

The reaction of 3,4-dihydro- β -carbolines with nitrile imines

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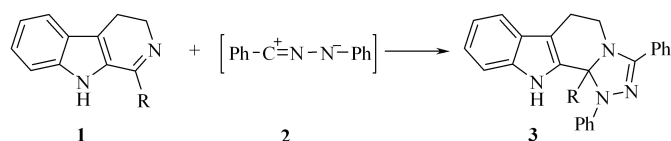
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The tetrahydro- β -carboline ring system can be found in numerous natural and synthetic compounds having interesting biological activities. We aimed at the syntheses of novel tetracycles (harmicine-derivatives) containing two or more heteroatoms in the five-membered D ring. The reaction of 3,4-dihydro- β -carbolines (**1**) with an *in situ* generated 1,3-dipole seemed to be useful to establish the D ring of the tetracycle. In 2009 we studied the reaction of 3,4-dihydro- β -carbolines with 4-fluoro-*N*-hydroxybenzenecarboximidoyl chloride and synthesised oxazaharmicines in quantitative yield [1-2]. In 2010 we examined this reaction of the β -carboline-derivatives (**1**) with a suitable

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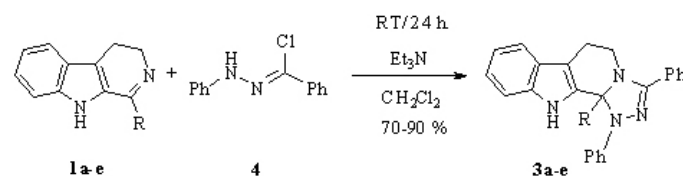
nitrile-imine (**2**) (Scheme 1).



R = H, alkyl, aryl group

Scheme 1 The planned reaction of 3,4-dihydro- β -carbolines with the *in situ* generated nitrile imine

By the reaction of 3,4-dihydro- β -carboline derivatives (**1a-e**) with *N*-phenylbenzene-carbohydrazonoyl chloride (**4**) in the presence of triethylamine in dichloromethane, the product (**3a-e**) was formed in good yield (**Scheme 2**). The structure of the isolated products was proved by NMR and mass spectroscopy and single crystal X-ray analysis.



R = H(a), Me(b), Pr(c), *i*-Pr(d), Ph(e)

Scheme 2 The reaction of 3,4-dihydro- β -carbolines with *N*-phenylbenzene-carbohydrazonoyl chloride

In these reactions we didn't observe the isomerisation of the product nor a second addition of the reagent as in case of nitrile-oxides [1-2].

Studying the reaction mechanisms, it was found, that similarly to the nitril-oxides, it is not an 1,3-dipolar cycloaddition, that is a typical reaction of 1,3-dipoles, but rather an ionic type non concerted stepwise addition reaction.

References

- 1 Ábrányi-Balogh P, *The reaction of 3,4-dihydro- β -carbolines with nitrile oxides*, Periodica Polytechnica **54** (2010), 47.
- 2 Milen M, Ábrányi-Balogh P, Dancsó A, Körtvélyesi T, Simig Gy, Keglevich Gy, *Reaction of 3,4-dihydro- β -carbolines with 4-fluorophenyl-nitrile oxide*, Letters in Organic Chemistry **7** (2010), 377-382.

Soil amelioration and nutrient supply by ashes from biomass combustion

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Combustion of biomass in power stations and heating centres results in the formation of more than 30 thousand tons of ash annually in Hungary, which generates great waste management problems.

By an innovative technology using 80-90 °C water it is possible to extract most of the ash's K content in order to produce mineral fertilizer. The aim of my work was to study the potential agricultural utilization of the ash residue that arose after this process, more exactly to certify the soil conditioning, nutrient supplier and toxic element stabilizing effect of the material out of consideration for the environmental risk of the usage.

According to the nature of the problem, we had to work out a specific integrated technology monitoring, therefore we used both physical-chemical (soil chemistry, nutrient content, toxic metal content) methods and environmental toxicology during our work – by latter we could gain information about the possible toxicity of the material and additionally, some organisms were able to sign the productivity of the soil.

The experiment was set up on two different soils, a sandy soil and a clay soil – they had strictly different colloid contents and chemical reactions. Based on these soils we generated four mixtures including contamination by toxic metals. We dosed 1, 3 and 5 mass percent ash residue to them leaving one unattended variant in each four cases. All the tests took place after eight weeks of incubating, to assure time to the conformation of a soil chemical balance.

The ash residue proved to be a good liming material – on one pH=4 sandy soil mixture 1 mass percent was enough to reach neutral reaction. Due to its high K and P content it is perfect for nutrient supply. Hopes of the toxic metal stabilizing effect were not confirmed – out of nine toxic elements, stabilization came true only in the case of Zn.

