

NEW MOLECULAR SYSTEMS FOR ADVANCED ENVIRONMENT-FRIENDLY APPLICATIONS BENEFICIAL FOR HUMAN HEALTH

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1. Introduction

On October 23, 2008, a conference of PhD students of chemistry departments of Faculty of science of Charles University in Prague took place. It was focused on the participation of PhD students in the research program “New molecular systems for advanced environment-friendly applications beneficial for human health” (funded by the Ministry of Education, Youth and Sports of the Czech Republic, project MSM 0021620857) and it was organized in cooperation with the Division of Analytical Chemistry of the Czech Chemical Society. High quality of contributions presented in English language, friendly atmosphere and usefulness of this conference as a suitable model for similar conferences promoting the participation of young generation on activities of Czech Chemical Society prompted us to prepare this paper summarizing the results of participants in this interesting and promising field.

2. The Aim of the Project

The contemporary world trends in chemical research emphasize chemical aspects of the sound development of human society and the environment. The natural limits for successful development are stability of the ecosystem on one hand and the exhausting of both unrecoverable and

recoverable resources of raw materials and energy on the other hand. Specific complex tasks in this area are aimed at sustainability. The most important challenges are: (a) design and elaboration of non-waste technologies and complex processes that secure total recycling of wastes and by-products, (b) design and development of new “intelligent” (stimuli-responsive) materials that can simultaneously fulfill several functions and meet conditions for minimization and enhanced efficiency of technical devices and consumer goods, (c) implementation of new means for treatment of serious civilization diseases (efficient side-effects-free drugs and other medical materials) and means for alleviating consequences of natural and human-caused catastrophes and for prevention and suppression of epidemics and (d) satisfaction of basic human needs for nourishment, health care and hygiene. The above mentioned research plan “New molecular systems for advanced environment-friendly applications beneficial for human health” is focused on the first two areas, but it concerns and partially implements all the above items. It is a multidisciplinary, however compact project of closely related research activities, which form a logical sequence of steps of any purposeful chemical research: (a) elaboration of environment-friendly methods for preparation of new compounds and molecular systems for advanced applications and technologies beneficial for human health and life, (b) design, development and optimization of methods for their analysis and characterization and (c) study of the structure, dynamics and function of molecular systems. The following examples can demonstrate these approaches and their results. The full versions of presentations in MS PowerPoint are available on web pages quoted in references.

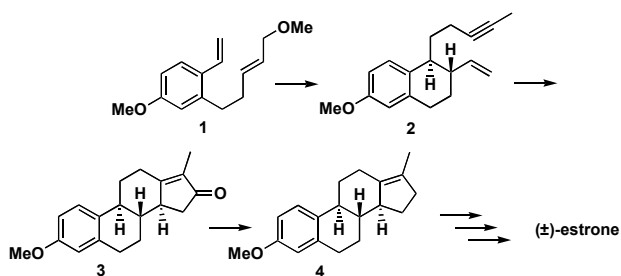
3. Overview of the Results

The following selection of presented results of PhD students are based on abstracts of those presentations. Individual paragraphs and their titles correspond to individual presentations available on web pages of our journal. The great variety of addressed problems within this project is reflected in great variety of the following paragraphs. We are fully aware that this is rather unusual paper but we are convinced it can be useful as a model for future activities of young members of the Czech Chemical Society.

3.1. New Formal Total Synthesis of Estrone

Efficient total synthesis of natural compounds is one of the goals of organic synthesis. One of the aims of the above mentioned project is development of a new synthesis of *ent*-derivatives of steroids based on transition metal reactions. This approach was used for preparation of de-

