XX Polish Peptide Symposium

Programme and Abstracts

September, 6th-10th, 2009
Władysławowo

Department of Bioorganic Chemistry
Faculty of Chemistry, University of Gdańsk

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The Organizing Committee would like to emphasize the participation of **The Foundation for the Development of Gdańsk University** in organization of XX Polish Peptide Symposium.

History of the Polish Peptide Symposia

Peptide research in Poland commenced in early 50-ies at the Technical University of Gdańsk by professor Emil Taschner was continued by his successors, i.e. professors: Andrzej Chimiak (Technical University of Gdańsk), Gotfryd Kupryszewski (University of Gdańsk), Bogdan Liberek (University of Gdańsk), Barbara Rzeszotarska (University of Opole), Teresa Sokołowska (Technical University of Gdańsk) and Czesław Wasielewski (Technical University of Gdańsk). Almost at the same time, peptide research began in three other centres: at the Warsaw University by professor Stefania Drabarek, at the Technical University of Łódź by professor Mirosław T. Leplawy, and at the Wrocław University by professor Ignacy Z. Siemion. At present, more than 200 research workers from about twenty groups are involved in peptide research. Their interests focused on the chemical synthesis, structure, pharmacology, biochemistry and immunology of biologically active peptides. All aspects of peptide and amino acid chemistry and biology are presented and discussed on the Polish Peptide Symposia which have become the main forum for exchanging views by the Polish peptide chemists. The first Polish Peptide Symposium was held by G. Kupryszewski in Gdańsk in 1967. Since the second symposium, which took place in 1973 in Wdzydze near Gdańsk, the Polish Peptide Symposia are organized every second year alternatively to the European Peptide Symposia. Usually about 150 peptide chemists and biologists take part. Also foreign colleagues participate in these meetings. Since the XVI Polish Peptide Symposium official language is English. The list of the Polish Peptide Symposia, their locations, dates and organizers are given below.

Contributed by Krzysztof Rolka

Number	Location	Year	Organizer
I	Gdańsk	1967	Gotfryd Kupryszewski
II	Wdzydze near Gdańsk	1973	Andrzej Chimiak
III	Warsaw	1975	Stefania Drabarek
IV	Oleśnica	1977	Ignacy Z. Siemion
V	Bronisławów near Łódź	1979	Mirosław T. Leplawy
VI	Serock	1981	Wincenty Kwapiszewski
VII	Turawa near Opole	1983	Barbara Rzeszotarska
VIII	Jastrzębia Góra near Gdańsk	1985	Zbigniew Grzonka
IX	Puławy	1987	Jan Izdebski
X	Polanica Zdrój	1989	Przemysław Mastalerz
XI	Łódź	1991	Mirosław T. Leplawy
XII	Karpacz	1993	Danuta Konopińska and Henryk Kozłowski
XIII	Nadole near Gdańsk	1995	Bernard Lammek
XIV	Polanica Zdrój	1997	Barbara Lejczak and Paweł Kafarski
XV	Waplewo near Olsztyn	1999	Elżbieta Kostyra
XVI	Kraków - Przegorzały	2001	Jerzy Silberring
XVII	Łódź	2003	Janusz Zabrocki
XVIII	Wrocław	2005	Zbigniew Szewczuk
XIX	Pułtusk near Warsaw	2007	Aleksandra Misicka
XX	Władysławowo near Gdańsk	2009	Adam Lesner and Jarosław Ruczyński