The results of environmental toxicology were dual. The num-

ber of microbes extremely rose by means of the added nutrients. On the *Vibrio fischeri* luminescence inhibition test no toxicity was shown. Alteration of the length of mustard rudiments (*Sinapis alba*) (Figure 1) showed that in 1 mass percent dosage the material calls forth exhortation, but in 3 and 5 mass percent the unexplained toxicity of the material counteracts.

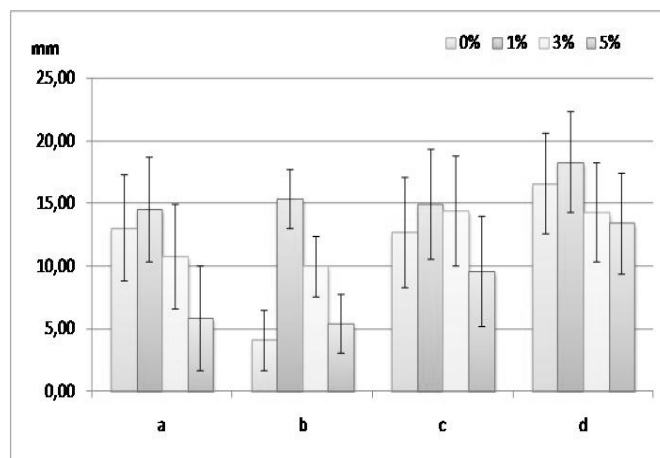


Fig. 1. Length of mustard rudiment stems (letters indicate different soil mixtures)

Cyclodextrin complexation of faradiol-esters extracted from marigold *Calendula officinalis* L. with supercritical carbon-dioxide

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The marigold is a commonly used medicinal herb that has several positive effects assigned. My aim was to develop a procedure to form complexes of the triterpene anti-inflammatory agents of marigold (faradiol-esters that have a negligible solubility in water which limits their reachable *in vivo* concentration and efficiency) and cyclodextrins. The fact that the raw material was not a pure substance but a complex herbal extract that contains several hundreds of components differentiates my work from the various procedures for complexation that can be found in the literature.

In addition to the already described procedures experiments were performed with a new method that can be summarized by mixing the extract with a carrier with a high specific surface, pumping carbon-dioxide through it that re-solubilizes the components. Then, the fluid containing the extract is pushed through a cyclodextrin bed where the components form a complex with

the cyclodextrin. This procedure enables us to operate it semi-continuously, and the product does not need any post-processing in opposition with the ones described so far.

The efficiency of three fundamental technologies, the atmospheric pressure water suspension, the batch and the dynamic reactors, were compared under supercritical carbon-dioxide pressure with a strong emphasis put on differentiating the adsorbed and the really complexed faradiol-esters. HPLC was used to analyse the samples.

After interpreting the results it turned out that the batch supercritical technology can surpass the dynamic method with the applied circumstances. Fortunately, the supercritical fluid technology, that is still spreading and can be considered as a green technology, proved to be more efficient than the conventional suspension procedure. It can be stated that the β -cyclodextrin is capable of forming complexes in the order of magnitude of 1000 ppm while the γ -cyclodextrin is not in the applied circumstances. The aim set, to increase the apparent solubility of the key components in water with several orders, has been achieved by the produced complexes.

Fabrication of complex coatings by Langmuir-Blodgett and sol-gel techniques

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There are several methods for film deposition onto solid substrates. Langmuir-Blodgett (LB) method is suitable to prepare nanoparticulate coatings with a planned structure and morphology. The practical applications of nanoparticulate LB films, however, are limited by their poor mechanical stability. Dip-coating technique based on sol-gel processes is a relatively simple method for preparing thin coatings with high durability. The properties of the films can precisely be controlled by changing the conditions of deposition and/or the composition of the precursor materials.

Our aim was to combine the advantageous features of the aforementioned methods for fabricating nanostructural, mechanically stable coatings with desirable morphology. LB films of Stöber silica nanoparticles having diameter of *ca.* 130 nm were coated with silica or titania precursor sols. Microscope glass slides were used as solid substrates. The mechanical stability, the structure and the surface morphology of the combined coatings were investigated.

The combined coatings, demonstrated by the lift-off tests, showed an improved mechanical stability compared to the original LB films. The optical properties were investigated with scanning angle reflectometry and UV-Vis spectroscopy methods and the resulting reflectance and transmittance curves were evaluated in terms of different thin layer optical models. The optical studies and atomic force microscopy investigations gave information about the structure and morphology of coatings. Investigations revealed surfaces with different nanomorphology which was mainly dependent on the thickness of the sol-gel layer deposited onto the nanoparticulate film. For comparison, combined coatings of microparticulate LB films and sol-gel layers were also studied.

