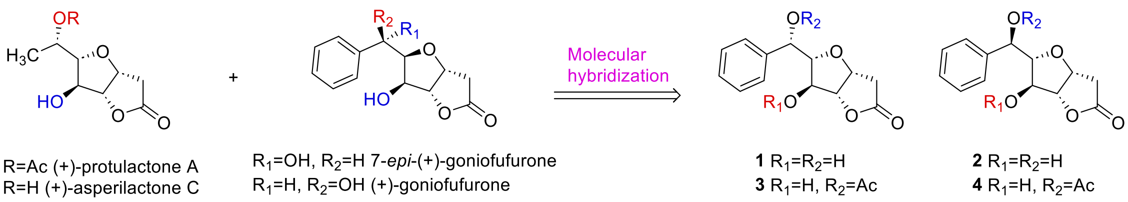

Figure 1. Design of new hybrid antitumour agents.

Acknowledgements. This work was co-funded by the European Union through the Interreg VI-A IPA Cross-border Cooperation Programme Croatia-Serbia, project Development of Anticancer Agents for Drug-Resistant Cancers DAADRAC HR-RS00053 and Ministry of Science, Technological Development and Innovation of the Republic of Serbia (Grants No. 451-03-137/2025-03/ 200125 & 451-03-136/2025-03/ 200125.

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MECHANOCHEMICAL ANION-TEMPLATED SYNTHESIS OF CYCLOPEPTIDES

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Cyclopeptides have emerged as a sought-after class of molecules possessing favorable properties such as proteolytic resistance and binding affinity largely due to their macrocyclic structure.[1] Synthesis of cyclopeptides via head-to-tail macrocyclization of their linear analogues presents a considerable challenge, especially in the case of smaller peptides due to the unfavorable conformation required to bring the reactive termini in spatial proximity.[2] Previously, we have found that anions can act as promoting agents for the lactamization step and, in the case of tetra-, penta- and hexapeptides, the use of salts such as TBACl and TEACl gave the corresponding cyclic peptides in moderate to high yields.[3] To prevent undesired oligomerization and polymerization processes, the reaction had to be carried out in high dilution in DMF over a course of 3 – 5 days, which were the main drawbacks of the method in addition to poor solubility of unprotected peptides in organic solvents. To circumvent these problems, we have envisioned a mechanochemical approach to cyclize oligopeptides by milling the linear peptide precursor with a chloride salt and potassium carbonate as a base, followed by addition of the coupling reagent DEPBT and further milling. Two cyclopeptides, cyclo[Phe3-Gly2) and cyclo[Phe4-Gly2], were prepared by the solution-based and mechanochemical approach. Molecular dynamics simulations gave insight into the role of the chloride salt used.[4] Additionally, the cyclic products were evaluated for biological activity.

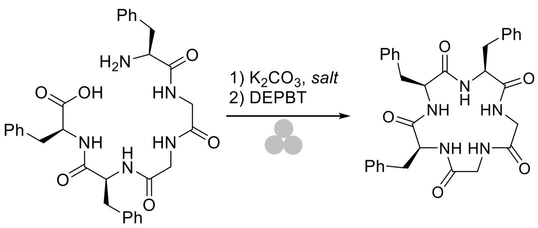

Figure 1. Macrocyclization of linear peptides.

Acknowledgements. This work has been supported by The European Union – NextGenerationEU project ToSiAn NPOO.C3.2.R2-I1.06.0043 and by the Croatian Science Foundation project MicroSynTotal IP-2024-05-5352

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TWENTY YEARS OF THE GIFT OF SCIENTIFIC COLLABORATION WITH PROF VIKIĆ-TOPIĆ

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In 1979, we both began our careers, I began my life career with my birth, and Prof Vikić-Topić began his scientific career with his employment at the Ruđer Bošković Institute. Our scientific paths crossed 27 years later and since then we have successfully elucidated numerous chemical structures and conformations of newly synthesized or extracted compounds using NMR spectroscopy. The topics of the work were diverse and included, for example, research into the structure of synthesized N-acylhydrazone derivatives of vitamin B6 and pyridine-4-carbaldehyde drugs,[1] and the determination of the structure of isonicotinamides obtained by eco-friendly quaternization in deep eutectic solvents.[2] In the chemistry field of transition metal complexes, we investigated the structures of coumarin-palladium(II) complexes,[3] mono- and dihalopyridinesilver(I) nitrate complexes,[4,5] and picolinamide complexes of Ni(II), Zn(II), Cd(II) and Hg(II) nitrates.[6] We also achieved good results in researching the chemical composition of sage, rosemary and laurel essential oils,[7] as well as pectin from tomato waste.[8] Some of the topics mentioned above were also the subject of several diploma theses that I supervised together with Prof Vikić-Topić.

It is a great gift to have a supervisor, manager, and colleague from whom you can learn about chemistry every day during small talks, about geography and environment while travelling to conferences, and about life in general while observing his relationship with family, animals, and the people around him.

Dear Prof Vikić-Topić, your age is a treasure chest of time where you are keeping a rich and well-guarded collection of knowledge and memories. Keep collecting and searching for the new gems of your life!

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NEW MULTICOMPONENT CO_2 FIXATION REACTIONS BY USING PROPARGYLIC SUBSTRATES AND ALLYL HALIDES: FROM ALKYNE ACIDITY PREDICTION TO CATALYSIS

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The continued release of CO_2 through fossil fuel combustion has raised urgent concerns, prompting the need for innovative strategies for its capture and transformation. One promising strategy in sustainable chemistry is the direct utilization of CO_2 as a renewable carbon feedstock for the synthesis of value-added organic compounds.[1] However, its high thermodynamic stability and kinetic inertness pose significant challenges to its efficient chemical activation.

Among the most attractive transformations is the catalytic or mediated incorporation of CO_2 into propargylic substrates such as alcohols and amines to afford cyclic α-alkylidene carbonates or carbamates.[2] These structural motifs are prevalent in pharmaceuticals, polymers, chiral auxiliaries, and polar aprotic solvents. Moreover, combining CO_2 fixation with carbon–carbon bond-forming reactions enables streamlined access to structurally complex products that typically require multi-step synthetic sequences.[3]

In this presentation, we will discuss our recent progress in developing copper(I)-catalyzed or -mediated multicomponent carboxylative cross-coupling reactions involving propargylic amines or alcohols, allyl halides, and CO_2 to synthesize functionalized oxazolidinones and dioxolanones under mild conditions. Particular emphasis will be placed on the observed stereospecificity of the transformation, as well as the stereoelectronic influence of aryl substituents including both inductive and resonance on the reactivity of alkyne substrates. The proposed reaction mechanism, supported by both experimental and computational studies, will also be presented and critically evaluated.