rivatives of 16-ketoestrone based on the repetitive cyclization of α,ω -dienes with Cp_2ZrBu_2 , followed by reactions with allylhalogenides. A similar principle was used also for formal total synthesis of estrone. However, because of synthetic problems in certain steps, it was necessary to modify this approach by using other reactions. The new strategy described in the contribution of Robert Betik and Martin Kotora¹ is based on cyclization of diene **1** with Cp_2ZrBu_2 followed by CuCl-catalyzed reaction with 2-bromobuta-2,3-diene leading to one step formation of enyne **2**. Crucial step, in which C and D steroid ring is formed, is $\text{Co}_2(\text{CO})_8$ -mediated Pauson-Khand reaction of enyne **2**, which yields almost quantitatively 16-keto-17-methyl estratetraene derivative **3**. Finally, the reduction of the keto group gave known intermediate **4** that can be converted into estrone in 2 steps. The overall synthesis of intermediate **4** was accomplished in 7 steps from commercially available material.



3.2. Fluorene Based Copolymers with Electron Transporting Moieties and Enhanced Stability

This contribution² was devoted to fluorene based polymers and copolymers, which represent promising group of blue emitting materials, due to their high fluorescence efficiency, comparatively high chemical stability of polymer backbone, facile functionalization of fluorene at C-9 position and high quantum yields also in the solid state. Nevertheless, fluorene based polymers and copolymers have some disadvantages. They suffer from undesired red shifted emission, which is caused by formation of keto defects and/or excimer formation. This disadvantage could be overcome by two strategies. a) by using 9,9-diaryl substituted fluorenes, b) by copolymerization with stabilizing co-monomers. One of the monomers with stabilizing effect is anthracene³. Oxadiazole as an electron transporting group is also electron withdrawing and should decrease LUMO level and increase HOMO level of the polymer⁴. Polyfluorene was found to have LUMO and HOMO level 2.12 eV and 5.8 eV, respectively. Value for electron injection (2.12 eV) is comparatively distant from the value of applicable cathodes. Incorporation of electron withdrawing group will shift both the energy levels but practically do not affect the energy gap and thus the blue emitting properties are preserved. Bondarev et al.² synthesized and characterized series of fluorene based copolymers. Enhanced thermal stability of materials was

achieved by replacing alkyl side chains with aryl ones or introduction of anthracene units into the main chain. All the polymer samples are soluble and processable materials with strong fluorescence in blue to cyan blue region.

3.3. Voltammetric and Amperometric Determination of Selected Agrochemicals on Amalgam Electrodes

The environmentally-friendly working electrodes based on silver solid amalgam can successfully substitute hanging mercury drop electrode (HMDE). The wide potential window comparable with HMDE, practically non-toxic electrode material, good mechanical stability, simple handling and regeneration are main advantages of silver solid amalgam electrodes (AgSAEs). The AgSAE can be used both in voltammetric batch methods and in flow systems for amperometric detection (HPLC, FIA or CZE). Daňhel et al.⁵ designed, constructed and employed AgSAE for a thin-layer flow cell and wall-jet detector for HPLC. This newly designed HPLC-ED system was used for the determination of 2-nitrophenol, 4-nitrophenol, 2,4-nitrophenol, 2-methoxy-5-nitrophenol (or more precisely their sodium salts) contained in mixture in the world-wide applied plant growth regulators (for example Atonic, Chaperone, N-fenol MIX). The optimal conditions of the separation by RP-HPLC at a column Nova-Pack C18 (150 × 3.9 mm) and of the detection by the flow cell based on AgSAE working electrode were found [mobile phase: 0.05M phosphate buffer pH 6.0 in mixture with methanol (7:3, v/v), flow rate 1.3 mL min⁻¹, respective 1.0 mL min⁻¹ (using wall-jet detector) and working electrode potential -1.0 V vs. Ag/AgCl (resp. -1.5 V)]. Under these conditions, the above given nitrophenols can be separated in 10 min and determined in concentration range 5–2500 μmol L⁻¹. Wide negative potential range, simple preparation and easy electrochemical pretreatment are main advantages of AgSAE. Relatively low limits of detection of reducible organic compounds as well as its low price are to be mentioned as well.