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Carbonized flax and hemp fibres

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Plant based carbon fibres can be used for soil improvement and – in activated form – as adsorbents. These fibres are carbon-neutral (the amounts of carbon dioxide developing during their combustion and fixed by the plant through photosynthesis, respectively, are equal), moreover, using of these carbon products (“biochar”) for soil amendment is a method for carbon sequestering.

The quality of the carbonized plant material is an important issue in application. According to the literature the properties of carbonized plant depend not only on the ratio of the plant's components, but on the interaction of the components as well.

Carbonization of flax and hemp fibres, advantageously cultivated in Hungary, was investigated. Differences between the two types of cellulose source, the pre-treatment of fibres and carbonization conditions influencing the quality of the product were investigated. Native fibres were modified by alkali/ultrasound

treatment used generally for partial disintegration of the fibrils. Parameters of carbonization were selected according to the TG-MS results. Effects of the selected carbonization parameters on the yield and density were determined. The nitrogen adsorption capacities of native, modified, and carbonized fibres were measured, porosity was calculated, SEM pictures of samples were taken.

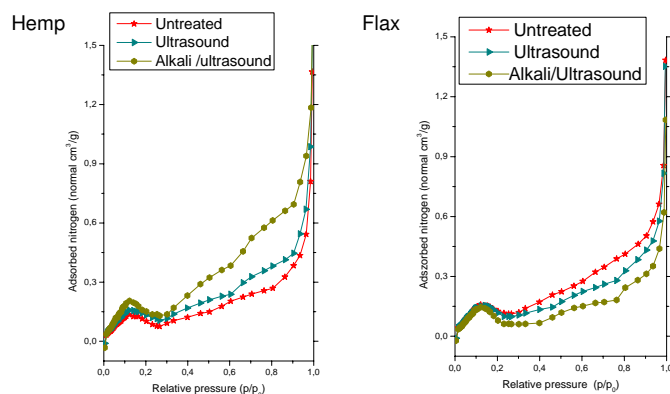


Fig. 2. Nitrogen adsorption isotherms of various flax and hemp fibres

The pre-treatment modified the pore structure of both flax and hemp fibres (Fig.): the nitrogen adsorption capacity of flax decreased, while that of hemp increased. In both cases, the effect of a combined alkaline/ultrasonic treatment was the highest.

The changes of native fibres were reflected by the properties of the *carbonized fibres*. In case of hemp both types of pre-treatments increased the pore volume of the carbonized fibre; alkali/ultrasound combined technology multiplied the pore volume by almost three. The pore volume of flax was decreased by simple sonication, while combined alkali/ultrasound treatment increased it, although in a smaller degree than in the case of hemp.

The changes of fibre properties due to the pre-treatments are different for flax and hemp. It calls attention on the potential contradictions of ultrasound treatments.

Production of extracts from garlic *Allium sativum* L. with ethyl alcohol, the scale-up of the procedure and product development based on the extracts

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Putting the medicinal herbs in order based on their importance

in the history of humankind, garlic has always been among the ten most important ones. Nowadays it plays an increasing role in the treatment of the number one illness of the modern age, the heart and circulatory diseases. With a wide spectrum of agents, garlic can reduce the blood pressure, reduce the level of the harmful LDL cholesterol and prevent formation of thrombi. According to the similar opinions of professionals the most important components of the garlic are the alliin and its metabolites that are formed during the activity of the alliinase enzyme that decomposes the alliin and causes the characteristic and well-known smell.

My aims were to uncover the factors influencing the extraction of alliin, to choose an excellent raw material that is rich in alliin and to map the extraction parameters of the chosen raw material. That work was based on earlier experiments. My recent aims were to develop an economical process for garlic extraction, to scale it up by one order of magnitude and to develop a dietetic supplementary product based on the extract.

Firstly, a multi-stage lab-scale extraction process was developed bearing economic aspects in mind. By further experiments, a method to measure the total alliin content in the garlic raw material was developed.

The second part of the work started with planning the pilot-scale equipment. After building it the adaptation of the lab-sized process could begin. I performed test experiments in order to find the adequate settings of the device. In further experiments the desired process in the pilot-scale experiment was reproduced even with better results than expected. In this phase of job several production experiments were performed producing solid extracts of an excellent quality and concentrated solutions of the extracts.

In the third phase, we performed the product development based on the extracts produced in the pilot-scale experiments in a close co-operation with our industrial partners. The product designed has a high alliin potential, moreover we have solved the molecular trapping of the smell produced during the process.

Growth pattern analysis in cell cycle mutants of fission yeast: Linear or bilinear?