In parallel, the acidity of terminal alkynes, critical for understanding reactivity in CO_2 activation was investigated. Through a comprehensive computational study, we assessed various acidity descriptors including proton transfer energies, hydrogen exchange barriers, and NMR parameters. Two strategies showed excellent agreement with experimental pK_a values: energy barriers for hydrogen exchange and acetylenic proton chemical shifts. These models were further applied to predict acidity in bioactive alkynes such as ethynylestradiol and ethynyluracil.

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STRUCTURAL CHARACTERIZATION OF HETEROCYCLIC LIGANDS AND THEIR METAL COMPLEXES WITH ANTIPROLIFERATIVE ACTIVITY

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Bioorganometalic complexes have attracted considerable interest and have shown promise for potential application in the treatment and diagnosis of cancer and bioimaging agents, some acting as theranostic agents. The accumulation of the Re(I) benzimidazo[1,2-a]quinolone complex in the lysosomes of colorectal carcinoma CT26 cells indicated the site of its bioactivity, thus making this complex a potential theranostic agent.[1] Bis(2-picolyl)amine (bpa), iminodiacetamide (imda), mono- and bis-1,2,3-triazole (bta) ferrocene ligands (L) with and without aliphatic linker were prepared by multistep synthesis. The *cis-fac*, *trans-fac*, or *mer* stereochemistry of their ML2 complexes with Ni(II), Cu(II), Cd(II), and Zn(II) was studied.[2,3] Some quinoline- and coumarin-derived 1,2,3-triazole and isoxazole analogs and their Re(I) and Ru(II) complexes showed pronounced antiproliferative activity.[4,5] The prepared ligands and their metal complexes are fully characterized by NMR and single crystal X-ray diffraction (Fig. 1).

a) b)

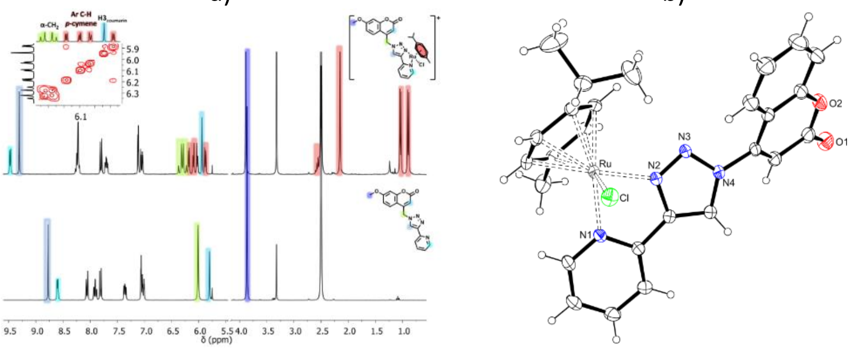

Figure 1. a) Comparison of proton NMR spectra of coumarin ligand and its Ru(II) complex; b) crystal structure of coumarin Ru(II) complex.

Acknowledgements. This work has been supported by the Croatian Science Foundation under the project HRZZ-IP-2022-10-9420.

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NMR AS AN ESSENTIAL DETECTIVES' TOOL FOR SOLVING CHALLENGES FROM INDUSTRY AND PRACTICAL USES

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This talk showcases several examples where NMR is used to solve challenges from the industry, including component identification, product and method development. Other practical uses of NMR in polymer chemistry like calculation of reactivity ratios and determination of monomer content are discussed. Beside NMR results, results of other techniques are shown to complete the holistic approach to solving different challenges.

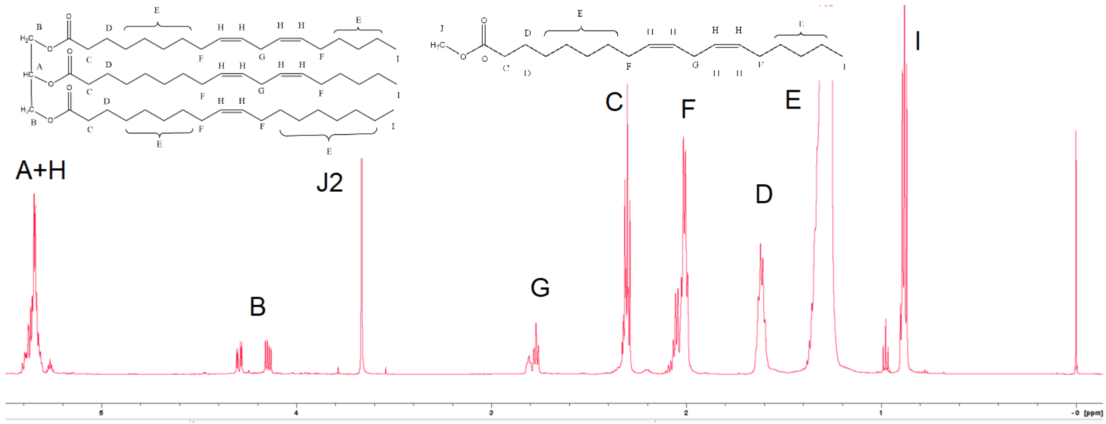

Figure 1. ^{1}H NMR of triglyceride and methyl fatty acid ester

Acknowledgements. This work has been supported partially by the project „Development and characterization of complex prolonged release drug delivery systems based on biodegradable polymers" (NPOO.C3.2.R3-I1.04.0126) funded by NextGenerationEU.

BINDING INTERACTIONS OF NOVEL PEPTIDOMIMETIC INHIBITORS WITH BUTYRYCHOLINESTERASE

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Cholinesterases are recognized as important biological targets for the regulation of cholinergic transmission, and their inhibitors are currently utilized in the treatment of Alzheimer's disease.[1] Several various strategies were followed to design new cholinesterase inhibitors, including the modification of compounds from a previously developed library, structure-based and fragment-based design, and target-guided synthesis.[2,3] Thus, a diverse collection of drug-like molecules (peptidomimetics) was constructed using an efficient four-component reaction, which was further optimized with innovative mechanochemical and microwave techniques. A novel strategy was employed in which the cholinesterase enzyme itself was used to guide the creation of potential inhibitors. Subsequently, advanced computer modelling and machine learning[4] were employed to correlate a molecule's structure with its inhibitory activity, allowing for the prediction of activity based on its energy profile. Large-scale computer simulations were conducted to explore the binding of these molecules within the enzyme's active site, and the potential for multiligand binding was revealed. Target-guided synthesis facilitated a more effective screening process leading to enhanced affinity of new inhibitors. Furthermore, STD NMR spectroscopy study was utilized for characterizing small molecule interactions with the macromolecule, allowing for the identification of key intermolecular contacts in the bound state.