Programme

September 6th, Sunday

19:15	welcome together meeting

September 7th, Monday

8:30-9:00	***opening Ceremony (Bernard Lammek Rector of Gdansk University, Jerzy Błażejowski Head of the Supreme Council of Higher Education, Dean of Chemistry Faculty, Adam Lesner, Jarosław Ruczyński)***	
9:00-11:40	***Perspective in peptide science (Zbigniew Grzonka, Jan Izdebski)***	
9:00-9:40	Jean Martinez	Amino acids and derivatives as attractive starting materials for the synthesis of active biomolecules
9:40-10:20	Ettore Bendetti	Molecular approaches for the design of new angiogenesis modulators
10:20-11:00	Michael Przybylski	Ion mobility- mass spectrometry and affinity-mass spectrometry: New molecular tools for the elucidation of oligomerisation and truncation structures of neurodegenerative target proteins
11:00-11:40	Zofia Lipkowska	Design of novel membrane active tetra-branched peptide dendrimers: biological activity and structural investigations
11:40-12:00	Coffee break	
12:00-14:00	***Biology of peptides (Aleksandra Misicka, Ettore Benedetti)***	
12:00-12:40	Grzegorz Bulaj	New peptide drugs for pain and epilepsy: from conotoxins to galanin
12:40-13:00	Katalin Uray	Effect of epitope modification on the secondary structure and antibody binding of MUC2 epitope peptide
13:00-13:20	Aneta Szymańska	The effect of amyloidogenic peptides on the dimerization of human cystatin C
13:20-13:40	Małgorzata Iwan	The influence of exogenous opioid peptides from human and cow's milk on proliferation of human peripheral blood T cells
13:40-14:00	John Podgórski	A comparison of mass spectrometry techniques for the analysis of peptides
14:00-15:00	Lunch break	

15:00- 17:20	***Enzymes substrates and inhibitors (Krzysztof Rolka, Jean Martinez)***	
15:00–15:40	Józef Oleksyszyn	Design, synthesis and activty of α-aminoalkylphosphonate diaryl esters as serine proteinase inhibitors
15:40-16:00	Bożena Spichalska	Design, chemical synthesis and kinetic investigation of substrates of transmembrane serine proteases
16:00-16:20	Marcin Sieńczyk	Design and synthesis of new α-aminoalkylphosphonates as human neutrophil elastase inhibitors
16:20-16:40	Timo Burster	Application of specific cell permeable cathepsin inhibitors in antigen processing of the MHC class II pathway
16:40-17:00	Justyna Majchrzak	Design and synthesis of libraries of artificial isomerases
17:00-17:20	Elżbieta Jankowska	HIV-1 Tat based peptides as allosteric inhibitors of proteasome activity

September 8th, Tuesday

8:30-10:50	***Peptides and proteins identification and functions (Paweł Kafarski, Michael Przybylski)***	
8:30-9:10	Jerzy Silberring	Peptidomics - filling a gap between proteomics and metabolomics
9:10-9:30	Marek Cebrat	Histdine-containing analogues of oxytocin and vasopressin analysed by HR-ESI-MS
9:30-9:50	Monika Kijewska	The selective detection of peptide-glucose conjugates
9:50-10:10	Paulina Czaplewska	Human cystatin C binding sites to different proteins revealed by epitope-excision-extraction mass spectrometry
10:10-10:30	Marx Ute C.	Latest developments in enhancing sensitivity and resolution in biomolecular NMR spectroscopy
10:30-10:50	Ardt Ingendoh	The role of increased resolution and scan speed of jon traps for top-down proteomics with ETD/PTR
10:50-12:00	Coffee break Poster Session I	

12:00-14:00	***Peptides as drugs (Jerzy Silberring, Zbigniew Szewczuk)***	
12:00-12:40	David Andreu	NrTP, a novel type of cell penetrating peptide that exquisitely targets the nucleolus of tumoral cells
12:40-13:20	Andrzej Lipkowski	Neuropeptide analogues as prospective adjuvants of cancer pain treatment
13:20-13:40	Aleksandra Misicka	Neuropeptide analogs as prospective selective carriers of platinum ion in anticancer therapy
13:40-14:00	Wioletta Kowalczyk	Strategies and limitations in synthetic immunodendrimer preparation. The influenza virus M2e epitope as a case study
14:00-14:20	Katarzyna Gach	The influence of selected opioids on the level of cancer markers in cancer cells
14:20-15:30	Lunch break	
15:30-16:30	Poster session II	
	Sport	Sport competition (*e.g.* football or basketball game) will be arranged during the Symposium
19:15	Concert	