The silver solid amalgam paste electrode (AgSA-PE) is similar to carbon paste electrode with the advantage of wider negative potential window and possibility to renew the electrode surface which solves the problem in the case of passivation of electrode surface. The pastes can be made from fine powder of the silver solid amalgam mixed with a suitable organic pasting liquid. These pastes can be stuffed into a Teflon body of the electrode and there is a possibility to easily renew the electrode surface by pushing the paste out and wiping off the surface of the paste. In combination with an electrochemical pretreatment of the electrode, it provides a good stability and reproducibility of voltammetric signals. Various types of silver amalgams with various pasting liquids were successfully tested and applied for the determination of trace amounts of 4-nitrophenol as a model electrochemically reducible environmental pollutant⁵.

3.4. Novel Materials for Second Harmonic Generation

Systematic search for new materials exhibiting non-linear optical properties (e.g. second harmonic generation, SHG) and desirable chemical and physical properties is an important research goal in non-linear optics. These materials serve as optical elements in devices for optical signal processing, optical communication and data processing and storage. Very promising is the group of salts of polarizable organic cations with delocalized π -electrons. Fridrichová et al.⁶ paid attention to materials in which the cationic part, mainly responsible for the non-linear optical properties of the material, is a guanidine derivative (scale of derivatives from guanylurea to substituted phenylbiguanides), connected with the anionic part by hydrogen bonds. To be capable of non-linear optical properties, the crystal structure of the material must be assembled non-centrosymmetrically. The anionic part can be inorganic or organic. Generally, the inorganic anion can notably contribute to favorable chemical, mechanical and thermal properties and the organic anion, if chiral, can even prevent the centrosymmetric structure assembly at all. The authors prepared 18 new guanidine derivative salts and characterized them (if possible, also X-Ray structures were determined); the SHG efficiency was measured by the Kurtz and Perry powder method. Four of compounds prepared exhibited high efficiency of SHG. One of these four promising materials proved, due to its easy crystallization and its chemical and physical stability, as suitable for further testing for potential applications. Full assignment of vibrational spectra of this interesting material (employing quantum-chemical computational methods) and more detailed study of SHG efficiency of powdered samples with different particle size (phase matching) were performed. The systematic „scanning“ of groups of novel materials with detailed study focused on interesting ones is an effective method of this branch of materials research.

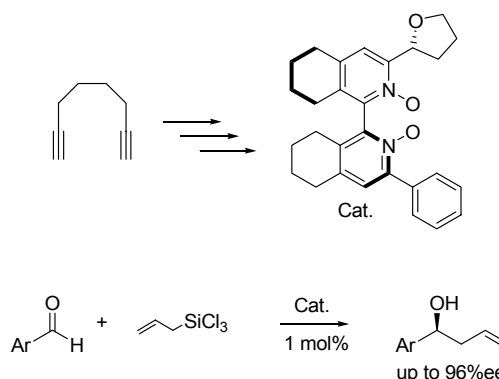
3.5. Thermal Effects in Electrophoresis

All electrophoretic techniques are driven by electric field that inevitably generates Joule heating, which causes an increase of temperature in a capillary. A magnitude of the temperature increase is a result of amount of generated heat, which is driven by applied power, and efficiency of the cooling. In practice CE instruments always have a certain part of the capillary, which is out of the cooled space (10–60 % of length). It implies that axial distribution of temperature along capillary is heterogeneous, which can complicate evaluation of experimental data by creation of one or more disturbing effects (e.g. oscillations of concentrations or current instability in time). The change of the temperature has a strong influence on electric conductivity of a solution (increase around 1.5–2.5 % per 1 °C) therefore it is a key quantity for examination of temperature changes and the efficiency of the cooling system. Since

high speed of analysis is usually required for routine applications the applied electric field is high (high voltage and a short length of the capillary) and therefore the temperature increase can be significant (even several tens of °C). Hruška and Gaš⁷ derived a set of fitting functions for experimental plots of conductivity vs. voltage, current or power for characterization of actual cooling efficiency of a given instrument. This provides numerical information about the efficiency of cooling and the conductivity of an electrolyte (BGE) free of Joule heating. These data are input parameters for electrophoretic simulations of thermal effects in a modified version program Simul 5, which has been developed in Gaš laboratory. It is a numerical simulator of electrophoretic processes based on a set of continuity equations for each constituent in the system. Such modification enables dynamic simulation of two or more differently cooled parts of the capillary and it visualizes changes in the temperature profile and concentration profiles. Program also calculates actual electric current and the profile of the intensity of the electric field.