Acknowledgements. This work was supported by the Croatian Science Foundation under the project number HRZZ-IP-2022-10-9525.

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METABOLOMICS OF SPONTANEOUSLY FERMENTED BEERS: INSIGHTS FROM NMR AND LC-MS

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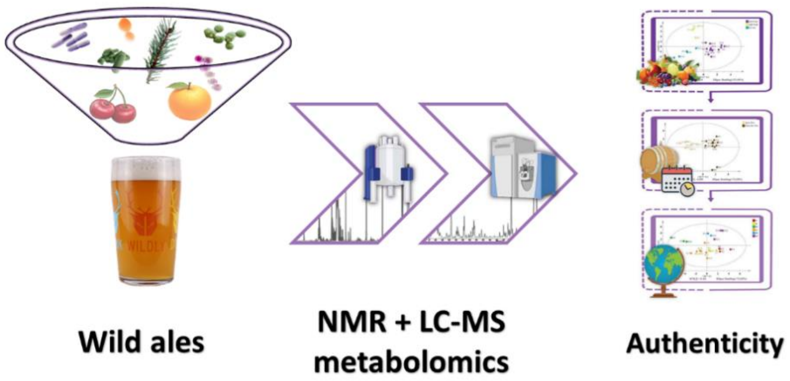
Wild ales are spontaneously fermented beers characterized by their diverse ingredients and complex microbial activity. In this study, we used NMR spectroscopy, LC-MS, and spectrophotometric assays to analyze the molecular composition and antioxidant potential of wild ales from six different countries. NMR proved to be a robust platform for quantifying key metabolites, including organic acids, amino acids, alcohols, nucleosides, nucleobases, saccharides, and phenolic compounds. LC-MS analysis extended the molecular coverage, partially overlapping with the NMR results and captured a broader range of specialized metabolites, including flavonoids, hydroxybenzoic and hydroxycinnamic acids, flavan-3-ols, procyanidins, bitter acids, and prenylated phenols. Multivariate analysis revealed that the addition of different fruits and the duration of aging significantly influenced the beers' chemical profiles and antioxidant activity. Correlations between specific metabolites and antioxidant assays identified compositional markers relevant to beer functionality. These results demonstrate the usefulness of NMR-based metabolomics for characterizing complex fermented beverages and linking composition to processing variables.



Acknowledgements: This work was supported by the Bulgarian National Science Fund, Project KP-06-M79/2. This investigation used research equipment, which forms part of the Distributed Research Infrastructure INFRAMAT, supported by the Ministry of Education and Science, and equipment purchased through project No BG16RFPR002-1.014-0001 (BIORESOURCES BG).

ANALYTICAL STRATEGIES FOR WINE ORIGIN AND AUTHENTICITY VERIFICATION

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Wine authentic analysis uses advanced chemical and sensory methods to verify a wine's label claims regarding origin, grape variety, and production process by analyzing its unique chemical fingerprint and known compounds. Key techniques include spectroscopic methods (like IR and UV-Vis) for fingerprinting, inductively coupled plasma mass spectrometry (ICP-MS) for mineral wine analysis, NMR spectroscopy for detailed chemical profiling, and chromatographic techniques to identify specific compounds. Sensory evaluations remain important, but chemical analyses, often combined with multivariate statistical methods, provide a more comprehensive and objective approach to detecting adulteration and counterfeiting. So, one of the main research activities is to improve already existing methods of authenticity monitoring as well as creating the new ones.

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NMR SPECTRA AS WINE FINGERPRINTS

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Various NMR techniques and their application in determining various parameters influencing a wine's quality will be discussed, such as the investigation on geographical origin, variety and vintage. Ranging from one-dimensional ^{1}H NMR with solvent suppression for water and ethanol signals, quantitative inverse-gated ^{13}C NMR, to the other less represented nuclei such as ^{31}P NMR, which can be used to determine glycerol content in wine. NMR spectroscopy has several advantages over other conventional techniques such as simple sample preparation and the absence of need for calibration standards. ^{1}H NMR can be used in both targeted and untargeted analysis, as well as for quantitative determination of wine metabolites.[1-3] Lastly, the vast amount of information contained within a single NMR spectrum of a wine can act as a fingerprint when coupled with advanced statistical methods such as deep reinforcement learning.[4]

A selected example of using DOSY (Diffusion-ordered) NMR spectroscopy in the investigation of sparkling wines will be presented. It can provide information on hydrodynamic radii, which in turn gives insight into the molecule size, and can aid in the analysis of complex mixtures. In sparkling wine investigation, DOSY NMR can help better resolving signal overlapping observed in ^{1}H NMR, which usually hinders useful information.

The potential of NMR spectroscopy in wine analysis even further increases by using modern statistical approaches like deep reinforcement learning protocols for analyzing experimental data providing reliable models for wine classifications, sensory property determination and fraud detection.

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POSTERS

THERMODYNAMICS OF THE COMPLEXATION OF MELOXICAM WITH β-CYCLODEXTRINS IN AQUEOUS SOLUTION

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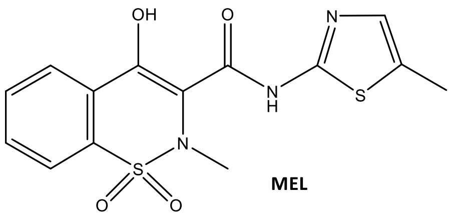
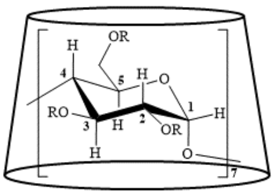
Meloxicam (MEL), a nonsteroidal anti-inflammatory drug, has a long history of use in the treatment of osteoarthritis, rheumatoid arthritis and various pain syndromes of skeletomuscular origin (e.g. low back pain).[1] It is poorly soluble in water and is classified as a class II drug in the Biopharmaceutical Classification System (BCS). The low aqueous solubility of MEL can be improved by complexing it with cyclodextrins (CDs) and their derivatives.[1] CDs bind lipophilic drug moieties into their hydrophobic central cavity, form inclusion complexes and thus improve the aqueous solubility and bioavailability of drugs. In this study, the thermodynamics of complexation of MEL by β-cyclodextrin (β-CD) and its derivatives (hydroxypropyl-β-CD (HP-β-CD), randomly methylated β-CD (RM-β-CD) and sulfobutylether β-CD sodium salt (SBE-β-CD)) in aqueous medium at pH 6.8 was investigated using isothermal titration calorimetry. The formation of 1:1 (MEL:CD) inclusion complexes was observed for all derivatives and the corresponding reactions were thermodynamically characterized by determining and discussing the complex stability constants (and derived standard reaction Gibbs energies), standard reaction enthalpies and entropies. The analysis of the thermodynamic parameters provided an insight into the molecular interactions driving the complexation process.



