PERIODICA POLYTECHNICA SER. CHEM. ENG. VOL. 48, NO. 2, PP. 97–115 (2004)

ABSTRACTS OF RECENT PHD THESES

2004

ABSTRACTS

ENZYMES IN THE CELLULOSE BIOLOGICAL UTILIZATION: β-GLUCOSIDASE AND PHOSHPOGLICERATE KINASE

Beáta FLACHNER

Supervisor: Katalin Réczey
Department of Agricultural Chemical Technology

Cellulose, the most abundant biomass on the earth, is a polymer of glucose with β-1,4 linkage. Because it is reproduced by photosynthesis, its importance increased recently in the context of sustainable growth. Constructing and furniture industry use cellulose in its original form together with lignin and hemicellulose. Textile and paper industries utilize it also in polymer structure, but separated from the accompanying polymers. Decomposition of cellulose into monomeric sugar has increasing importance, as glucose is a carbon source for many industrial processes. Several enzymes cooperate in the enzymatic degradation of cellulose, from which two were studied in the present thesis. β-glucosidase, which breaks down cellobiose and 3-phosphoglicerate kinase (PGK), which is one of the key enzymes of glucose utilization.

I had two distinct goals in the thesis, reflecting two different aspects of two different laboratories: Department of Agricultural Chemical Technology, Budapest University of Technology and Economics and Institute of Enzymology, BRC, Hungarian Academy of Sciences. I have studied two different enzymes both playing role in carbohydrate metabolism and degradation.

In my PhD thesis β-glucosidase production and characterization were performed in both soluble and immobilized form. In these experiments crude fermentation supernatants and microbial pellets were used. With these experiments we intended to provide results for applied enzymology and industrial application. Four Aspergillus strains were studied for β-glucosidase fermentation. The fermentation supernatants were examined to describe the kinetical behavior of the non-purified enzyme and were found suitable for enzyme supplementation of enzymatic hydrolysis of cellulose. Stabilization of *Aspergillus phoenicis* QM 329 pellets was

performed with glutaraldehyde according to the experimental design and the optimal treatment (1.2% (v/v) glutaraldehyde concentration and the 24 h stabilization time) was determined. In consecutive cellobiose hydrolysis series I managed to improve the operational stability of optimally treated pellets.

I have also studied the structure-function relationship of 3-Phosphoglicerate kinase (PGK), one of the key enzymes of glucose utilization. My research with PGK deals with the problems of domain closure, the nucleotide binding mode and the role of the metal ion, i.e. the questions of general interest for kinases. I have found that domain motion and substrate antagonism, characteristic properties of PGK are closely related and can be explained by a conformational change through the interdomain region from 3-PG-site to nucleotide site. This process may be the main domain closure and the catalysis and it leads to the weakening of Mg^{2+}-mediated link of the nucleotide phosphates. My experiments supported the different binding modes of ATP and MgATP, derived from the crystal structures of the respective binary complexes. Thus, the metal ion is essential in formation of the substrate-like interaction with the nucleotide. The phosphate chains of MgAMP-PCP and MgAMP-PNP, analogues of MgATP, bind two different sites on the enzyme. I assume, that during the catalytic cycle the phosphate chain of MGATP fluctuates between these two alternative sites and thereby regulates the operation of the molecular hinge and assist in domain closure.

References

[1] FLACHNER, B. – RÉCZEY, K., β-Glucosidase Production and Characterization of Some *Aspergillus* Strains, Subbmitted to *Chemical and Biochemical Engineering Quarterly* in September 2003.

[2] FLACHNER, B. – BRUMBAUER, A. – RÉCZEY, K., Stabilization of β-Glucosidase in Aspergillus Phoenicis QM 329 Pellets, *Enzyme and Microbial Technology*, **24** (1999), pp. 362–369.

[3] MERLI, A. – SZILÁGYI, A. N. – FLACHNER, B. – ROSSI, G. L. – VAS, M., Nucleotide Binding to Pig Muscle 3-Phosphoglycerate Kinase in the Crystal and in Solution: Relationship Between Substrate Antagonism and Interdomain Communication, *Biochemistry*, **41** (2002), pp. 111–119.

[4] KOVÁRI, Z. – FLACHNER, B. – NÁRAY-SZABÓ, G. – VAS, M., Crystallographic and Thiol-Reactivity Studies on the Complex of Pig Muscle Phosphoglycerate Kinase with ATP and Analogues: Correlation Between Nucleotide Binding Mode and Helix Flexibility, *Biochemistry* **41** (2002), pp. 8796–8806.

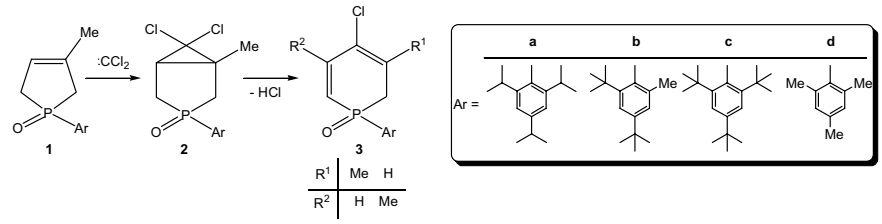
[5] FLACHNER, B. – KOVÁRI, Z. – VARGA, A. – GUGOLYA, Z. – VONDERVISZT, F. – NÁRAY-SZABÓ, G. – VAS, M., Mg^{2+}-Induced Differences in the Interactions of ATP and ADP Phosphates with 3-Phosphoglycerate Kinase: Solution Binding Data and New Crystallographic Structures with ATP and MgATP, Submitted to *Biochemistry* in June 2003.