3.6. New Bipyridine *N,N'*-Dioxides as a Catalyst in Allylation of Aldehydes

Bipyridine *N,N'*-dioxides serve as very useful catalysts in enantioselective allylations of substituted aromatic aldehydes⁸. Kadlčíková a Kotora⁹ developed a short and attractive route for the preparation of new unsymmetric bipyridine *N,N'*-dioxides starting from 1,7-octadiyne. The bipyridine framework built-up is based on „one-pot“ cyclootrimerization of a tetrayne with benzonitrile and chiral tetrahydrofurancarbonitrile catalyzed by $\text{CpCo}(\text{CO})_2$ under microwave irradiation^{10,11}. The obtained bipyridine was oxidized with *m*-CPBA affording two diastereomeric bipyridine *N,N'*-dioxides that were readily separated by a simple column chromatography on alumina. These new chiral bipyridine *N,N'*-dioxides appeared to be very efficient catalyst (1 mol.%) in enantioselective allylation of variously substituted benzaldehydes. *Ee*'s of the obtained homoallyl alcohols were up to 96 %.



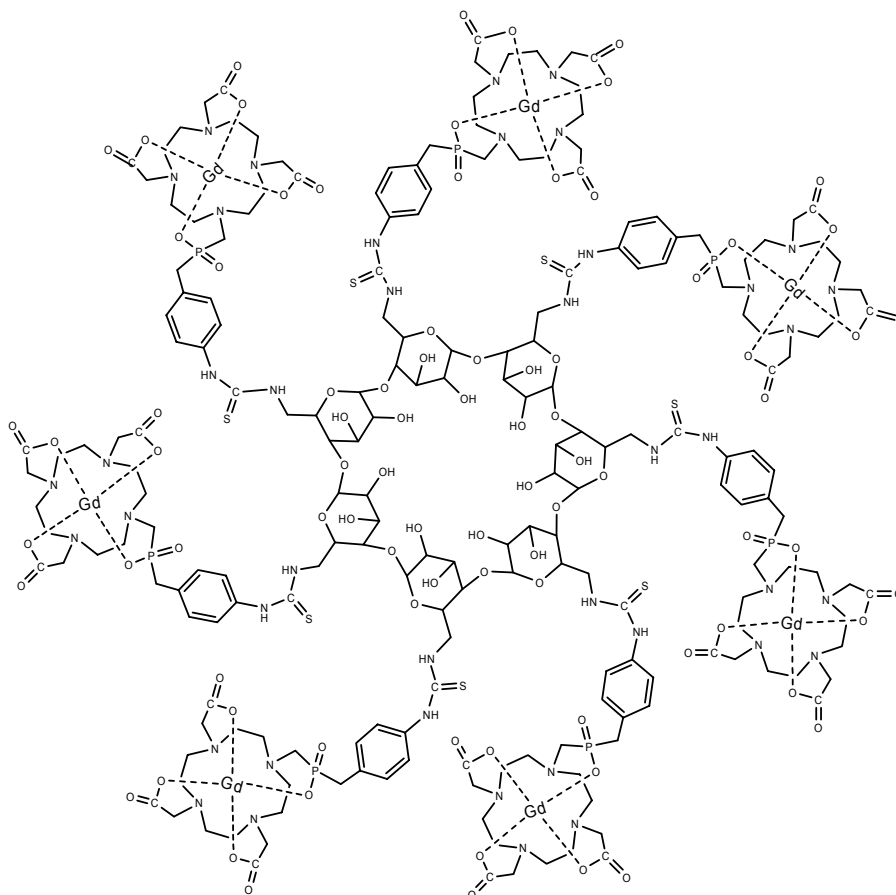
3.7. Magnetic Resonance Imaging Contrast Agents Based on Cyclodextrins