MEL



β-CD: R = H_{21}
HP-β-CD: R = H_{21-n} or $(–CH_2CH(OH)CH_3)_n$, n = 2–8; DS = 4.5
RM-β-CD: R = H_{21-n} or $(–CH_3)_n$, n = 9–15; DS = 12
SBE-β-CD: R = H_{21-n} or $(–(CH_2)_4SO_3Na)_n$, n = 3–7; DS = 6.5

Figure 1. Structures of meloxicam and β-cyclodextrins.

Acknowledgements. This work has been supported by the Croatian Science Foundation (project IP-2022-10-6033).

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HYDROLYSIS OF ENVIRONMENTALLY RELEVANT ANTICANCER DRUGS MONITORED BY NMR SPECTROSCOPY

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When placed in an acidic or neutral aqueous solution, anticancer drugs cyclophosphamide (**CPA**) and ifosfamide (**IFO**) undergo hydrolysis reactions. Phosphorous-nitrogen bonds are broken, giving final acyclic products. There are multiple possible reaction pathways that yield the same final compounds, but with different intermediate products [1,2].

Using various 1D-(^{1}H, ^{13}C, and ^{31}P) and 2D-NMR techniques we monitored the hydrolysis reactions and determined the reaction pathway and its intermediates for both compounds. The coupled 1D-^{31}P, ^{1}H, as well as HMBC spectra of the intermediates were acquired and analysed. The splitting patterns of multiplets, as well as the HMBC coupling suggest that the reactions first proceed by opening the ring, followed by further hydrolysis of the linear intermediate product.

Since **CPA** hydrolyses much slower than **IFO** the signals of the intermediate could only be seen when the initial concentration of **CPA** was high, and consequently the reaction accelerated, allowing for the sufficient accumulation of the intermediate. Both reaction rates were determined using corresponding ^{31}P integrals.

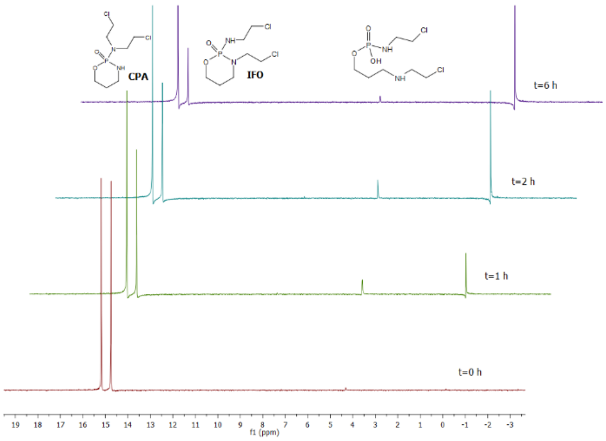

Figure 1. Stacked ^{31}P{^{1}H} NMR spectra of the reaction mixture (ifosfamide/cyclophosphamide/HOCl/D_2O)

Acknowledgements. This work has been supported by Croatian Science Foundation (projects IP-2022-10-2634 (“Pharma-Eco”) and DOK-NPOO-2023-10-7238).

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SYNTHESIS AND CHARACTERIZATION OF POROUS ORGANIC POLYMERS CONTAINING AZO AND AZODIOXY LINKAGES

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The continuous rise in atmospheric carbon dioxide (CO_2) concentration is one of today's most concerning ecological and economic challenges. Due to the lack of efficient technologies for the selective capture and storage of CO_2, generated by fossil fuel combustion, there is a growing need to develop new materials capable of serving as CO_2 adsorbents in industrial settings. Porous organic polymers (POPs) incorporating nitrogen-containing linkages, such as azo, have emerged as promising candidates for this purpose. [1]

In this study, novel POPs bridged by azo and azodioxy linkages were synthesized using various aromatic nitro and amino monomers and several synthetic strategies under conventional and microwave heating.[2–6] The structural characteristics of the resulting POPs were examined using ^{13}C CP/MAS NMR spectroscopy, IR spectroscopy, powder X-ray diffraction, and elemental analysis. Thermal stability was evaluated by thermogravimetric analysis (TGA) under nitrogen atmosphere, while porosity parameters were determined from nitrogen adsorption-desorption isotherms. CO_2 adsorption capacity of POPs was measured using TGA in a CO_2 atmosphere. The findings indicate that POPs containing azo linkages exhibit higher Brunauer-Emmett-Teller (BET) surface areas and enhanced CO_2 adsorption capacities compared to POPs with azodioxy linkages.

Acknowledgements. This work has been fully supported by Croatian Science Foundation under the project IP-2020-02-4467.

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RUDIMENTARY INSTRUMENTAL SETUP FOR IN SITU STUDY OF PHOTOSWITCHING BY NMR SPECTROSCOPY: CASE STUDY OF BRIDGEHEAD NITROGEN HETEROCYCLIC AZOBENZENE

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The integration of *in situ* LED (light-emitting diode) illumination has significantly advanced the application of NMR spectroscopy in the investigation of reaction mechanisms. This approach leverages the inherent strengths of NMR spectroscopy, enabling the real-time monitoring of photochemical reactions with high specificity and sensitivity. [1] In accordance with this methodology, the photokinetic behavior of 7-dimethyl-6-(phenyldiazenyl)pyrazolo[1,5-a]pyrimidine was examined, focusing on the E to Z isomerization induced by ultraviolet irradiation at a wavelength of 365 nm (Figure 1). NMR experiments were conducted on a Bruker Avance III 500 MHz spectrometer equipped with a 5-mm inverse broadband (^{1}H, X) z-gradient probe head. Spectra were acquired at a controlled temperature of 298 K. Photocatalytic conversion was performed using an external UV light source (365 nm LED lamp), single-mode and multimode fiber optic patch cable (600 µm diameter, 039 NA, 5 m), inserted into a 3 mm NMR tube immersed into 5 mm NMR tube containing a 4 mg/mL solution of investigated compound.

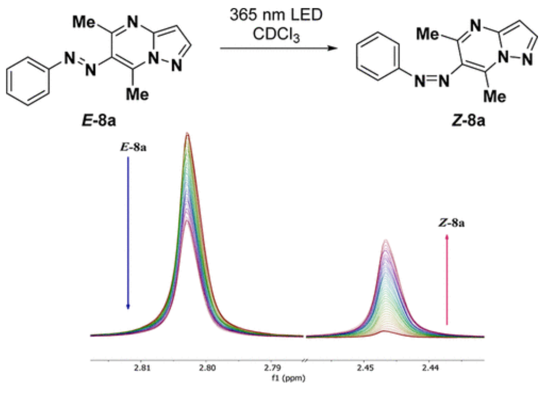

Figure 1. Photoisomerization of 7-Dimethyl-6-(phenyldiazenyl)pyrazolo[1,5-a]pyrimidine (*E*-8a) monitored by NMR spectroscopy.