REACTION OF HETEROCYCLIC TRIALKYLPHENYLPHOSPHINE OXIDES AND DIMETHYL ACETYLENEDICARBOXYLATE

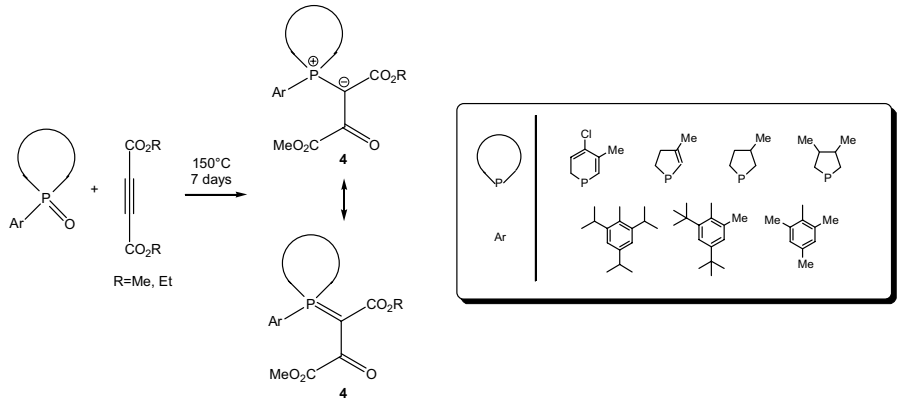
Henrietta FORINTOS

Supervisor: György Keglevich
Department of Organic Chemical Technology

We synthetised 5- (*1-2*) and 6-membered (*3*) *P*-heterocycles with sterically demanding substituents as tri-*iso*propylphenyl (**a**), di-*tert*-butyl-methylphenyl (*b*), tri-*tert*-butylphenyl (*c*), and trimethylphenyl (*d*) on the phosphorus atom.

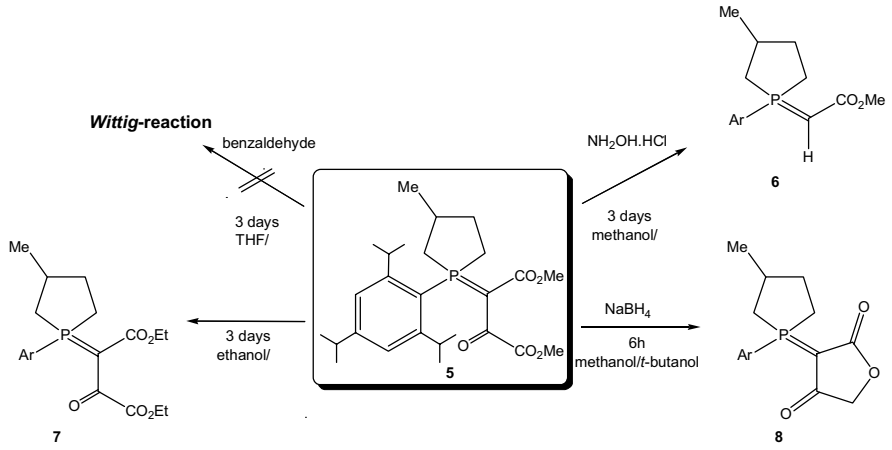


We found that the reaction of dihydrophosphinine oxides and DMAD at 150°C (in a bomb) lead to phosphonium ylide (*4*). The reaction is of general value and was extended to 5- and 6-membered cyclic phosphine oxides with different trialkylphenyl substituents on the phosphorus atom.



The strong delocalization has an effect on the reactivity of the stabilized phosphonium ylide (*5*) in Witttig reaction thus, (*5*) would not enter into reaction with benzaldehyde. Aiming at the synthesis of the corresponding oxime, (*5*) was reacted with hydroxylamine hydrochloride. The result of the reaction was, however,

another phosphorane/ylide (*6*). The reaction may have been the consequence of the attack of hydroxylamine on the β-keto group, or took place to the effect of hydrochloric acid. Phosphonium ylide (*5*) was subjected to reduction by an excess of sodium borohydride. To our surprise, a (phospholane-1-ylidene)-furan-2,4-dion (*8*) could be isolated from the reaction mixture.



References

[1] KEGLEVICH, GY. – KESERŰ, GY. M. – FORINTOS, H. – SZÖLLŐSY, Á. – LUDÁNYI, K. – TŐKE, L., *J. Chem. Soc., Perkin Trans.*, **1** (1999), p. 1801; *Magyar Kémiai Folyóirat*, **107** No. 12 (2001), p. 544.

[2] KEGLEVICH, GY. – FORINTOS, H. – SZÖLLŐSY, Á. – TŐKE, L., *Chem. Commun*., (1999), p. 1423.

[3] KOLLÁR, L. – BERENTE, Z. – FORINTOS, H. – KEGLEVICH, GY., *Tetrahedron Asym*., **11** (2000), p. 4433.

[4] KEGLEVICH, GY. – FORINTOS, H. – KESERŰ, GY. M. – HEGEDŰS, L. – TŐKE, L., *Tetrahedron*, **56** (2000), p. 4823.

[5] KEGLEVICH, GY. – FORINTOS, H. – SIPOS, M. – DOBÓ, A. – LUDÁNYI, K. – VÉKEY, K. – TUNGLER, A. – TŐKE, L., *Heteroatom Chem.*, **12** No. 6 (2001), p. 528.

[6] KEGLEVICH, GY. – KÖRTVÉLYESI, T. – FORINTOS, H. – TAMÁS, A. – LUDÁNYI, K. – IZVEKOV, V. – TŐKE, L., *Tetrahedron Letters*, **42** (2001), p. 4417.

[7] KEGLEVICH, GY. – FORINTOS, H. – KÖRTVÉLYESI, T. – TŐKE, L., *J. Chem. Soc. Perkin Trans. 1*., **26** (2002).

[8] KEGLEVICH, GY. – KÖRTVÉLYESI, T. – FORINTOS, H. – LOVAS, S., *J. Chem. Soc. Perkin Trans.*, **2** (2002), p. 1645.