September 9th, Wednesday

8:30-11:20	***Amino acid/peptide synthesis (Janusz Zabrocki, Marianna Kańska)***	
8:30-9:10	Alicja Kluczyk	Novel heterocyclic amino acids in peptides - recent advances and developments
9:10-9:40	Katarzyna Guzow	Synthesis and biological activity of 3-(2-benzoxazol-5-yl)alanine derivatives
9:40-10:00	Małgorzata Ratajska	Novel benzimidazole-containing side chains in peptides
10:00-10:20	Renata Perlikowska	Synthesis and biological activity of endomorphim and (Dmt1)endomorphin analogs with six-membered proline surrogates in position
10:20-10:40	Kamil Różniakowski	Design, synthesis and application of *N*-triazinylammonium sulphonates as coulping reagents for peptide synthesis
10:40-11:00	Krzysztof Kaczmarek	Short nociceptin analogues containing 4-aminopyroglutamyl residue
11:00-11:20	Peter Dakin	Fast conventional synthesis of chemokine SDF-1α (1-68) on the Symphony®
11:20-11:50	Coffee break	

11:50-13:30	***Peptide synthesis and modifications (Piotr Rekowski, Krystyna Midura-Nowaczek)***	
11:50-12:10	Ryszard Ostaszewski	Chemoenzymatic approach to the synthesis of bioactive tripeptide mimetics
12:10-12:30	Grażyna Relich	Glycosylation of peptides
12:30-13:10	Jan Izdebski	Cyclic enkephalin-deltorphin hybrids containing a carbonyl bridge
12:50-13:30	Alex Shöner	Microwave peptide synthesis
13:30-14:30	Lunch break	
14:30-16:30	***Peptide/protein structural studies (David Andreu, Jerzy Ciarkowski)***	
14:30-15:10	Cezary Czaplewski	Simulations of protein folding thermodynamics using UNRES physics-based coarse-grained force field with replica exchange molecular dynamics
15:10-15:30	Piotr Stefanowicz	Electron capture dissociation as a tool for spatialy resolved monitoring of ubiquitin high pressure denaturation
15:30-15:50	Aneta Buczek	Conformational properties of *N*-Acetyl-(*E*)-dehydrophenylalanine *N*',*N*'- dimethylamide
15:50-16:10	Maria Kwasiborska	NMR studies of cyclic dynorphin analogues
16:10-16:30	Ewa Rudzińska	Phosphorylated quinines as novel polyfunctional chiral auxiliaries for NMR spectroscopy
16:30-17:30	Poster session III	
19:00	Grill and music	

September 10th, Thursday

8:30-10:10	***Peptidomimetics (Michał Zimecki, Andrzej Lipkowski)***	
8:30-9:10	Paweł Kafarski	Peptidomimetics
9:10-9:30	Katarzyna Pułka	α-Amino aldehydes as components for 1,2,3,4-tetrahydro-β-carbolines synthesis
9:30-9:50	Aleksandra Walewska	Selenopeptides and the integrated oxidative folding: technological advances in studying disulfide-rich peptides
9:50-10:10	Dominika Wilczyńska	α,β-hybrides of opioid peptides containing β3-homo-amino acids
12:00	Closing ceremony	
13:00	Lunch	

Plenary Lectures

Amino acids and derivatives as attractive starting materials for the synthesis of active biomolecules

Miramon Hélène, Cavelier Florine, Declerck Valérie, Lamaty Frédéric, Cristau Michèle, Cantel Sonia, Subra Gilles, Farran Daniel, Dewynter Georges, Martinez Jean

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Amino Acid Derivatives are important starting materials for the synthesis of biomolecules. Among these derivatives, N-Carboxyanhydrides (NCA) as well as diketopiperazines (DKP) present interesting reactivity.

We have used NCA for the synthesis of:
(i) Homopolymers of Amino Acids that were used as « elicitors » to stimulate the natural defences of plants *(F. Cavelier, M. Bénard, & J. Martinez Patent 2 832 409 n° 01 14084 published April 2006)*;
(ii) Peptides in « solid » media, without using solvent (*V. Declerck, F. Lamaty, & J. Martinez, Patent N° 0753970 Filed March 2007*);
(iii) Supported reagents for the formation of disulfide bonds in peptides (*M. Cristau, S. Cantel, G. Subra, & J. Martinez, US Patent Filed June 2006*).