Acknowledgements. This work has been supported by the Ministry of Science, Technological Development and Innovation of Republic of Serbia (Contract number: 451-03-136/2025-03/200168, 451-03-136/2025-03/200288 and 451-03-136/2025-03/200026

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INFLUENCE OF POTASSIUM POLYASPARTATE AND YEASTS ON CROATIAN SPARKLING WINES MONITORED BY NMR SPECTROSCOPY

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The production of sparkling wines has significantly increased in the last two decades which has also increased the need for their efficient analysis.[1] NMR spectroscopy is one such technique that has frequently been used to detect wine metabolites with the advantages of non-destructive sample preparation and the possibility of both targeted and untargeted analysis.[2]

Sparkling wines are a special type of wines characterized by effervescence and foam, which contain endogenous carbon dioxide originating from the second fermentation. Yeasts play an important role in the fermentation processes because they release various compounds in the process of autolysis.[3] Meanwhile, the biopolymer potassium polyaspartate (KPA) is added to sparkling wines in order to prevent the precipitation of tartaric salts at the bottom of the bottles. While KPA links the potassium cation, it might link to other compounds present in the sparkling wine, necessitating further investigations.[4]

The aim of this research was to evaluate the effect of using KPA agent and various yeasts on the composition of sparkling wines of different vintages and varieties. The NOESY presaturation (1D-^{1}H-NOESY) technique was used to obtain one-dimensional ^{1}H NMR spectra with water and ethanol suppression. Furthermore, the DOSY NMR pseudo-two-dimensional technique, which gives information regarding translational diffusion coefficients, was used to increase spectral resolution without the need of physical separations of the compounds. Inverse-gated ^{13}C NMR spectroscopy was also applied to provide quantitative data.[1,5]

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P-06

RECENT ADVANCES IN TITANIUM DIOXIDE (TiO_2) NANOLAYERING FOR ENHANCED MEDICAL DEVICE PERFORMANCE IN DENTISTRY

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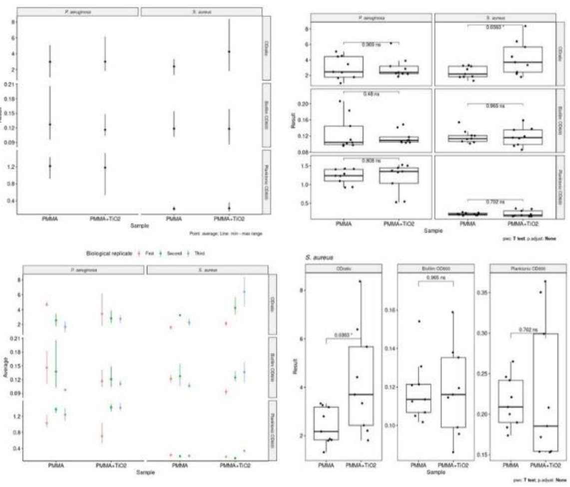
Polymethyl methacrylate (PMMA) is widely used in dentistry and medicine, but its susceptibility to microbial colonization and biofilm formation presents a major limitation. To address this, we applied Atomic Layer Deposition (ALD) to deposit highly uniform and conformal titanium dioxide (TiO_2) coatings onto PMMA substrates. ALD enables precise thickness control at the nanometer scale, optimizing surface properties for biomedical applications. All prepared samples were subjected to both microbiological assays and Atomic Force Microscopy (AFM) analysis, allowing direct correlation between antimicrobial and mechanical performance. Microbiological evaluation assessed bacterial adhesion, growth inhibition, and biofilm reduction on TiO_2-coated surfaces. AFM provided nanoscale insights into surface roughness, hardness, and elasticity, revealing the mechanical reinforcement achieved through ALD coatings. The combined findings demonstrate that ALD TiO_2 nanocoatings improve both antimicrobial activity and mechanical resilience of PMMA, highlighting their potential for safer and longer-lasting dental and biomedical prosthetic materials.



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BAYESIAN FRAMEWORK FOR OVERCOMING SCARCE NMR DATA: CASE STUDY OF THE HALDANE SYSTEM BONO

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Broadband NMR is a technique used to probe numerous solid-state systems across a wide range of frequencies, temperatures, magnetic fields, pressures, and other parameters. However, this versatility often comes at the cost of reduced signal quality, which necessitates longer signal acquisition times and, in general, limits the number of data points in the parameter space of interest. In this work, we address the resulting data scarcity problem by applying Bayesian inference modelling to analyse both ^{1}H spectral evolution and nuclear relaxation data in the Haldane system BoNO.

3,5-bis(N-tert-butylaminoxyl)-3'-nitro-1,1'-biphenyl, commonly referred to as BoNO, is a model S=1 quasi-one-dimensional system realised in a purely organic crystal. As such, BoNO lacks the intrinsic spin Hamiltonian anisotropies that have complicated earlier Ni-and V-based organometallic systems, making it an ideal testbed for theoretical predictions.[1] ^{1}H NMR data were collected across the entire phase diagram, at temperatures ranging from RT down to 300mK, and in magnetic fields up to 35 T. Subsequent Bayesian analysis shows an excellent agreement with the theoretical simulations, which was challenging to quantify using the standard ordinary least square (OLS) approach.

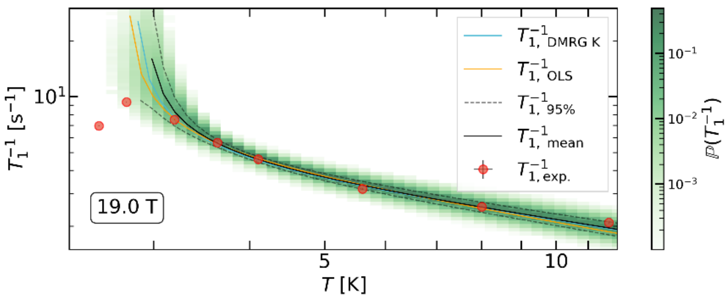

Figure 1. ^{1}H nuclear relaxation data fitted using Bayesian and OLS method(s).

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BIOACTIVE METABOLITES ISOLATED FROM *HERICIUM ERINACEUS* (BULL.) PERS.