[9] KEGLEVICH, GY. – FORINTOS, H. – SZELKE, H. – TAMÁS, A. – VASKÓ, Á. GY. – KOVÁCS, J., *Phosphorus, Sulfur and Silicon*, (2002).

[10] KEGLEVICH, GY. – KÖRTVÉLYESI, T. – FORINTOS, H. – VASKÓ, Á. GY. – VLADISZLAV, I. – TŐKE, L., *Tetrahedron*, **58** (2002), p. 3721.

CLASSIFICATION OF REVERSED-PHASE HPLC STATIONARY PHASES USING BIFFERED MOBILE PHASES BY CONVENTIONAL COMPARISON AND PRINCIPAL COMPONENT ANALYSIS

Péter FORLAY-FRICK

Supervisors: Jenő Fekete
Department of General and Analytical Chemistry
Károly Héberger
Chemical Research Center Hungarian Academy of Sciences

In this work the retention (capacity) factors, the theoretical plate numbers and the symmetry factors were determined using four test compounds in case of seven silica-based stationary phases. The interactions on the surface determine the above chromatographic parameters. The following effects were also investigated: effect of mobile phase buffers, injection volume, molecule size and organic content of mobile phase (methanol, acetonitrile). The examinations reveal whether the results of test compounds of small molecular mass can be extrapolated to more complicated molecules (larger molecules with more functional groups). In addition, I tried to use the popular principal component analysis (PCA) for process of my measurement data and drawing the conclusions.

The physical-chemical properties of tested stationary phases were very different. End-capped and non end-capped stationary phases were studied. Among tested columns the Chromolit SpeedROD (distributed by Merck) was also found, that is a porous monolithic silica rod made from high purity silica gel. The experiments aimed to establish the usefulness and classification of this phase, too. Four test compounds were used: benzoic acid, N,N-dimethyl-aniline, Ciprofloxacin and Vancomycin. The latter 2 compounds are often used antibiotics in the pharmaceutical industry. They have higher molecule weight and several polar groups (especially Vancomycin). The concentrations of KH_2PO_4 and triethylamine buffers were 25 mM. The pH of these buffers was adjusted to 'optimum' value in the case of all compounds, which was calculated using *Pallas 3.0* and *Marvin Sketch* pK_a and lgD prediction softwares. The mobile phases contained acetonitrile or methanol as apolar component. The organic content of mobile phase was adjusted, that the lgk (logarithmic of relative retention factor) of tested compounds should change in the range of 0.1–1.3 in the case of all stationary phases and test compounds. The plate number and symmetry factor, which form the basis of comparison of stationary phases, were calculated according to European Pharmacopoeia in all measuring points.

In addition to stationary phase examinations performed by a deductive method, principal component analysis (PCA) was also used for classification of measurement data and drawing the conclusions. PCA is known to be a non-supervised pattern recognition method. Plate numbers and symmetry factors at all retention

values (between $\lg k = 0.1 - 1.3$ with 0.1 units) were subjected to principal component analysis. The input data were 13×28 data-matrixes in case of all compounds and separately for plate numbers and symmetry factors. The principal component analysis was performed by *Statistica release 6 for Windows* software. The number of principal components was determined defining a certain percentage of explained variance as an empirical criterion in every case. Consequently, the retained principal components carry not less than 95.0% of total variance.

I verified, among other things, that the monofunctional test compounds can only be used for primary screening of stationary phases, even if these phases are designed for separation of compounds having more functional groups and high molecule weight. I determined using principal component analysis which stationary phases (mobile phase compositions) and which buffers give same-similar chromatographic parameters with use of the four different kinds of compounds. Hereby, possibility for replacement of columns was disclosed. I also verified that the reduction of number of variables can be possible without loss of information.

References

[1] FORLAY-FRICK, P. – NAGY, Z. B. – FEKETE, J., Comparative Study of Reversed-Phase Columns for Evaluation of Monolithic Stationary Phase, *J. Liq. Chrom. & Rel. Technol.,* **25** No. 9 (2002), pp. 1431–1445.

[2] FORLAY-FRICK, P. – FEKETE, J., Comparison of Selected Stationary Phases for Determination of Vancomycin and Ciprofloxacin Using Buffered Mobile Phases, with and without Triethylamine, *J. Liq. Chrom. & Rel. Technol.*, **27** No. 1, (2004), pp. 119–138.

[3] FORLAY-FRICK, P. – NAGY, Z. B. – FEKETE, J. – KETTRUP, A. – GEBEFUGI, I., Reversed-Phase High-Performance Liquid Chromatographic Method for Determination of Vancomycin in Influenza Vaccine, *J. Liq. Chrom. & Rel. Technol.*, **24** No. 4 (2001), pp. 497–507.

[4] FORLAY-FRICK, P. – NAGY, Z. B. – FEKETE, J., Determination of Ciprofloxacin in Influenza Vaccine by Reversed-Phase High-Performance Liquid Chromatography, *J. Liq. Chrom. & Rel. Technol.*, **24** No.6 (2001), pp. 827–836.

THE RELATIONSHIP BETWEEN THE MOLECULAR STRUCTURE AND THE RESPONSE OF FLAME IONIZATION DETECTOR

Mariann KÁLLAI

Supervisor: József Balla
Department of General and Analytical Chemistry

It is well known that flame ionization detector gives a signal proportional to the carbon number at measuring n-hydrocarbons. In a case when a measured component contains a heteroatom as well, there can be seen a signal reduction characteristic to the heteroatom. To characterize this phenomenon at first Sternberg and his colleagues introduced the definition of effective carbon number (ECN), that gives the signal intensity of the molecule containing a heteroatom related to the n-hydrocarbon with the same carbon number. By definition ECN is proportional to the relative sensitivity of the molecule related to the n-hydrocarbon, in this way it can be used in quantitative measurements.