On the other hand, N-protected DKP were used for the stereoselective synthesis of Pyrrolidine-2, 4 Diones through the Transannular Rearrangement of Activated Lactams (TRAL reaction) that was discovered in our laboratory (*G. Dewynter, D. Farran, J. Martinez, Brevet n°0753973, 21 Mars 2007; Farran et al., Angew. Chem. Int. Ed. 2007; Farran et al. Org. Letters, 2007*).

These syntheses as well as some of their applications will be described and discussed.

Molecular approaches for the design of new angiogenesis modulators

Benedetti Ettore, D'Andrea Luca D., Del Gatto Annarita, Pedone Carlo, Saviano Michele, Zaccaro Laura

Department of Biological Sciences, University of Napoli "Federico II", Institute of Biostructures and Bioimaging, IBB- CNR and DFM scarl, via Mezzocannone 16, 80134, Naples, Italy

Angiogenesis plays a role in several pathologies of significant medical as well as social impact; in fact, there is a tremendous interest rising from the possibility of modulating this phenomenon pharmacologically. According to the angiogenesis foundation at least 184 and 310 million patients in the western nations can benefit, respectively, from anti-angiogenic and proangiogenic therapy. Currently, more than 200 biotech companies, as well as large pharmaceutical companies, have programs aimed at the development of new pharmaceuticals which act on the angiogenic process and it has been estimated that so far the research and development of angiogenesis-based molecules is one of the most well-financed field of medical research. Antibodies, peptides and small molecules targeting active endothelial cells represent an innovative tool in therapeutic and diagnostic fields. In our laboratories, we have developed new peptides and peptidomimetics for the modulation of angiogenesis, using molecular approaches and rational design techniques. The results obtained on the design of new molecules to modulate two specific protein systems, the vascular endothelial growth factor and its receptor and integrins, and their application in the therapy and for selective in vivo imaging will be presented.

[1] L.D. D'Andrea, G. Iaccarino, R. Fattorusso, D. Sorriento, C. Carannante, D. Capasso, B. Trimarco, C. Pedone (2005) Proc Natl Acad Sci U S A, 102, 14215–14220.
[2] A. Del Gatto, L. Zaccaro, P. Grieco, E. Novellino, A. Zannetti, S. Del Vecchio, F. Iommelli, M. Salvatore, C. Pedone, M.Saviano (2006) J Med Chem, 49, 3416–3420.

Ion mobility - mass spectrometry and affinity-mass spectrometry: New molecular tools for the elucidation of oligomerisation and truncation structures of neurodegenerative target proteins

Przybylski Michael[a], Vlad Camelia[a], Manea Marilena[a], Perdivara Irina[a], Cozma Claudia[a], Moise Adrian[a], Paraschiv Gabriela[a], Slamnoiu Stefan[a], Dragusanu Mihaela[a], Marquardt Andreas[a], Danzer Karin[b], Hengerer Bastian[b]

[a]Department of Chemistry, Laboratory of Analytical Chemistry and Biopolymer Structure Analysis, University of Konstanz, 78457 Konstanz, Germany [b]Boehringer Ingelheim Pharma, ZNS Research Department, Biberach, Germany

A large variety of cellular processes are based on the formation and dynamics of multi/supramolecular protein assemblies, and several diseases, previously thought to be unrelated, such as cancer and neurodegenerative diseases, are characterised by "misfolded" protein aggregates. Chemical structures and conformation-dependent reaction pathways of pathophysiological protein aggregates are only poorly characterised and understood at present. "Soft-ionisation" methods of mass spectrometry, particularly electrospray-MS (ESI-MS), have enabled substantial progress in the characterisation of chemical structures and their modifications, e.g. in proteome analysis; however, the application of HPLC-ESI- MS is unsuitable to direct "insitu" analysis of conformational states and reaction intermediates in protein assemblies. Most recently, **ion mobility mass spectrometry** (IM-MS) is emerging as a new molecular tool to probe complex protein structures from solution phase structures, due to the potential of IMMS for separation of protein mixtures by conformation state, and spatial shape and topology. In first applications of IM-MS to neurotoxic oligomeric aggregates of alpha-synuclein, a target protein for Parkinson's disease, two conformationally distinct truncation and oligomerisation products could be separated and their structures identified. IM-MS was also successfully applied to the analysis of oligomers and aggregates of ß-amyloid (Aß), the key neurotoxic peptide fragment in Alzheimer's disease (AD) plaques. Recent studies towards the development of immuno-therapeutic methods for AD have yielded (i), therapeutic antibodies by immunisation with Aß(1-42), that disaggregate Aß-plaques, and (ii), physiological Aßautoantibodies in serum capable of eliciting a protective effect to inhibit the formation of Aßplaques. **Affinity-mass spectrometry**, using proteolytic excision of the immobilized Aß-antigen-immune complex in combination with high resolution mass spectrometry, provided the identification of the Aß-plaque-specific N-terminal epitope Aß(4-10) [1, 2], as well as the epitope recognised by Aß-autoantibodies in serum. This epitope, located on the Aß(21-37) sequence, was identified as the molecular basis for the Aß-oligomer-specific neurotoxic response [3]. The differential epitope structure determination of Aß-specific antibodies from healthy non-AD individuals and AD patients provide a breakthrough for the development of (i), new immuno-therapeutic approaches with Aß-specific antibodies, and (ii), new diagnostic tools for AD with absolute specificity [2, 3]. These studies illustrate IM-MS and affinity-MS as powerful tools for the molecular elucidation of aggregation structures and conformational intermediates of target polypeptides for neurodegenerative diseases.