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Hericium erinaceus is considered one of the most promising medicinal fungi for the treatment of neurodegenerative diseases, containing a wide variety of bioactive compounds, including hericenones, erinacines, and many other related molecules, which exhibit significant neurotrophic and neuroprotective properties. Previous studies demonstrated that erinacine-enriched mycelium of *H. erinaceus* can delay neuronal cell death in animal models of neurodegenerative conditions such as Alzheimer's disease and ischemic stroke. [1] Our research focus on the isolation of a diverse range of bioactive compounds from employing conventional chromatographic techniques, followed by their structural characterization predominantly through nuclear magnetic resonance (NMR) spectroscopy. Hericene A was isolated from the ethyl acetate extract of the *H. erinaceus* fruiting bodies by performing gravitational chromatography, followed by additional purification on semi-dry flash chromatography. Furthermore, erinacines A and C were isolated from the ethyl acetate extract of the mycelium. The extract was initially processed through semi-dry flash chromatography, followed by subsequent purification utilizing preparative high-performance liquid chromatography (HPLC). The structures of isolated compounds were determined using 1D (^{1}H NMR and ^{13}C) and 2D (COSY, J_{RES}, HMBC, HSQC and NOESY) NMR experiments. These purification methodologies demonstrated high efficiency and cost-effectiveness, resulting in the isolation of pure natural compounds appropriate for subsequent pharmacological investigations. Future investigations will prioritize the optimization of isolation protocols for various metabolites derived from both the mycelium and fruiting bodies of *H. erinaceus*, alongside comprehensive characterization and systematic evaluation of their biological activities, with the aim of elucidating potential therapeutic applications in the treatment of neurodegenerative disorders, particularly Alzheimer's disease.

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DETECTION AND QUANTIFICATION OF ADULTERANTS IN BEESWAX USING ^{1}H NMR SPECTROSCOPY

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Beeswax is a natural secretion produced by honey bees, primarily utilized for the construction of honeycombs in which honey is synthesized and stored. Chemically, beeswax comprises a complex mixture dominated by esters of long-chain fatty acids and alcohols, with smaller proportions of long-chain hydrocarbons and free fatty acids. Comprehensive analysis of beeswax is critical for ensuring product authenticity, quality control, and consumer safety, especially within cosmetic, pharmaceutical, and food industries. Adulteration of beeswax is prevalent, commonly involving lower-cost natural waxes such as carnauba, palm, and candelilla waxes, as well as petroleum-derived paraffins, stearic acid, or synthetic waxes. Such adulterations compromise both product quality and regulatory compliance.[1] Beewax ranks among the most frequuently adulterated commercial products, and traditional verification methods include assessment of physicochemical parameters such as melting point, penetrability, ester and saponification numbers and advanced instrumental techniques including high-temperature gas chromatography and, more recently, Fourier-transform infrared (FTIR) spectroscopy. FTIR spectroscopy offers rapid, sensitive, and reliable detection of adulterants by identifying characteristic vibrational bands, such as aliphatic aliphatic C–H st at ~2915 and 2849 cm^{-1} and ester C=O st in range of 1730-1745 cm^{-1}) which distinguish genuine waxes from synthetic or low-cost substitutes. However, as the extent of adulteration increases, FTIR detection becomes more challenging due to overlapping bands between authentic waxes and adulterants. In contrast, nuclear magnetic resonance (NMR) spectroscopy privides enhanced detection capabilities by delivering detailed molecular structural information. The presence or absence of specific ester signals and relative intensities of aliphatic proton resonances serve as definitive markers of authenticity when compared with reference spectra. [2] A major advantage of NMR is compatibility with internal standards such as TMS, DITMS or 1,4-dinitrobenzene, facilitating quantitative analysis. Characteristic NMR chemical shifts, notably δ_H 4.0–4.2 corresponding to CH_2–O protons of ester groups in triglycerides, provide critical insights into beeswax purity and adulteration extent.

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COMPLEXATION REACTIONS OF SOFT METAL CATIONS WITH THIOAMIDE CALIX[4]ARENE DERIVATIVE

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Calixarenes are macrocyclic oligomers consisted of phenolic residues linked by methylene bridges in the *ortho* position.[1] Depending on the size and functionalization of upper and/or lower rim, these compounds can be very efficient or even selective binders of various ionic and neutral chemical species.[1,2] Among them, those bearing sulphur-functionalities form stabile complexes with low-charge density and soft metal cations.[3] In the scope of this work, thioamide calix[4]arene derivative **L** (Figure 1) was synthesized and its binding affinity towards alkali and soft metal cations in acetonitrile was investigated. Thermodynamic parameters of complexation were determined by means of NMR spectroscopy, isothermal titration calorimetry, and spectrophotometry. The obtained results were discussed regarding the structural characteristics of the ligand, as well the size and charge density of the cations.

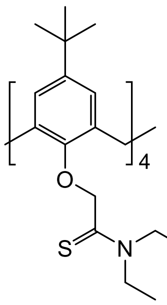
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Figure 1. Structure of thioamide calix[4]arene derivative **L**.

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METABOLOMIC ANALYSIS OF *TRIFOLIUM PANNONICUM* USING NMR SPECTROSCOPY AND CHENOMX: ADVANTAGES AND LIMITATIONS

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Hungarian clover (*Trifolium pannonicum* Jacq.), a previously underutilized forage legume of growing agricultural importance, was investigated for its metabolomic complexity and antioxidant potential.[1] Two genotypes—Panon (a cultivated variety) and a natural Balkan population—were compared to *T. hybridum* (Swedish clover) as a control. A total of 52 samples, collected at the budding stage optimal for forage use, were extracted with solvent mixture (MeOD: buffer KH_2PO_4 in D_2O, v/v = 1:1) and analyzed by ^{1}H NMR spectroscopy. Multivariate analyses (PCA and OPLS-DA) revealed clear genotype-level separation. Combined 1D and 2D NMR experiments (COSY, HSQC, HMBC), supported by spectral database comparison (Chenomx), led to the identification of 24 metabolites, including amino acids, sugars, and phenolic acids—most notably eucomic and hydroxyeucomic acids. Special attention was given to evaluating the advantages and limitations of the Chenomx platform. Chenomx offers robust tools for metabolite identification and quantification, particularly in complex biological samples. Its capacity to resolve overlapping signals through the utilization of an integrated spectral library facilitates relatively accurate compound profiling in the absence of pure standards.[2] The software's simplicity allows even users without advanced NMR expertise to navigate it effectively. However, its performance strongly depends on spectral quality and database coverage; unidentified compounds and spectral overlap remain major limitations. Moreover, given that the software is optimized for 1D ^{1}H NMR, its utility in resolving structurally complex metabolites is limited. In this study, the characterization of eucomic and hydroxyeucomic acids in complex mixtures required verification through 2D NMR experiments. While Chenomx enabled initial spin system identification, full structural elucidation was only possible using complementary 2D NMR experiments.