Over the last two decades, magnetic resonance imaging (MRI) has grown into one of the most useful diagnostic method in medicine. The quality of the images is increased by using of contrast agents (CA) which are based on Gd(III) complexes of polydentate ligands. As the efficiency of clinically used CA, expressed by its relaxivity, is very low, there is a continuous interest in the development of more efficient contrast agents. The relaxivity of CA is governed by several parameters, mainly residence time of water molecule coordinated to Gd(III) ion (τ_M), and rotational correlation time of the molecule (τ_R). Kotková, Kotek and Herman¹² have paid attention to the design of complexes allowing a simultaneous optimization of both these parameters. Optimal τ_M was guaranteed by using of macrocyclic ligand with one phosphorus acid pendant arm, 1,4,7,10-tetraazacyclododecane-4,7,10-triacetic-1-{methyl [(4-aminophenyl)-methyl]phosphonic acid}) and optimal τ_R by conjugation of the 6 (7 or 8) ligand molecules to a rigid per-6-amino- α (β or γ)-cyclodextrin core through a thiourea bridge. These conjugates were characterised by size exclusion HPLC and ¹H and ³¹P NMR spectroscopy. Structures of the conjugates were estimated by means of NMR spectroscopy and molecular modelling in Hy-

perchem. The Y(III), resp. La(III) complexes of the conjugates were characterized by MS, and Eu(III) complexes by NMR spectroscopy and luminescence measurements. Relaxometric properties were tested on Gd(III) complexes. It was confirmed that the parameters τ_M and τ_R are in optimal ranges which results in unexpectedly high relaxivity and, therefore, a high efficiency of these compounds as potential CAs. The presence of several efficiently relaxing Gd(III) centers within one molecule of a moderate size leads also to a high relaxivity confined to a small molecular space (high density of relaxivity). This is useful especially in the field of cell imaging. In spite of the high charge of the complexes, these agents are not cytotoxic and are easily internalized in cells.

3.8. Cyclodextrin- and Cellulose-based Chiral Stationary Phases for Enantioseparation of Substituted Binaphthyls

Loukotková et al.¹³ studied retention and enantioseparation behavior of 2,2'-disubstituted or 3,2,2'-trisubstituted 1,1'-binaphthyls and 8,3'-disubstituted 1,2'-binaphthyls (10 analytes in total) on two types of chiral stationary phases (CSP): (i) CSPs based on β -cyclodextrin (β -CD) and hydroxypropylether- β -cyclodextrin (HP- β -CD), and



(ii) *tris*(3,5-dimethylphenylcarbamate) cellulose-based CSP. The effects of the mobile phase composition in normal- (NP) and reversed-phase (RP) separation modes were investigated.

The NP mobile phases contained *n*-hexane and propane-2-ol at various volume ratios on both studied types of CSPs. Reversed-phase separation mode was created by mixing of methanol (MeOH) and water or 0.5% triethylamine acetate buffer (TEAA), pH 3.0 and 6.0, on CD-based CSPs. On cellulose-based CSP the RP mobile phases were composed of acetonitrile (ACN) and water or 20 mM phosphate buffer of pH 3.0 or 6.0. The RP separation mode has been found more suitable for enantioresolution of the majority of analytes. (No enantioseparation was observed on CD CSP while NP was employed). With the respect to the enantioseparation, better results (better peak symmetry, higher resolution values) were obtained on the HP- β -CD CSP with MeOH/water mobile phases containing lower portion of the organic modifier (10–40 % of MeOH). Three analytes were partially enantioseparated in MeOH/water 30/70 (v/v) mobile phase on CD CSPs. On *tris*(3,5-dimethylphenylcarbamate) cellulose-based CSP, lower content of acetonitrile (40 vol. %) in the ACN/water mobile phase provided sufficient retention of the binaphthyl derivatives to enable enantioseparation of the majority of these analytes with resolution values higher than 1.2. Application of phosphate buffer instead of water also influenced the retention and slightly chiral separation. The best enantioseparation of the bulk of analytes (7 from total number of 10) was observed in the mobile phase composed of ACN/20 mM phosphate buffer, pH 3.0, 40/60 (v/v).