With the help of ECN quantitative result can be given at such substances that can not be obtained as pure standards, in this way experimental determinations of relative sensitivity would be impossible.

From the early days of using detectors until today many scientists have been working on making ECN into a form which can be used in practice, but real practice shows that this method still, has uncertainties with which ECN can not meet the requirements of today's analytics.

In my work, after searching for the causes of these uncertainties by examining them systematically, I have developed a new method, with the help of which counting of the relative sensitivity of a molecule became possible with less then 1 error percentage related to the measured sensitivity.

As a first step in my work, with the help of the modern measuring techniques (dedicated equipment), I have determined the contribution of 8 different heteroatoms and functional groups by using their homologous series. By extending my measurements to components with higher carbon number (> 10), I could form a picture of the signal forming characteristics of components with bigger molecule size.

By analysing the influencing effect of molecule structure, I have determined and compared the signal decreasing effect of heteroatoms at aromatic (benzene derivatives) and open chain saturated compounds without changing any other condition.

As a second step, during measuring the influencing effects of measurement conditions I have studied those conditions (the mode of injection, injector, column, temperature of the detector, applied concentration, choosing the reference substances) that, by causing a change in signal ratio of the measurable substance and the reference substance (n-hydrocarbons) related to each other, affect the ECN value indirectly in this way.

On the basis of my results I have established, that apart from the content the size and the structure of the molecule, the quality of the bonding between the carbon structure and the heteroatom also influence the ECN value, which can be measured as well. Furthermore, except for the effect of the reference substance, the examined measurement conditions affect ECN only in a less degree. Concerning results, choosing the reference substance is crucial.

Completing my work, using the results of my studies I could develop a new ECN measuring method, that takes into consideration the effect of the newly developed affecting factors as well. Thus, using this method without standard substance the quantity of the measurable components can be determined by calculation with less than 1% difference compared to the experimentally determinable (with using standard) value.

References

[1] KÁLLAI, M. – VERES, Z. – BALLA, J., Response of Flame Ionization Detectors to Different Homologous Series, *Chromatographia*, **54** (2001), pp. 511–517.
[2] KÁLLAI, M. – BALLA, J., The Effect of Molecular Structure upon the Response of the Flame Ionization Detector, *Chromatographia*, **56** (2002), pp. 357–360.
[3] KÁLLAI, M. – MÁTÉ, V. – BALLA, J., Effects of Experimental Conditions on the Determination of the Effective Carbon Number, *Chromatographia*, **57** (2003), pp. 639–644.

DETERMINATION OF PHYSICO-CHEMICAL PARAMETERS INFLUENCING THE ANALYTICAL AND THERAPEUTIC PROPERTIES OF VINPOCETINE AND RELATED COMPOUNDS

Károly MAZÁK

Supervisor: Béla Noszál
Semmelweis University of Budapest

The acid-base properties of vinpocetine and its eleven derivatives were characterized by macroscopic and microscopic protonation constants. The macroconstants were obtained by extrapolation from apparent macroconstants determined by UV-pH titrations in methanol-water mixtures. The microconstants were calculated by deductive methods. The differences in the basicity of the compounds were discussed and several structure-basicity relationships were set up. The protonation constants were used in the planning of the capillary electrophoresis separation of seven compounds. For the first time in the literature we proved that partly aqueous protonation constants are able to predict electrophoretic mobilities based on Offord's equation. The lipophilicity of the ten ester derivatives was characterized by a reversed-phase thin-layer chromatographic method. The calculated log *P* values were verified by the stir-flask log *P* determination of some of the compounds. The differences in the lipophilicity of the compounds were discussed and several structure-lipophilicity relationships were set up. The *in vitro* results of our basic research on the basicity and lipophilicity of these compounds, after converting them to the *in vivo* conditions in the compartments of the human body, can contribute to understanding and therapeutically influencing biological processes at the molecular level. The incorporation of these log *K* and log *P* values into structure-activity relationships provides the opportunity of developing new, more potent drug molecules.

References

[1] NOSZÁL, B. – MAZÁK, K. – SZAKÁCS, Z., Gyógyszervegyületek mikroegyensúlyai, *Acta Pharmaceutica Hungarica*, **3** (1999), pp. 108–114.

[2] MAZÁK, K. – NEMES, A. – NOSZÁL, B., Proton Speciation and Microspeciation of Vinpocetine and Related Compounds in Aqueous and Biomimetic Media, *Pharmaceutical Research*, **16** (11) (1999), pp. 1757–1763.

[3] MAZÁK, K. – SZAKÁCS, Z. – NEMES, A. – NOSZÁL, B., Capillary Electrophoresis Separation of Vinpocetine and Related Compounds: Prediction of Electrophoretic Mobilities in Partly Aqueous Media, *Electrophoresis*, **21** (2000), pp. 2417–2423.

[4] MAZÁK, K. – VÁMOS, J. – NEMES, A. – RÁCZ, Á. – NOSZÁL, B., Lipophilicity of Vinpocetine and Related Compounds Characterized by Reversed-Phase Thin-Layer Chromatography, *Journal of Chromatography A*, **996** (2003), pp. 195–203.

OVERPRESSURED LAYER CHROMATOGRAPHY (OPLC) WITH SPECIAL EMPHASIS ON THE SEPARATIONS IN THE SORBENT LAYER SEGMENTED BY FLOWING ELUENT WALL

Emil MINCSOVICS

Supervisor: Ernő Tyihák
Department of General and Analytical Chemistry; OPLC-NIT Ltd.
e-mail: emil.mincsovics@axelero.hu

Column and layer liquid chromatographic techniques as supplementary techniques, due to their arrangements, have always been characteristically developed in constant mutual interaction. Hence it is not surprising that the intensive development of high-performance column liquid chromatography (HPLC) entailed the need for the fundamental renewal of the most popular planar layer liquid chromatographic technique, TLC. In light of this it can also be understood that the latest efforts aimed at the further development of layer liquid chromatography are characterized by the desire to introduce sophisticated instrumental techniques similar to HPLC.