Acknowledgements. This work has been supported by the Ministry of Science, Technology and Innovation, contract number: 451-03-136/2025-03/200026, 451-03-136/2025-03/200168, and contract number 451-03-136/2025-03/200217.

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STRUCTURAL CHARACTERIZATION OF DISULFIDE-CONTAINING NITROSOARENES BY VARIABLE TEMPERATURE NMR

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Nitrosoarenes are a versatile class of organic compounds that can exist in three distinct forms: as monomers and as *Z*- or *E*-azodioxides.[1] In the solid state, *E*-azodioxides are typically the dominant species, whereas in solution, an equilibrium is established between nitroso monomers and azodioxides. At ambient conditions, monomers prevail, but lowering the solution temperature shifts the equilibrium toward azodioxides. Solution-state NMR spectroscopy has proven to be a powerful tool for elucidating the structural properties, thermodynamics, and kinetics of these equilibria, providing valuable insight into the behavior of nitrosoarenes under different conditions.[2,3] Sulfur-containing nitrosoarenes are of special interest due to their ability to adsorb onto gold (111) surface through S–Au covalent bond, enabling the formation of azodioxy bilayer or multilayer thin films.[4,5] The photochromic and thermochromic behavior of the azodioxy/nitroso system in the solid state[6] could enable external control of azodioxy bond disassembly and reassembly in films via UV radiation and heat, respectively. Aromatic dinitroso derivatives have recently emerged as promising organic semiconductors, and their integration into azodioxy films could serve as a foundation for constructing organic electronic devices.[7]

The current work focuses on thorough structural characterization of the two newly synthesized nitrosoarene derivatives with disulfide headgroups, which can self-polymerize into azodioxy oligomers or polymers on gold surface.[8] The obtained azodioxy thin films show promise for applications in molecular electronics and organic electronic devices. Solution-state structures and dimerization/oligomerization of disulfide-containing nitrosoarenes were investigated by variable temperature ^{1}H and 2D NMR (*e.g.*, COSY, ^{1}H-^{13}C HSQC and ^{1}H-^{13}C HMBC) experiments. Nitroso monomers and azodioxy species were identified by detailed analysis of acquired spectra.

Acknowledgements. This work has been fully supported by the Croatian Science Foundation under the project IP-2020-02-4467.

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ENCAPSULATING LATANOPROST: AN NMR-BASED STUDY

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Latanoprost, a prostaglandin F2α analogue, is widely used in the treatment of glaucoma due to its ability to lower elevated intraocular pressure.[1] However, its efficacy is limited by adsorption to container surfaces and susceptibility to hydrolysis, both of which reduce its concentration in solution.[2] To overcome these issues, surfactants such as polyoxyl castor oil can be employed. This study investigates the interaction between latanoprost and polyoxyl castor oil, a non-ionic surfactant capable of forming micelles, and further evaluates the influence of sodium hyaluronate on the encapsulation. Results of ^{1}H, NOESY, and DOSY NMR analyses indicated a strong interaction between latanoprost and polyoxyl castor oil. Chemical shifts perturbation suggested binding, NOE interactions confirmed micellar encapsulation, and reduced diffusion coefficients corroborated the incorporation of latanoprost into the micellar system. In contrast, sodium hyaluronate produced no detectable perturbation of NMR signals and showed no evidence of interaction with either latanoprost or polyoxyl castor oil. These results demonstrate that polyoxyl castor oil can efficiently encapsulate latanoprost in the presence of sodium hyaluronate, supporting its potential application for stabilisation of ophthalmic formulations.

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MECHANISTIC INVESTIGATIONS OF A COPPER-MEDIATED CARBOXYLATIVE C-C CROSS-COUPLING REACTION

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Incorporation of carbon dioxide (CO_2) into organic compounds is particularly challenging due to its kinetic and thermodynamic inertness. However, its use as a C1 synthon represents an attractive approach in modern organic synthesis for the preparation of highly valued organic carbonates and carbamates.[1,2]

CO_2 capture followed by cyclization and C-C cross-coupling reactions can overcome the thermodynamic limitations, and provide a direct access to complex products.[3,4] We are presenting a three-component reaction on various propargylic alcohols mediated by inexpensive copper salts. Our efforts are focused on the synthesis of allylated carbonates employing allyl halides, propargylic alcohols, and CO_2 under atmospheric pressure (Figure 1). The optimization of the reaction conditions has been performed in combination with the elucidation of the reaction mechanism by experimental and computational methods, i.e., control experiments, NMR monitoring and density functional theory (DFT) calculations.

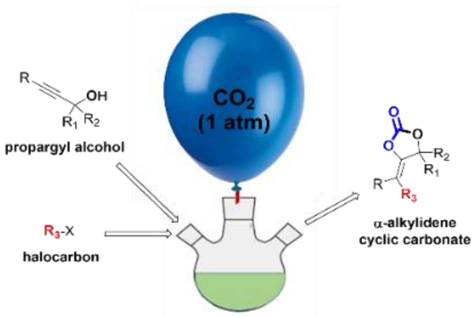

Figure 1. Illustration of a three-component carboxylative C-C cross-coupling reaction.

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NMR ANALYSIS OF LIGNIN EXTRACTED FROM HEMP POMACE

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Lignocellulosic materials are the most promising alternative feedstocks for the production of biofuels and various bio-based products. Hemp pomace is a by-product of hemp oil production and after oil extraction, hemp pomace contain insoluble fibre, lipids, proteins and sugars.[1] Fibres are composed of polymers cellulose (39–61%), hemicellulose (11–15%) and lignin (8–16%).[1] Lignin has a very complex heteropolymeric structure, mainly composed of three different phenylpropanoid units: p-coumaryl, sinapyl and coniferyl alcohol which are interconnected via numerous different linkage types.

NMR spectroscopy has been widely used for over three decades for the structural and compositional analysis of lignocellulosic biomass and it can provide various qualitative and quantitative information on functional groups, molecular architecture, and the degree of polymerization. [2]

In this study, the effect of biological treatment of lignin extracted from hemp pomace with *Thermomyces lanuginosus* via SSF (solid state fermetation) on the chemical composition was investigated by NMR spectroscopy. Lignin isolated from hemp pomace prior to and during SSF (after 4, 7 and 10 days of fermentation) has been analysed by ^{1}H and DOSY NMR spectroscopy. Significant changes in composition of lignin were observed.