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In the first half of the 20th century yeasts have become model organisms in cell cycle studies. The cylindrically shaped fission

yeast cells grow exclusively at their tips almost from birth to division by maintaining a constant diameter, therefore cell length is approximately proportional to cell volume. Length growth patterns may therefore indicate connections between volume changes and cell cycle events. The classical method to study the growth of individual cells is time-lapse microphotography; cells are growing on the surface of an agar pad in a thermostated photomicroscope, and later on, the growth pattern of cell length can be simply studied by a projector. A size control acts in every cell cycle to maintain homeostasis of cell size. With further analyses of growth patterns we are able to estimate the position of this size control.

In different cell types, there is considerable controversy concerning the exact growth profile of size parameters during the cell cycle. Linear, exponential and bilinear (i.e., two linear segments with a Rate Change Point (RCP)) models are commonly considered, and the same model may not apply for all species. The bilinear model is namely a linearised biexponential model, which makes possible a smooth, continuously differentiable transition between two linear segments. Selection of the most adequate model to describe a given data-set requires the use of quantitative model selection criteria, like Akaike Information Criterion (AIC), which are suitable for comparing differently parameterised models.

I have analysed the length growth patterns of 180 fission yeast cells (60 *cdc2-3w* mutants, 60 *cdc2-3w cdc25Δ* double mutants and 60 *cdc2-3w cdc25Δ pyp3Δ* triple mutants). The above mentioned model selection criteria were used for discriminating among linear, exponential and bilinear models and selecting the most adequate one in the case of all these cells' length growth patterns. Although relatively small differences were found in several cases, essentially all the quantitative selection criteria considered here indicated that the bilinear model was generally more adequate than either the exponential or the linear ones. At least the half of the cells had a bilinear growth pattern. In these cases the slopes of the two linear segments were also compared with *t*-test (Statistica® software, *Homogeneity of slopes* function). "Average cells" were also constructed from all the individual cells' data for all strains, whose patterns were definitely found to be bilinear by any criterion used. In *cdc2-3w cdc25Δ* double and *cdc2-3w cdc25Δ pyp3Δ* triple mutants size control seems to act after RCP, in late G₂. Furthermore, in *cdc2-3w* mutant cells, size control probably operates in both G₁ and G₂ phases. This is in sharp contrast to wild type cells, but confirms our former results.

Acknowledgement

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The synthesis of phosphinates in an environmentally friendly manner

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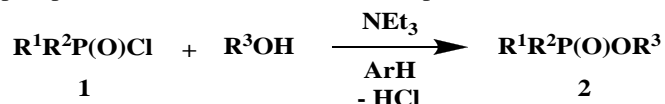
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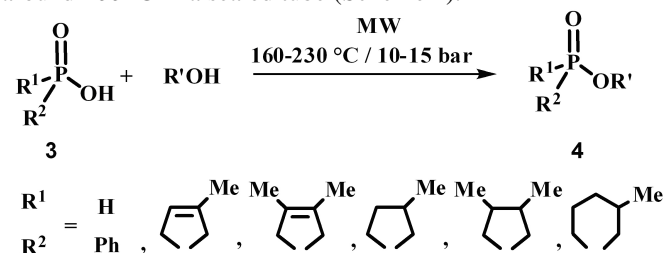
As phosphinic acids cannot undergo direct esterification with alcohols, phosphinates are usually prepared by the reaction of phosphinic chlorides with alcohols and phenols (Scheme 1).



Scheme 1. A general route for the preparation of phosphinates

Although this method has drawbacks from the point of view of "green" chemistry and costs, it is widely applied in industry.

It was found that the otherwise impossible direct esterification of phosphinic acids takes place under MW conditions by irradiating the mixture of the cyclic phosphinic acids selected as model compounds and the alcohol (used in ca 15-fold excess) at around 200 °C in a sealed tube (Scheme 2).



Scheme 2. Novel method for the preparation of phosphinates

Under such conditions, the cyclic phosphinates could be obtained, in most cases, in about 45-60% yields. The only criterion is that the alcohol must not be too volatile to avoid a pressure > 15 bar.

The novel reaction is the consequence of the beneficial specific MW effect that was proved by comparative thermal experiments.

Our method, that is an environmentally friendly approach, and which can be accomplished without the use of phosphinic chlorides, is of novelty and of general value. For this, we make efforts for further extensions and applications.

Molecular mechanism of enzymatic evolution – the Kemp eliminase

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The origin of enzymatic catalysis still remains to be elucidated [1]. The goal of our work was to uncover the major driving forces of enzymatic reactions and to design even more efficient enzymes using these principles.