The first successful step to a real planar layer version of HPLC was the development of a pressurized ultramicro chamber, the basic instrument of overpressured layer chromatography (OPLC), using a pump system for admission of the eluent into the sorbent layer. The infusion and transfusion off-line and on-line operating modes in OPLC and their combination, as well as the parallel and serial coupled multi-layer systems, are basic technical versions of OPLC. Automated OPLC 50 system provides a user-friendly, automatic, accurate and sensitive solution of the original technique.

A new OPLC separation procedure has been developed for single and multi-channel separation using a non-segmented sorbent bed and flowing eluent wall (FEW) for operating segmentation. The FEW detaches the sorbent bed into active and non-active parts regarding separation during the process. Only mobile phase is introduced into the non-active part, while for the active part, eluent and also the sample can be admitted, thus the non-homogeneous part of the sorbent bed is excluded from the separation process. The FEW helps the elimination of the edge effect of overpressured layer chromatography (OPLC) in case of single sample injection and abolition of the sample mixing effect of neighbouring lanes in the case of a multi-channel separation process. In case of dirty samples, the one-channel FEW OPLC system is well-suited for quick isolation in different preparative ranges using preparative chromatoplates. The multi-channel solution will be a tool for high throughput analysis using efficient fine, superfine, or monolithic layers. The four-channel version can be applied for high throughput multi (parallel) analysis as well as micro- and semi-preparative parallel isolation using efficient analytical or preparative layers. The FEW provides the possibility for real multi-channel liquid chromatographic separation on a non-segmented sorbent bed.

References

[1] TYIHÁK, E. – MINCSOVICS, E. – KALÁSZ, H., *J. Chromatogr.*, **174** (1979), pp. 75–81.
[2] MINCSOVICS, E. – TYIHÁK, E. – KALÁSZ, H., *J. Chromatogr.*, **191** (1980), pp. 293–300.
[3] KALÁSZ, H. – NAGY, J. – TYIHÁK, E. – MINCSOVICS, E., *J. Liquid Chromatogr.*, **3** (1980), pp. 845–890.
[4] TYIHÁK, E. – MINCSOVICS, E. – KALÁSZ, H. – NAGY, J., *J. Chromatogr.*, **211** (1981), pp. 45–51.
[5] TYIHÁK, E. – MINCSOVICS, E. – KÖRMENDI, F., *Hung. Sci. Instr.*, **55** (1983), pp. 33–40.
[6] MINCSOVICS, E. – TYIHÁK, E. – SIOUFFI, A. M., in: *Proc. Int. Symp. TLC with Special Emphasis on Overpressured Layer Chromatography (OPLC)*, Szeged, Hungary, 1984 (E. Tyihák, Ed.), Labor MIM, Budapest, 1986, pp. 251–264.
[7] TYIHÁK, E. – MINCSOVICS, E., *J. Planar Chromatogr.*, **1** (1988), pp. 6–19.
[8] MINCSOVICS, E. – TYIHÁK, E. – SIOUFFI, A. M., *J. Planar Chromatogr.*, **1** (1988), pp. 141–145.
[9] MINCSOVICS, E. – TYIHÁK, E., *J. Planar. Chromatogr.*, **1** (1988), pp. 309–312.
[10] MINCSOVICS, E. – TYIHÁK, E., in: *Recent Advances in Thin-Layer Chromatography* (F. A. A. Dallas, H. Read, R. J. Ruane, and I. D. Wilson, Eds.), Plenum Press, New York and London, 1988, pp. 57–65.
[11] TYIHÁK, E. – MINCSOVICS, E. – SZÉKELY, T. J., *J. Chromatogr.*, **471** (1989), pp. 375–387.
[12] TYIHÁK, E. – MINCSOVICS, E. – SIOUFFI, A. M., *J. Planar Chromatogr.*, **5** (1990), pp. 121–125.
[13] MINCSOVICS, E. – GARAMI, M. – TYIHÁK, E., *J. Planar Chromatogr.*, **4** (1991), pp. 299–303.
[14] FERENCZI-FODOR, K. – MINCSOVICS, E. – TYIHÁK, E., Overpressured Layer Chromatography, in: *Handbook of Thin-Layer Chromatography*, (Eds.: Sherma J. and Fried B.), Marcel Dekker Inc., New York, 1991, pp. 155–181.
[15] TYIHÁK, E. – PETRO-TURZA, M. – KARDOS, K. – MINCSOVICS, E., *J. Planar Chromatogr.*, **5** (1992), pp. 376–382.
[16] MINCSOVICS, E. – FERENCZI-FODOR, K. – TYIHÁK, E., Overpressured Layer Chromatography, in: *Handbook of Thin-Layer Chromatography*, 2^{nd} Edition, (Eds.: Sherma J. and Fried B.), Marcel Dekker, Inc., New York, 1996, pp. 171–203.
[17] MINCSOVICS, E. – GARAMI, M. – KECSKÉS, L. – TAPA, B. – VÉGH, Z. – KÁTAY, G. – TYIHÁK, E., *J. AOAC Int.*, **82** (1999), pp. 587–598.
[18] MINCSOVICS, E., in *Proc. Planar Chromatogr. 2000*. Lillafüred, Hungary, Sz. Nyiredy (Ed.) Res. Inst. Medicinal Plants, Budakalász, 2000, pp. 109–119.
[19] TYIHÁK, E. – MINCSOVICS, E., Overpressured-Layer Chromatography (Optimum Performance Laminar Chromatography) (OPLC), in: *Planar Chromatography-A Retrospective View for the Third Millennium*, (Sz. Nyiredy, Ed.), Springer, Hungary, 2001, pp. 137–176.
[20] MINCSOVICS, E. – SÁRDI, É. – VELICH, I. – KÁTAY, GY. – TYIHÁK, E., *J. Planar Chromatogr.*, **15** (2002), pp. 280–285.
[21] MINCSOVICS, E. – FERENCZI-FODOR, K. – TYIHÁK, E., Overpressured Layer Chromatography, in: *Handbook of Thin-Layer Chromatography*, 3^{rd} Edition, Revised and Expanded, (Eds.: Sherma J. and Fried B.), Marcel Dekker Inc., New York, 2003, pp. 175–205.
[22] MINCSOVICS, E. – MANACH, M. – PAPILLARD, D., In: *Proc. Planar Chromatography 2003*, Budapest, Hungary, Nyiredy Sz. (Ed.), Res. Inst. Medicinal Plants, 2003, pp. 163–178.
[23] MINCSOVICS, E. – MANACH, M. – KECSKÉS, L. – TAPA, B. – PAPILLARD, D. – TYIHÁK, E., *J. Liq. Chromatogr. & Rel. Technol.*, **26** (2003), pp. 2593–2609.
[24] PAPILLARD, D. – MANACH, M. – LAROCHE, S. – TRUCHY, C. – BRYSON, N. – MINCSOVICS, E., *LC GC EUR, Suppl. Appl. Book*, Sept., 2–3 (2003).

