Chair of Organic Chemistry 2008-2012

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In organic chemistry the current research interests are clearly focused on investigations into organic reaction mechanisms and reactivity. These research topics have clear roots in traditions and history of organic chemistry in Tartu, and are supported by contemporary analytical technologies and computational facilities. These fundamental interests are efficiently complemented by application and development of various methods of organic synthesis and materials chemistry.

During the last decade an important part of this research has been focused on synthesis of bioactive compounds and study of principles of their molecular recognition by biological target sites, and their involvement in allosteric mechanisms of protein regulation. On the other hand, several other basic issues of physical organic chemistry have been considered, including investigations into reactivity at reaction centers, which are different from carbon atom. More recently, several new aspects of solvent effects have been explored in binary aqueous-solvent systems, keeping in mind possibility of application of water as the most “green” reaction medium in technologies, which use organic reactions. Computer modeling, first-hand based on different applications of molecular dynamics calculations, is supporting all the recent research activities.

And finally, some new areas of materials science have attracted our attention, mostly focused on chemical modification of existing materials for their technological applications. The following part of this survey was addressed to all these aspects of our research interests, and is accompanied with list of publications from years 2008 – 2012.

Biomimetics

Term “biomimetics” refers to human-made substances, materials and processes that imitate nature. Most often these compounds consist of fragments of natural compounds linked by non-natural synthetic chemical structures. These linking structures significantly modify stability and other properties of biomimetics, if compared with their natural analogs, and could be interesting chemical tools or precursors for more systematic development of biomaterials and drugs.

Firstly, we have been focused on development of peptides and their analogs, either modified at side groups or in backbone structure. One series of peptidomimetics was designed and synthesized proceeding from structure of glutathione (γ-L-glutamyl-L-cysteinyl-glycine), where the naturally occurring γ-L-glutamyl group was replaced by α-L-glutamyl residue and different amino acids were added to the N- and C termini. These derivatives of glutathione could be interesting as free radical scavengers and their activity was tested in several model systems and also in bioassays [1-3]. This part of research was carried out in cooperation with Prof. Ursel Soomets and his group at Medical Faculty of Tartu University.

Further, series of peptides with aza-amino acids were prepared and tested as substrates of protein kinase A. This enzyme was selected to model molecular recognition of aza-peptides by a protein binding site. Depending on the parent
compounds, we have investigated analogs of both \(\alpha\)-amino acids and \(\beta\)-amino acids. In aza-amino acids the nitrogen-carbon fragment \((N-C^{\alpha})\) of the peptide backbone was replaced by nitrogen-nitrogen fragment \((N-N)\), as shown below in the case of a short model peptide (left - natural amino acid; right aza-amino acid).

![Peptide backbone comparison](image)

This minor change in peptide backbone structure affects significantly chemical reactivity and conformational properties of peptidomimetic compounds, but still the aza-peptides can be phosphorylated by protein kinase A (Figure 1).

![Computer modeling of binding](image)

**Figure 1.** Computer modeling of binding of peptide substrate RRASVA (left panel) and peptidomimetic substrate aza-\(\beta\)-3-RRASVA (right panel) with ATP - protein kinase A complex. In both cases the serine OH-group is close to the \(\gamma\)-phosphate group of ATP and can be efficiently phosphorylated.

Due to changes in chemical stability aza-amino acids cannot be prepared as individual compounds, and for their introduction into structure of peptidomimetics stable precursors of these compounds should be used. On the other hand, if already included into structure of peptide analogs, the presence of the aza-amino function may significantly increase biological stability of these peptidomimetics. At the same time introduction of aza-group removes chirality center of the parent amino acid and the influence of this change on ligand recognition was addressed in the case of peptidomimetic compounds studied [4-9]. Parts of this synthetic project were obliquely supported by other studies in hydrazine chemistry [10-18].