As a model system, an artificial enzyme, the Kemp eliminase was used [2]. This enzyme is resulted by a rational design process in an effort to find an optimal scaffold for an active site that was obtained by quantum chemical methods. The original, native enzyme showed very good agreement with the computational model, but its activity was far behind the natural enzymes. Therefore it was subjected to an *in vitro* evolution experiment, which could improve the catalytic rate by 200 fold. We aimed to simulate the reaction mechanism of both the native and the evolved enzyme and compare the major catalytic factors. The activation barriers obtained by the Empirical Valence Bond (EVB) method [3] were in accord with the experimental data. We found that in course of the laboratory evolution the reorganization energy was optimized significantly, meanwhile the activation barrier only modestly decreased. This observation was corroborated by analyzing the individual residue contributions to the catalysis. Almost all mutations in the evolved variant were accompanied by reduction of the reorganization energy, while the electrostatic energies did not exhibit a uniform trend. Based on this observation, we have developed a screening programme that computes reorganization energy contributions of the studied mutations. This code is applicable for high throughput screening and was tested on almost 200 virtual mutations. The computational results were compared to all available mutants that appeared during the *in vitro* evolution experiments. In all cases, where the mutant enzyme was experimentally available, i.e. selected by evolution, the change in reorganization energy was either negligible or considerably decreased. This verified our conclusion that this quantity is optimized during evolution. Kinetic parameters of the K221A mutant were determined

and exhibited excellent agreement with the computational data. Testing of other designed mutants is ongoing.

References

- 1 Warshel A, Florian J, *Computer simulations of enzyme catalysis: finding out what has been optimized by evolution*, Proc. Natl Acad. Sci. U.S.A., 1998, pp. 5950-5955, DOI 10.1073/pnas.95.11.5950.
- 2 Khersonsky O, *Evolutionary optimization of computationally designed enzymes: Kemp eliminases of the KE07 series*, J Mol Biol **396** (2008), 1025-1042, DOI 10.1016/j.jmb.2009.12.031.
- 3 Hwang J K, Warshel A, *Semiquantitative calculations of catalytic free energies in genetically modified enzymes*, Biochemistry **26** (1987), 2669-2673, DOI 10.1021/bi00384a003.

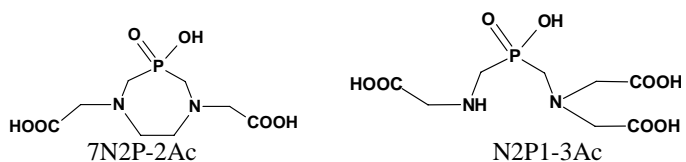
Copper(II) complexes of two bis(aminomethyl)phosphinicacid derivatives studied by EPR spectroscopy

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There is increasing interest in the research of new chelating ligands for use as molecular imaging agents of metallic radionuclides in medical diagnostic methods (e.g. ^{64}Cu $t_{1/2}=12,7$ h, in positron emission tomography, PET). Up till now, macrocyclic polyaza polycarboxylate and polyphosphonate ligands (with terminal carboxylate and phosphonate groups) were commonly used but nowadays emphasis were made on the preparation and investigation of ligands containing phosphinate groups in a ring or in open chains. Here we report on the complexation study of two new chelating ligands, (1) 2,2'-(3-hydroxy-3-oxo-1,5,3-diazaphosphhepan-1,5-diil)-diaceticacid (7N2P-2Ac) and (2) (N-methyl-glycino)(N-methyliminodiacetic)phosphinate (N2P1-3Ac) (Scheme 1).



Scheme 1: Structures of the ligands studied

These chelating ligands must achieve several criteria – the most important is the thermodynamic stability of their complexes. Our goal was to investigate the stabilities and structures of the paramagnetic copper(II) complexes of the above ligands using Electron Paramagnetic Resonance spectroscopic (EPR) methods. A series of EPR spectra were recorded at room temperature in different pHs and ligand to metal concentration ratios and were simulated simultaneously with a „two-dimensional” simulation program [1]. By the decomposition of the measured spectra the program can provide the isotropic EPR parameters

as well as the formation constants of the components formed in the overlapping equilibria. EPR spectra at 77 K were also measured at each pH-point, which were simulated by the EPR program [2] to obtain the anisotropic EPR parameters. In order to facilitate the interpretation of our data geometry optimization of the complexes and calculation of the theoretical EPR parameters were performed by DFT quantum chemical calculations.

References

- 1 Rockenbauer A, Szabó-Plánka T, Árkosi Zs, Korecz L, J. Am. Chem. Soc. **123** (2001), 7646-7654.
- 2 Rockenbauer A, Korecz L, Appl. Magn. Reson **10** (1996), 29-43.