RAPID AND PRECISE THERMOCHEMICAL CALCULATIONS BY QUANTUM CHEMICAL METHODS

Adrienn RUZSINSZKY

Supervisor: Gábor I. Csonka
Department of Inorganic Chemistry

Estimation of correlation energy plays a central role in theoretical chemistry. The electron correlation is the error of the Hartree-Fock method and the correlation energy is equal to the difference of the complete non-relativistic CI energy and the Hartree-Fock energy calculated numerically or extrapolated to the infinite basis set. This energy is only one percent of the total Hartree-Fock energy, however, it is critical for chemical applications. Due to this error (the average absolute error is about 220 kcal/mol for the 222 G3/99 test molecules) the non-corrected Hartree-Fock method is usually unsuitable for thermochemical applications.

Analysis of the exact correlation energy for free atoms shows that correlation energy depends on nuclear charge, on the number of electrons and on the spin pairing. Knowledge of the correlation energy of atoms cannot easily be transferred into the molecular environment because the spin system of the molecules differs from those of atoms.

Our goal is to develop a new and more rapid method than the earlier methods and to achieve the chemical accuracy (1–2 kcal/mol). The Rapid Estimation of the Basis set Error and Correlation Energy from Partial charges (REBECEP) method can be summarized as follows:

1. We partition the molecular correlation energy among atoms.
2. The correlation energy of an atom is calculated with the use of the partial charges by linear interpolation.
3. We add the correlation energy estimated from the sum of atomic parts to the Hartree-Fock energy calculated with a given basis.
4. The total energy, corrected by the zero-point vibration energy, the thermal correction and the atomic energies according to a known procedure, yields the non-relativistic enthalpy of formation of the molecule.

For developing and testing the method we constructed a database of 161 molecules that contains the molecular geometry, the atomic partial charges of the atoms of the molecules, the Hartree-Fock energy, the zero-point energies, and the experimental data. With the use of a multilinear equation system we determine the atomic correction factors reproducing best the experimental enthalpies of formation of the selected molecules. The largest disadvantage of this method is that it requires an expensive frequency analysis. For this reason we have developed a parameter system that can treat implicitly the zero-point energy and thermal corrections: Rapid Estimation of the Enthalpies of Formation from Hartree-Fock results (REEF-HF).

Calculations on 161 molecules show that our method can approximate the heat of formation even for large molecules with 1.5 kcal/mol average absolute error. This error is about 1 kcal/mol for the many thousand times more expensive G3 method. We compared our results for large molecules to the results obtained with the generally used B3LYP method; this latter considerably more expensive method reproduced the experimental standard enthalpies of formation by 7 kcal/mol average absolute error [1].

References

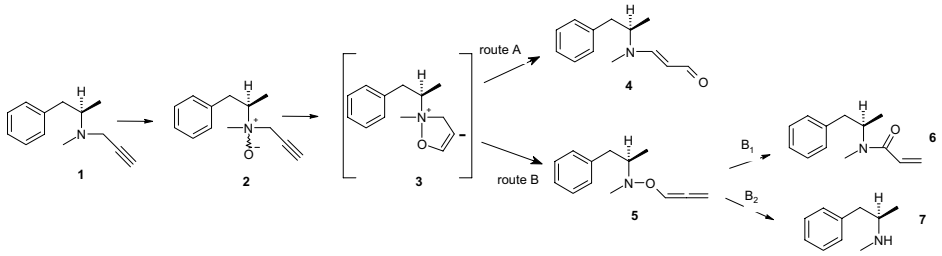
[1] RUZSINSZKY, A. – CSONKA, G. I., *J. Phys. Chem. A*, **107** (2003), pp. 8687–8695.

INVESTIGATION OF REARRANGEMENTS OF TERTIARY PROPARGYLAMINE-*N*-OXIDES

Anna SZABÓ

Supervisor: István Hermecz
Sanofi-Aventis Chinoin Ltd.

Certain propargyl-substituted tertiary amines are irreversible monoamine oxidase (MAO) inhibitors. One major metabolic degradation path of tertiary amines starts with oxidation of the tertiary nitrogen atom what can be followed by enzymatic and chemical transformations. The Meisenheimer [2, 3] sigmatropic rearrangement of tertiary propargylamine-*N*-oxides in aprotic medium to give *O*-allenylhydroxylamines has been described for cyclic-, aromatic- and benzylamine derivatives. We investigated the reactivity of Selegiline-*N*-oxide (*1*) in protic media, where two new products, the enamino-aldehyde (*4*) major, and the acrylamide (*6*) minor product were isolated. Formation of these new products was interpreted by assuming novel rearrangements.