Cellulose-based CSPs are in comparison with cyclodextrin-based ones much more convenient for enantioseparation of the set of studied analytes either in normal-, or in reversed-phase separation mode. Even semi-preparative separation mode could be employed on the cellulose-based CSP. Cyclodextrin-based columns could be useful for separations of mixtures of binaphthyls formed during the process of synthesis in reaction mixture.

3.9. 14-3-3 Protein Masks the DNA Binding Interface of Forkhead Transcription Factor Foxo4

This topic was investigated by Šilhán and co-workers¹⁴. The forkhead family of transcription factors shares a highly conserved 100-amino-acid large DNA binding (DBD) Forkhead box domain. These proteins display large functional diversity and play a wide range of roles in a number of physiological and pathological processes. Among the forkhead family, the FOXO class consists of four members that play a central role in cell-cycle control, differentiation, metabolism control, stress response and apoptosis¹⁵. Transcriptional activity of FOXO proteins is regulated through insulin-PI3K-AKT/PKB signaling pathway. The AKT/PKB-dependent phosphorylation of FOXO proteins generates two 14-3-3 binding sites.

These two motifs border the DBD, raising the possibility that the 14-3-3 proteins could participate in the disruption of FOXO binding to DNA. Such 14-3-3 protein-dependent inhibition of DNA binding has been suggested for DAF-16 (*C. elegans* FOXO homologue) and FOXO4 (ref.¹⁶). However, the exact mechanism of this 14-3-3-dependent inhibition of DNA binding is still unclear. Since the second AKT/PKB motif is embedded in the C-terminal part of forkhead DBD the 14-3-3 protein could interfere with the binding of this region to the DNA, mask other parts of FOXO DNA-binding interface or change the conformation of forkhead domain core. To better understand the mechanism of 14-3-3-dependent regulation of FOXO function, Šilhán et al.¹⁴ investigated which regions of forkhead DBD of FOXO4 transcription factor, apart from the C-terminal phosphorylated motif, physically interact with 14-3-3 protein. Four single cysteine-containing mutants of FOXO4 (human FOXO4, sequence 11-213) suitable for site-specific labeling of DBD with thiol-reactive fluorescence dye 1,5-IAEDANS were prepared. To map the interaction between the 14-3-3 protein and FOXO4-DBD, the time-resolved fluorescence intensity and anisotropy decay measurements of all four AEDANS-labeled Cys mutants of FOXO4 have been performed. It has been found that the mean lifetime $\bar{\tau}$ of all phosphorylated FOXO4 mutants significantly increases upon binding of the 14-3-3 protein. The authors suggest that the observed increase in $\bar{\tau}$ is a binding-induced decrease of polarity around the AEDANS moieties. The effects of 14-3-3 binding on segmental dynamics of the AEDANS-labeled FOXO4 mutants was also examined and it was found that 14-3-3 binding to any of the FOXO4 changes mode of AEDANS motion. Fluorescence anisotropy experiments strongly indicate that interaction of FOXO4 with 14-3-3 causes restriction of the AEDANS motion at all tested locations. The effects are likely caused by combination of the sterical hindrance and the binding-induced decrease of the protein segmental mobility. In conclusion, presented data indicate that 14-3-3 protein masks the DNA-binding interface of FOXO4 DBD.

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