QM/MM study of the bond cleavage of *Mycobacterium tuberculosis* dUTPase mutants

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According to the data of WHO tuberculosis is the most deadly infectious disease, which causes nearly 2 million deaths each year. The dUTPase enzyme that is in focus in our laboratory, is a potential drug target, since it is essential for *Mycobacterium tuberculosis*. The dUTPase plays a crucial role in DNA integrity, as it catalyses the hydrolysis of dUTP to dUMP and pyrophosphate, thereby it produces the precursor of dTTP biosynthesis and keeps the dUTP:dTTP ratio low. In the absence of dUTPase this ratio will be high, which causes DNA fragmentation and the so-called thymine-less cell death.

To understand the enzymatic mechanism mutant enzymes were previously generated, which differ from the wild type at the substrate binding C-terminal arm. The structures of two mutants – one with alanine instead of histidin, which stacks over the uracile ring and one, which lacks the full C-terminal arm – could be resolved and have been deposited to the PDB database with ID of 3LOJ and 3I93. To compare the substrate hydrolysis mechanism of the wild type and the mutants, *in silico* analysis have been performed at the NIH (National Institutes of Health, Bethesda, Maryland, USA).

Using a previously defined [1] reaction coordinate, the geometries have been minimized starting with the initial enzyme-substrate structures and following the reaction through the tran-

sition state to the product state. The substrate and the whole active site of the wild type and mutant enzymes were included in the quantum region, which was calculated using density functional theory, while the remaining part of the enzyme and the solvent within 20 Å from the substrate-bound Mg²⁺ ion was treated with classical molecular mechanics and was coupled to the quantum region using full electrostatic embedding.

The calculated activation energies are in good agreement with the experimentally observed catalytic rate constants. The moieties important to the efficient catalytic activity of the dUTPase enzyme can be identified according to the charge distribution of the active site within the obtained structures, and to the changes in relevant bond lengths and angles.

References

- 1 Barabás, 2010. under publication.

Modeling the response of single-nanopore electrochemical biosensors

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(Bio)chemical sensing with nanostructures is among the most sensitive bioanalytical methods as they enable in certain cases single molecule detection. Sensing with nanopores/nanotubes for molecular detection emerged recently in the forefront of nanostructure-based chemical sensors [1] due to its potential for: (a) ultrafast DNA sequencing; [2] (b) biosensing with selective receptor modified nanopores; [3] (c) macromolecule and nanoparticle counting [4]. Sensing with nanopores most often implies monitoring changes in the applied voltage driven ion flow across a nanopore that separates two electrolyte chambers as particles are passing through or residing within the nanopore sensing zone. The entering particles displace their own volume of highly conductive electrolyte, which leads to a clearly detectable increase in the transpore resistance if the particle size is comparable to the pore diameter. However, the interpretation of the detection events at nanoscale is prone to inconsistencies even regarding fundamental expressions, e.g., the theoretical expression of the current through a nanopore with known geometry. Therefore, we proposed to model the transpore current through nanopores of various geometries and surface properties as well as to extend it to determine the current modulating effect of species with different physical and chemical properties. When counting nanoscale entities with nanopores their translocation frequency is dramatically reduced in very diluted solutions. This problem can be alleviated by extending the capture zone of the nanopore in the solution bulk as much as possible by

establishing an electric field gradient in case of species with high electrophoretic mobility or inducing electroosmotic flow to capture uncharged species. To determine the relative extent of various transport mechanism and their efficiency to capture the targeted species and direct them into the nanopore sensing zone we solved the coupled Poisson, Nernst-Planck and Navier-Stokes differential equations using COMSOL Multiphysics finite element package. The results confirm the importance of considering the entrance resistance into the nanopores when correlating the transpore current and pore geometry as well as that both electrophoresis and electroosmosis can increase the translocation efficiency of the target species through the nanopore. The present study was successful in providing the theoretical predictive basis for the development of single-nanopore electrochemical sensors for virus and nanoparticle counting.

References

- 1 **Griffiths J**, *Analytical Chemistry* **80** (2008), no. 1, 23.
- 2 **Branton D, Deamer D. W, Marziali A, Bayley H, Benner S. A, Butler T, Di Ventra M, Garaj S, Hibbs A, Huang X. H, Jovanovich S. B, Krstic P S, Lindsay S, Ling X. S. S, Mastrangelo C. H, Meller A, Oliver J S, Pershin Y V, Ramsey J M, Riehn R, Soni G V, Tabard-Cossa V, Wanunu M, Wiggin M, Schloss J A**, *Nat. Biotechnol.* **26** (2008), no. 10, 1146-1153.
- 3 **Jágérszki G, Gyurcsányi R. E, Höfler L, Pretsch E**, *Nano Lett.* **7** (2007), no. 6, 1609-1612.
- 4 **Uram J D, Ke K, Hunt A J, Mayer M**, *Small* **2** (2006), 967-972.