Based on experimental results with properly substituted derivatives, isotope labelling, and relevant literature data, the possible mechanisms for these competing rearrangements were suggested. We have assumed that the enamino-aldehyde (*4*) is formed by an ionic mechanism, through isoxazolinium-ring intermediates, whereas the acrylamide (*6*) arises from the Meisenheimer rearrangement by further transformation of the primarily formed (*5*) *O*-allenylhydroxylamine.

Effect of the reaction conditions on the ratio of the competing rearrangements was investigated on a cyclic propargylamine-*N*-oxide model by reaction kinetic measurements. To understand the energy difference between the competing routes, theoretical method (DFT) was applied. Based on *ab-initio* studies, the possible structures of the transition states and intermediates were determined and the energy profiles of these transformations were drawn. In protic medium both rearrangements were found to go through an isoxazolinium-type, zwitterionic intermediate (*3*) stabilized by hydrogen bonds, which is formed in the rate determining, slow, ring-closure reaction step. Product distribution is determined by subsequent fast reactions: the enamino-aldehyde (*4*) is formed from the common intermediate

following protonation and ring-opening. Without protonation, (*3*) rearranges into the *O*-allenylhydroxylamine (*5*) Meisenheimer product. The acrylamide (*6*) and secondary amine (*7*) products arise from (*5*) by further transformations.

References

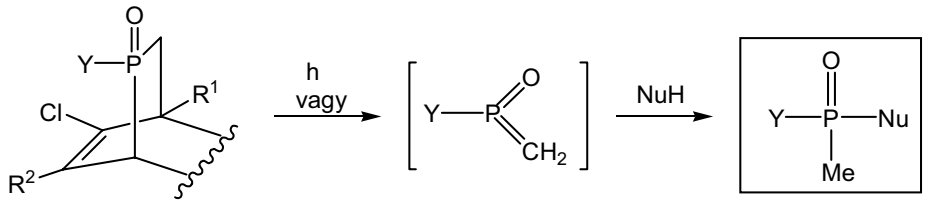
[1] GAÁL, J. – HERMECZ, I., in *Inhibitors of Monoamine Oxidase B*; I. Szelényi, Ed.; Birkhäuser: Basel, 1999, pp. 75–108.
[2] HALLSTRÖM, G. – B. LINDEKE, A. – KHUTHIER, H. – AL-IRAQI, M. A., *Tetrahedron Lett.*, (1980) pp. 667–670.
[3] SZABÓ, A. – HERMECZ, I., *J. Org. Chem.* **66** (2001), pp. 7219–7222.
[4] SZABÓ, A. – GALAMBOS-FARAGÓ, Á. – MUCSI, Z. – TIMÁRI, G. – VASVÁRI-DEBRECZY, L. – HERMECZ, I., *Eur. J. Org. Chem.*, (2004), pp. 687–694.
[5] MUCSI, Z. – SZABÓ, A. – HERMECZ, I., *J. Mol. Struc. THEOCHEM*, **666–667** (2003), pp. 547–556.

SYNTHESIS AND UTILIZATION OF BRIDGED P-HETEROCYCLES IN FRAGMENTATION-RELATED PHOSPHORYLATIONS

Helga SZELKE

Supervisor: György Keglevich
Department of Organic Chemical Technology

Our aim was to prepare bridged P-heterocycles (*1*) that are useful in the generation of methylenephosphine oxides (*2*). These reactive intermediates, generated by UV light and thermoinduced fragmentations, can be utilized in phosphonylations and phosphinylations.



1 **2** **3**

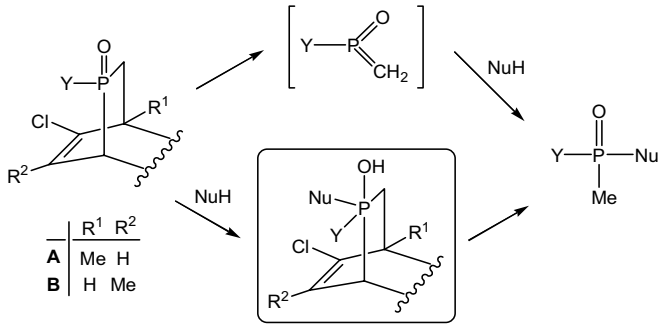
R^1, R^2 = H, Me

Y = Ph, Et, 4-MeC_6H_4, 2-MeC_6H_4

Nu = OMe, OEt, *i*PrO, *n*PrO, *i*PrNH, *n*BuNH

One of the advantages of the mild UV light mediated fragmentation-related phosphinylations/phosphonylations is the easy synthesis of P-derivatives with four different substituents (*3*). The efficiency of the phosphorylations through the thermally induced generation of methylenephosphine oxides (*2*) was somewhat decreased by the inevitable polymerization of the reactive intermediate.

While the thermoinduced phosphinylations obviously take place through an elimination–addition (EA) mechanism involving methylenephosphine oxide (*2*) as intermediate, the photochemically initiated phosphinylations may also involve a novel addition–elimination (AE) route via an intermediate with a pentavalent pentacoordinated phosphorus atom (*4*). We wished to utilize the newly synthesized aryl-precursors (*1*) to obtain new data on the mechanism of the photoinduced phosphinylations.



References

[1] KEGLEVICH, GY. – SZELKE, H. – NAGY, Z. – DOBÓ, A. – NOVÁK, T. – TŐKE, L., *J. Chem. Res.*, **35** (1999), p. 580.