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^{1}H NMR SPECTROSCOPY IN HIGH SCHOOL: COMPOSITION OF DIFFERENT CANDLE WAXES

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During a course in organic chemistry, second-year high school students learned that candle wax can be made from various alternative materials apart from paraffin. Four different types of candle wax were acquired or made: paraffin, beeswax, soy wax, and the used cooking vegetable oil wax – which was made in the school laboratory by following commonly found instructions on the internet.[1] After the initial literature search where similar research has been published,[2,3] the samples were analysed by Bruker Avance III HD 400 MHz/54 mm Ascend spectrometer.

The ^{1}H NMR spectra of different samples of waxes show significant differences compared to each other. The spectra of *alternative* waxes show more complexity compared to the simplest one — the pure paraffin. The largest peaks can easily be assigned to aliphatic, and methyl protons, which is expected since it is known that main components of all waxes are long saturated hydrocarbon chains. Apart from that, there is a strong indication of ketone, aldehydes or carboxyllic protons in traces. Some waxes show suspected presence of aromatic protons, which, depending on concentration, might create harmful products of pyrolysis. The acquired spectra have been compared to other research and no major discrepancies were found.[4–6] Further investigation of content of gaseous products of burning different waxes is planned.

Though the full analysis and assignation of wax components were above the students' level of comprehension, spectra showed enough visible differences between waxes. The students accepted the brief explanation of different parts of spectra and acknowledged that more theoretical background is needed for the full analysis.

Acknowledgement. This work could not be possible without resources provided by the NMR Laboratory of the Department of Chemistry, Faculty of Science, University of Zagreb. A special thanks goes to Dr. **Katarina Leko** for her advisement, guidance and measurements.

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^{1}H NMR AND GC–MS METABOLOMICS REVEAL HERBICIDE-INDUCED BIOMARKERS IN GRAPEVINE (*VITIS VINIFERA* L.)

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Metabolomics has become an essential tool in plant science, providing a systems-level understanding of physiological responses to environmental and chemical stressors. The goal of metabolomic analysis is to comprehensively assess metabolic changes and dynamics associated with disease states or stress exposure. Chromatographic and spectroscopic techniques are the most employed methods in metabolic studies.[1] Data processing is a critical step, particularly for multivariate analysis, where the removal of noise and preservation of relevant signals are essential to accurately capture true biological variation among samples.[2] In this study, metabolic changes induced by herbicide application in grapevine (*Vitis vinifera L.*) were investigated using a complementary analytical approach combining ^{1}H NMR spectroscopy and GC-MS. The objective was to identify potential metabolic biomarkers associated with herbicide-induced stress and to evaluate the feasibility of integrated metabolomics for monitoring grapevine health. Leaf and root samples were collected from both treated and untreated plants. Metabolites were extracted using a methanol/water (1:1) solution. For GC-MS analysis, samples underwent a two-step derivatization (methoximation followed by silylation), while NMR analysis (*noesypr1d*) was performed directly using CD_3OD/phosphate buffer in D_2O (pH = 6). Data obtained from GC-MS analysis was processed using the XCMS online platform to do peak selection, alignment, and deconvolution. The ^{1}H-NMR spectra were processed using the online program NMRProcFlow (v1.4.16) to calibrate the chemical shift, correct the baseline, align locally, and divide the spectrum into buckets using the intelligent binning method spectrum was divided into buckets. For the identification of the biomarkers in ^{1}H NMR spectra, Chenomx V11.0 software was used, as well as 2D NMR spectra. Multivariate data analysis was performed to differentiate metabolic fingerprints and identify potential biomarkers. This study highlights the utility of combining NMR and GC-MS metabolomics for the detection of herbicide-responsive biomarkers in grapevine and improving our understanding of plant biochemical adaptation.

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HOST–GUEST CHEMISTRY OF DIAMANTANE AMMONIUM SALTS AND MODIFIED β-CDS

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Cyclodextrins (CDs) are important host macrocycles in supramolecular chemistry since their deep, centrally positioned cavity enables the inclusion of hydrophobic guest molecules through non-polar interactions. Owing to their favorable properties and bioavailability, CDs have found widespread application in pharmacology, biotechnology, catalysis, and nanotechnology.[1] On the other hand, structural variation in substituent position and the degree of amine methylation on the diamantane guest skeleton provides valuable insight into the interplay of structural and electronic effects during complex formation with CDs. Furthermore, the use of peripherally derivatized β-CDs highlights how additional charges affect complex stability, since the substituents at positions 1, 3, and 4 of the diamantane framework carry positive charges (Figure 1). In this work we studied the binding of various diamantane ammonium salts[2,3] we have synthesized with such modified β-CDs. Stability constants of the complexes were determined using microcalorimetry and ^{1}H NMR titrations. The structures of the complexes in solution and the specific intermolecular interactions were further explored by NMR spectroscopy (ROESY, DOSY). Finally, computational methods provided deeper insight into the structural features of the formed complexes.

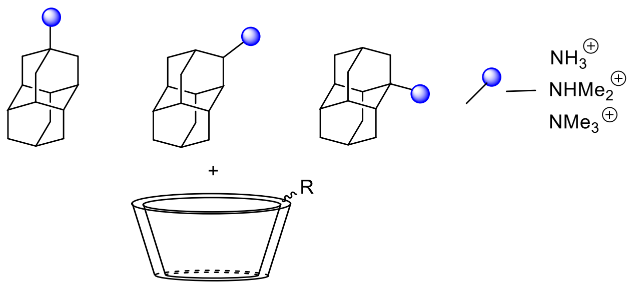

Figure 1. Diamantane guests and modified β-CD hosts investigated in this study.

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STEREOCHEMISTRY ELUCIDATION OF SOME 1,2-DIAZINE DERIVATIVES USING NMR

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1,2-Diazine (pyridazine) are organic small molecule azaheterocycles of great importance in medicine and pharmacy as drugs and biologically active compounds (possecing a largy variety of actione: antimicrobial, cardiovascular and antihypertensive, anti-inflammatory, analgesic, antinociceptive, antimicrobial,),[1] in agriculture as grow up factors, insecticides, pesticides,[2] in optoelectronics as semiconductors, LED/OLED devices,[3] sensors and biosensors,[4] etc.

In continuation of our continuous efforts in the field of azaheterocyclic derivatives, we present herein a thoroughly study cocerning stereochemistry os some 1,2-diazine derivatives.[5] The synthesis is stright and efficient, in two steps: N-alkylation of nitrogen atom from 1,2-diazine derivatives, followed by a reaction with hydrazine. The stereochemistry of the hydrazide 1,2-diazine derivatives was studied using the NMR exeperiments (1H, 13C, 2D HMBC, NoeDiff 1D) at room and hight temperature, and reveal a conformational equilibrium betwen a *Z-sc* (around 90%) conformer and *E-ac* (around10%) conformer.

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