Is there a biological cost of protein disorder: analysis of cancer associated mutations

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In the past two decades more and more evidence has accumulated supporting the view that intrinsically disordered proteins comprise a large fraction of eukaryotic proteomes. Although these proteins do not have a well-defined tertiary structure, they play key roles in essential processes such as cell-cycle control, regulation, signaling and apoptosis [1]. It was also observed that disordered proteins with disordered regions are often involved in cancer and other disease associated proteins [2]. Thus, it has been suggested that protein disorder entails a biological cost. To address this statement it is necessary to understand the associations between the structural properties of proteins and the mutations which are responsible for the development of disease.

The aim of my research was the detailed analysis of the frequency of cancer-associated point mutations and of polymorphisms in ordered and disordered protein segments and disordered binding regions by means of bioinformatics and statistical methods. The experimentally verified mutational data of cancer-associated proteins were taken from the Swiss-Prot database. In the statistical analysis of the distribution of mutations, both the random distribution and the distribution weighted by the observed polymorphisms were used as a reference.

The analysis of the polymorphisms showed that they are not evenly distributed in proteins and can be found more frequently in disordered regions. This points to the higher tolerance of disordered regions to mutations. The connection between cancer-associated mutations and the structural properties of proteins indicated a more complex picture. Contrary to the generally accepted view, in general, the cancer-associated mutations were more likely to fall into the ordered parts of the sequence than expected based on the random distribution, and this effect was even more significant when the uneven distribution of the polymorphisms was used as a reference. In addition, I presented a few examples that illustrated the connection between protein function and mutations in various ordered and disordered regions.

To summarize my findings, the presumption that protein disorder imposes a biological cost per se in the context of point mutations can be refuted. The results may contribute to the understanding of the role of protein disorder in cancer, which can be applied in a wide range of disciplines from basic research to rational drug design.

References

- 1 **Wright P E, Dyson H J**, *Intrinsically unstructured proteins: re-assessing the protein structure-function paradigm.*, *J. Mol. Biol.* **293** (1999), 321-31, DOI 10.1006/jmbi.1999.3110.
- 2 **Iakoucheva L M**, *Intrinsic disorder in cell-signaling and cancer-associated proteins.*, *J. Mol. Biol.* **323** (2002), 573-84, DOI 10.1016/S0022-2836(02)00969-5.

Enzyme catalysed kinetic resolution of *trans*-1,2-cyclohexanediol in a continuous high pressure reactor

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Diastereoselective azetidine synthesis

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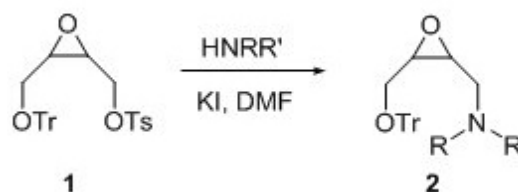
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The *trans*-1,2-cyclohexanediol (CHD) enantiomers and its derivatives are frequently used chiral building blocks during the synthesis of larger molecules. One possible way of the production of the optically pure enantiomers or enantiomerically pure derivatives of CHD is the enantioselective acylation of racemic CHD. In this reaction monoacetate and diacetate products are formed. *Candida Antartica* B lipase (CalB) enzyme catalyzes enantioselectively the formation of enantioselective *R,R*-diacetate. Therefore, by kinetic resolution, a mixture of *S,S*-monoacetate and *R,R*-diacetate can be synthesised in various solvents. Environmentally benefits of the supercritical carbon dioxide (scCO₂) extraction and the simple solvent - solute separation results in increasing number of applications of the supercritical solvents as reaction media as well, including the field of enzyme catalyzed reactions.

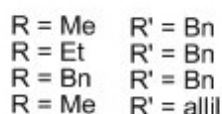
In earlier experiments the acylation of CHD was studied in a batch reactor in scCO₂ media. The reaction is fast, while the formation of monoacetate is only slightly enantioselective, in the second acylation step only *R,R*-diacetate is produced. Complete conversion was achieved at a pressure of 100 bar and temperature of 45 °C at a minimum of 10:1 acylation agent (vinyl acetate): CHD initial molar ratio. In the current work I implemented a continuous reactor, the depressurisation of the batch reactor was mostly responsible for the loss in enzyme activity, and in a continuous reactor the productivity might be increased significantly. A combined extractor – enzymatic reactor unit was developed. The scCO₂ dissolved the CHD in the extractor column and the flow was mixed with the vinyl acetate in a static mixer before entering the column filled with immobilised CALB. The optimised operation conditions of the batch reactor setup were applied in the continuous system. The aim of the study was to maximalise the productivity. By increase of CO₂ flow (and thereby reducing the residence time) the productivity increased linearly over a wide range, at optimal conditions with 500-fold compared to the best results obtained in the the batch reactor. With 13 seconds of residence time, 10:1 vinyl acetate: CHD molar ratio enantiopure products were produced. No loss of enzyme activity was observed during the 3-5 h long runs.