Finally, series of analogues of peptide neurotransmitter galanin was designed and studied through different bioassays, and this work has permitted development of specific ligands for individual galanin receptor subtypes [19-21]. This project was carried out in our cooperation with Prof. Ülo Langel and his group at Stockholm University and in the Institute of Technology of Tartu University.
Another type of bioactive compounds studied belongs to group of neurotransmitter analogs and blockers, acting at various G-protein coupled receptors and neurotransmitter transporter proteins of CNS. For several practical reasons we have been interested in synthesis of ligands, which are in use as tracer molecules for positron emission tomography (PET) that is a relatively new and fast developing field of non-invasive diagnostics. This work has been largely made in cooperation with our partner company Pharmasynth AS in Tartu Science Park (www.pharmasynth.eu), where the list of available ligands exceeds 50 entries. Our students and members of the research team have investigated many aspects of synthesis of these compounds and studied their interaction with appropriate target sites. For example, the presence of slow isomerization step in mechanism of interaction of effective dopamine transporter blockers with their target protein was discovered. Further, using kinetic methods of analysis proved the presence of alternative binding sites for agonists and antagonists of this transporter protein and possible pharmacological and medicinal implications of this phenomenon were discussed.

In general, these studies have advanced our previous results, demonstrating the presence of slow “isomerization” step in interaction of effective ligands with their target proteins, which all share trans-membrane location, but possess completely different structure [22-30]. Proceeding from these results new possibilities emerge to control ligand-binding mechanism through variation of ligand structure. Some parts of this project were carried out in cooperation with Prof. Christer Halldin and his Department at Karolinska Institute in Stockholm.

**Synthetic chemistry of hydrazines**

Hydrazine is an interesting backbone for synthesis of biologically active compounds, ligands for catalysts and building blocks for materials science. New strategies and methods were developed for getting differently substituted hydrazine derivatives by using hydrazine polyanions and stepwise synthesis strategy. Recently synthesis of heterocyclic compounds containing hydrazine moiety was successfully demonstrated by using ring-closing metathesis reactions and Pd-catalysts. Some prototypical chiral hydrazine ligands for Pd-catalysis were designed and successfully used in practice for synthesis of polymerizable ionic liquids and for linking of hydrazine derivatives to glasses, metal oxides and electrodes using sol-gel technology [10-18].

**Reactions at silicon atom**

Although correlation analysis has proved to be an efficient and useful tool for unraveling reaction mechanisms and prediction of rate or equilibrium constants in organic chemistry, overwhelming majority of these studies has been made for reactions occurring at carbon atom, and only in a few cases reactions at other atoms of the second and third period have been involved. Therefore we have started investigation into substituent effects in organosilicon chemistry, keeping in mind that importance of this element has significantly increased in different technologies. This analysis revealed that the classical steric parameters were not applicable and the classical resonance effect was insignificant in nucleophilic substitution reactions at the silicon reaction center. Moreover, the inductive effect of substituents at the silicon atom was expressed by complex parameters, which involve contribution of
electronegativity. In parallel with the theoretical analysis, also practical investigation of silicone reactivity was continued involving kinetic study of the Grignard reaction with silanes [31-35]. The practical outcome of this project may be significant for development of several aspects of materials science, as was shown by

**Organic reactions in aqueous binary solvents**

The analysis of solvent effects in water–solvent binary mixtures has been intertwined with somewhat earlier studies of ultrasound effect on rate of various organic reactions in water-alcohol mixtures. These studies revealed that ultrasound may affect also ionic reactions and depending on character of the reactants may either accelerate or inhibit these processes. These facts were by no means understandable in terms of the generally accepted cavitation theory of ultrasonic effects, and clearly pointed to necessity of some more general explanation of these phenomena [36-42]. Proceeding from structure-reactivity relationships in sonication effects, hypothesis was advanced that these phenomena could be connected with influence of ultrasound on hydrophobic solvation of reacting molecules in water-solvent systems. This hypothesis was confirmed by a more wide analysis of binary solvent effects in more than 100 organic reactions, investigated at wide concentration range of various binary solvents [38]. Significant part of experimental data for this comprehensive analysis was compiled from the “Tables of Rate and Equilibrium Constants of Heterolytic Organic Reactions” (V.Palm, Ed., volumes 1-5).

More recently the solvent effects were modelled by using the molecular dynamic simulations, where behaviour of reagent, molecules of organic solvent and water are analyzed at various temperatures [36]. The presence of preferential solvation of ethyl acetate with acetonitrile molecules in binary mixture of this solvent with water was illustrated in **Figure 2**.