[2] JANKOWSKI, S. – RUDZINSKI, J. – SZELKE, H. – KEGLEVICH, GY., *J. Organomet. Chem.*, **595** (2000), p. 109.

[3] KEGLEVICH, GY. – SZELKE, H. – TŐKE, L., *Heterocyclic Comm.*, **7** (2001), p. 365.

[4] KEGLEVICH, GY. – SZELKE, H. – DOBÓ, A. – NAGY, Z. – TŐKE, L., *Synth. Commun.*, **31** (2001), p. 1737.

[5] KEGLEVICH, GY. – SZELKE, H. – TAMÁS, A. – HARMAT, V. – LUDÁNYI, K. – VASKÓ, Á. GY. – TŐKE, L., *Heteroatom Chem.*, **13** (2002), p. 626.

[6] KEGLEVICH, GY. – FORINTOS, H. – SZELKE, H. – TAMÁS, A. – VASKÓ, Á. GY. – KOVÁCS, J. – KÖRTVÉLYESI, T. – KOLLÁR, L. – TŐKE, L., *Phosphorus Sulfur*, **177** (2002), p. 1681.

[7] KEGLEVICH, GY. – SZELKE, H. – BÁLINT, Á. – IMRE, T. – LUDÁNYI, K. – NAGY, Z. – HANUSZ, M. – SIMON, K. – HARMAT, V. – TŐKE, L., *Heteroatom Chem.*, **14** (2003), p. 443.

[8] KEGLEVICH, GY. – FORINTOS, H. – SZELKE, H. – KOVÁCS, J. – KÖRTVÉLYESI, T., *Magyar Kémiai Folyóirat*, (2004), in press.

[9] KEGLEVICH, GY. – SZELKE, H. – KOVÁCS, J., *Current Org. Synth.*, (2004), in press.

SYNTHESIS OF MASS EXCHANGE NETWORKS USING MATHEMATICAL PROGRAMMING

Zsolt SZITKAI

Supervisor: Zsolt Fonyó
Department of Chemical Engineering

The environmental impact of chemical facilities cannot be effectively mitigated by simple end-of-pipe treatments. The concept called process integration allows pollution prevention to be taken into account during the process synthesis step. To the analogy of heat integration and heat exchanger network synthesis (HENS), from 1989, the concept of mass integration and mass exchanger network synthesis (MENS) has been developed. With the developing algorithms and computer technology, from the early nineties, it became possible to solve large, realistic process integration problems using mathematical programming (MP) methods. The MP-based synthesis approach does not include trial and error elements, hence theoretically gives the opportunity to surpass the performance of the well established, heuristic or pinch design methods.

The thesis deals with the development of mixed integer nonlinear programming (MINLP) models for mass exchange applications.

It is revealed that handling the removably discontinuous Kremser equation in MINLP based MENS models is an important issue. A new, one binary variable formulation for handling removably discontinuous functions is suggested that results in simpler mathematical programs. Using the new formulation, existing MP-based models for MENS are compared, and various extensions to the best literature model are suggested. Topics such as generation of feasible initial values and calculation with integer stage numbers are touched upon.

A profound, example-problem-based comparison is made between the best available MP-based, and the most advanced, rival pinch design methods. It is shown, that the two competing approaches perform more or less equally, though theoretically, the MP-based approach should perform better. The reason for this is that the existing MP models for MENS consist mainly of nonlinear mass balances, that set up a nonconvex search space, and so the gradient-based optimization algorithms cannot find global optima for the problems. Therefore, a new, fairly linear MINLP model for the synthesis of MENs is developed.

The second half of the thesis deals with the development of rigorous MINLP models that allow the optimal design of industrial mass exchange applications. In this part, emphasis is given not to the network synthesis but to the detailed design of the individual mass exchange units. A model for designing distillation-pervaporation systems for solvent dehydration is developed, that allows process intensification of an existing plant. Finally, a rigorous, multiple-component model is suggested for the design of wastewater strippers.

References

[1] SZITKAI, Z. – LELKES, Z. – RÉV, E. – FONYÓ, Z., Handling of Removable Discontinuities in MINLP Models for Process Synthesis Problems, Formulations of the Kremser Equation, *Computers and Chemical Engineering*, **26** (2002), pp. 1501–1516.

[2] SZITKAI, Z. – MSIZA, A. K. – FRASER, D. M. – RÉV, E. – LELKES, Z. – FONYÓ, Z., Comparison of Mathematical Programming and Pinch-Based Techniques for Mass Exchange Network Synthesis, *Hungarian Journal of Industrial Chemistry*, **30** (2002), pp. 149–154.

[3] SZITKAI, Z. – LELKES, Z. – RÉV, E. – FONYÓ, Z., Optimization of Hybrid Ethanol Dehydration Systems, *Chemical Engineering and Processing*, **41** (2002), pp. 631–646.

[4] LELKES, Z. – SZITKAI, Z. – RÉV, E. – FONYÓ, Z., Rigorous MINLP Model for Ethanol Dehydration System, *Computers and Chemical Engineering*, **24** (2000), pp. 1331–1336.

[5] BENKO, N. – RÉV, E. – SZITKAI, Z. – FONYÓ, Z., Optimal Water Use and Treatment Allocation, *Computers and Chemical Engineering*, (1999), pp. S157–S160.

[6] RÉV, E. – EMTIR, M. – SZITKAI, Z. – MIZSEY, P. – FONYÓ, Z., Energy Savings of Integrated and Coupled Distillation Systems, *Computers and Chemical Engineering*, **25** (2001), pp. 119–140.

[7] FONYÓ, Z. – RÉV, E. – SZITKAI, Z. – EMTIR, M. – MIZSEY, P., Energy Savings of Integrated and Coupled Distillation Systems, *Computers and Chemical Engineering*, (1999), pp. S89–S92.