Nowdays, preparation of enantiopure compounds having two or more asymmetric centers is one among the most investigated fields of synthetic organic chemistry. Our group aimed to synthesise chiral trisubstituted azetidines starting from oxirane derivatives. The concept based on our earlier work where we elaborated 2,3 disubstituted oxirans to oxetans using lithium diisopropylamide activated by potassium *tert*-butoxide (LDA-KOR) [1].

The starting materials of the organometallic reaction were prepared by a multistep reaction sequence through oxirane **1**. Thus numerous new amino group containing oxirane derivatives (**2**) were synthesised.

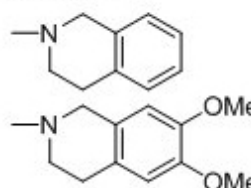


where

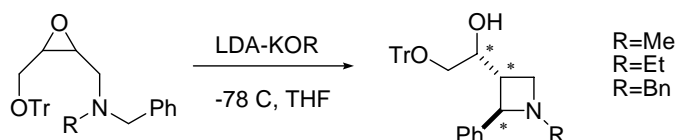


or

R-N-R' =



The superbases (LDA-KOR) induced rearrangements of compounds **2** resulted in the stereoselective formation of the desired *trans*-azetidine derivatives:



Steric arrangements of the ring substituents were confirmed in three cases (R = Me, Et, Bn) by two dimensional NMR measurements. In addition, *N*-Boc protected azetidine was prepared using the same superbases and an *N*-allyl azetidine derivative

was obtained by LiC-KOR induced rearrangement of the corresponding oxirane derivative.

References

- 1 Thurner A, Faigl F, Mordini A, Bigi A, Reginato G, Töke L, *A new base promoted rearrangement of (E)-1-benzyloxy-2,3-epoxyalkanes*, *Tetrahedron* **54** (1998), 11597-11602, DOI 10.1016/S0040-4020(98)00684-X.

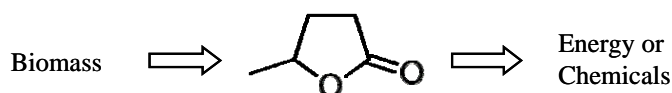
Kinetic study of the elementary reaction of OH radicals with γ -valerolactone in the gas phase using the fast discharge flow technique

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γ -valerolactone (GVL) is a naturally occurring C5 fruit lactone and a frequently used food additive. It can be produced with high efficiency from cellulose, as it has been demonstrated recently by a group of organic chemists at the ELTE University [1]. Its favourable physical and chemical properties make GVL a promising renewable source for the production of energy and for use as a basic material in the chemical industry to replace in a part fossil fuels and feedstocks.



Motivated by the scientific interest and potential practical significance of the subject, we have very recently launched a comprehensive research programme to study the photochemistry and reaction kinetics of GVL. As a first step, the rate coefficient of the reaction OH + GVL (1) has been determined. The OH-reaction plays an important role in the combustion of GVL, as it is the case for the combustion of all organics, and it is this reaction that determines the atmospheric lifetime of GVL and therefore its effect on the chemistry of the atmosphere.

Kinetics of the gas-phase elementary reaction between γ -valerolactone and OH have been studied by using the fast discharge flow technique, depletion of the concentration of OH radicals in the reaction has been detected by resonance-fluorescence (DF-RF). The OH radicals were produced by reacting H atoms with NO₂, H was obtained by microwave discharging of H₂. The reaction was performed under pseudo-first-order conditions in high excess of γ -valerolactone over hydroxyl radicals.

The following rate coefficient has been determined:
 $k_1(298\text{ K}) = (3.17 \pm 0.42) \times 10^{-12} \text{ cm}^3 \text{ molecule}^{-1} \text{ s}^{-1}$. To our knowledge, this is the first kinetic result on the reaction of

OH with GVL and even it is the first result on the reaction of OH radicals with a cyclic ester. k_1 is a high value and consequently the atmospheric lifetime of GVL is short, $\tau \approx 7$ days.

Acknowledgement

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References

- 1 Horváth I T, Mehdi H, Fábos V, Boda L, Mika T L, *γ -Valerolactone sustainable liquid for energy and carbon-based chemicals*, *Green Chemistry* **10** (2008), 238–242, DOI 10.1039/B712863K.