**Figure 2.** MD simulation of ethyl acetate molecule in water (left panel) and in water-ethanol binary mixture at 40 wt% (center). These calculations revealed significant preferential solvation of the reagent by organic solvent at 300°K, demonstrated by distribution curve of ethanol molecules (right panel, solid line) and water molecules (right panel, dotted curve) around the ethyl acetate molecule. Data were taken from Ref 36.
**Allosteric phenomena in ligand binding with proteins**

Analysis of molecular recognition mechanisms in ligand interaction with their target sites has always been used as efficient tool for bioactivity prediction. However, most recent developments in this field have demonstrated that binding effectiveness can be modulated by the presence of other ligands, which bind with the protein molecule in distinct binding site, and therefore the phenomenon is named as allostery. The simplest allosteric model can be interaction of protein kinase with its two substrates: the phosphorylatable peptide substrate and ATP as phosphate group donor. Thorough kinetic analysis of peptide phosphorylation reaction by protein kinase A allowed quantitative description of this allosteric system, and further the same principles were extended to interaction of different inhibitors with the enzyme [43-48]. Straightforward correlation was found between substrate binding effectiveness and the allosteric effect, and the principle “better binding – stronger allostery” was formulated. Currently we are able to describe the allosteric phenomena by using computer modeling of ligand docking with the enzyme.

Combining kinetic measurements with protein engineering several mutants of L-type pyruvate kinase were studied to determine the role of protein phosphorylation in regulation of activity of the enzyme. These experiments demonstrated that protein phosphorylation was a molecular switch of cooperativity of the enzyme, while activity of the non-phosphorylated enzyme was not cooperatively regulated by phosphoenolate concentration. These studies were also supported by computational modeling of ligand docking with the native enzyme and its mutants [49-55].

The main conclusion from these experiments demonstrated that ligand interaction with its biological target site cannot be discussed without taking into consideration possible allosteric modulation effects, excreted by other external or internal components of the system.

**Study of materials**

This part of our recent activity has been focused on two different topics. Firstly, possibilities of chemical modification of polyvinylidene fluoride surface were made, to allow stable coloring of this chemically inert and “non-sticky” material [56]. The surface of polyvinylidene spheres was activated and regularly covered with polystyrene brushes, as illustrated in Figure 3.

Secondly, sol-gel technology has been used as a unique low temperature method for preparation of different ceramic materials and composites. The size and structure of these ceramic materials was studied and conditions for preparation of nanoparticles, micro- and nano-fibers and thin films were worked out, including metal oxide micro and nano-rolls. The nano-colloidal SnO$_2$ water-sol was prepared as a very perspective material for surface chemistry. This work was done in collaboration with Institute of Physics and Institute of Technology of Tartu University and Tallinn University of Technology [57-59].
Figure 3. SEM images of surface of polyvinylidene particles that was chemically activated and thereafter modified through styrene polymerization. The polystyrene “brushes” formed on the surface were shown at different magnification. Data were taken from Ref 60.

Figure 4. Comparison of chemically treated wood samples (above) and not treated sample (below). Both samples were exposed to outdoor weather conditions during 5 years.

Thirdly, systematic study has been made for chemical modification of wood, aiming to increase stability of this material in outdoor conditions and preventing its decomposition by microbes and fungi [60]. These innovative formulations for wood protection, which are not based on application of metals and poisonous chemicals, are developed in cooperation with laboratory of Dr. Nasko Terziev in Swedish
University of Agricultural Sciences, Uppsala. More details about this promising technology will be available after solution of IP issues, connected with this R&D project. Samples of the chemically treated (above) and not treated (below) pieces of wood are compared in Figure 4. This new wood protection technology seems to be very promising for prolongation of life-time of wooden constructions in outdoor conditions, while the materials used for protection have no negative impact on nature after destruction of the material.

Some current projects
Staff of the Chair of Organic Chemistry is involved in implementation of one Estonian Target Financing Project (PI J.Järv), several Estonian Science Foundation Grants (PIs S.Salmar, D.Panov), FP7 project 2011-286933-E-SIGNAGE (subcontract, PI J.Järv) and Research Infrastructure Upgrading Project TAP30-8.

References


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