[1] McLaurin, J., et al. (2002) *Nature Med.* **8**: 1263-1269; Macht, M., et al. (2004) *Anal.Bioanal. Chem.* **378**, 1102-1111.
[2] Perdivara, I., et al. (2009) *J. Proteome Res.* **8**, 631-642; Perdivara, I., et al. (2009)*Glycobiology*, in press; Stefanescu, R., et al. (2007) *Eur. J. Mass Spectrom.* **13**, 69-75.
[3] Przybylski, M. et al./Univ. Konstanz & Budapest (2008) Eur. & US Patent Applications

Design of novel membrane active tetra-branched peptide dendrimers: biological activity and structural investigations

Urbańczyk-Lipkowska Zofia[a], Polcyn Piotr[a], Janiszewska Jolanta[b], Rajnisz Aleksandra[c], Solecka Jolanta[c], Szaniawska Bożena[d]

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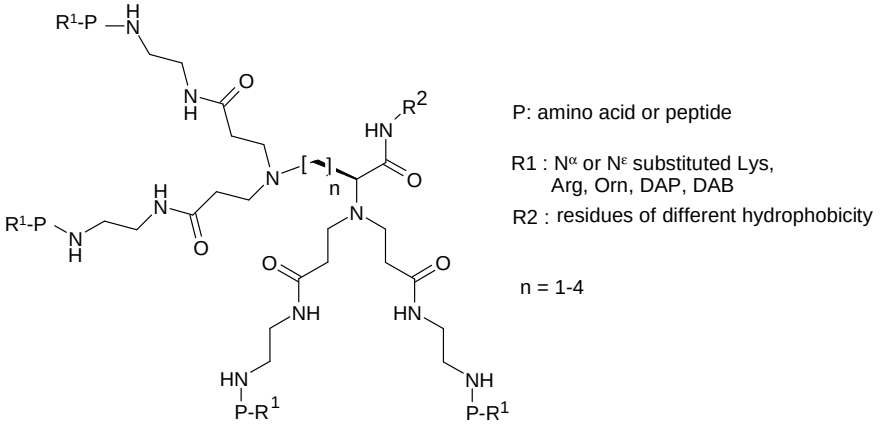
Novel type of peptide dendrimers based on highly branched tetra-arm core of amino acid origin (type **A)** were designed. They are branched analogs of natural cationic antimicrobial peptides and therefore, are characterized by amphiphilic structure and are positively charged. The tetra-branched dendrimeric peptides described here demonstrate higher activity against Gram(+) and Gram(-) strains including multi-drug resistant reference strains than similarly substituted respective tri-Lys scaffolds . The most flexible molecules are active against human melanoma cancer cells. Their activity against human fibroblasts and hemolytic properties strongly depend on structure and hydrophobicity of the R group.



Structure A

These compounds are fully characterized by NMR spectrometry. The secondary structure of dendrimers was estimated by CD spectroscopy in water, PBS buffer at various pH and anisotropic environment of SDS micelles.

Financial support from the European Community Program NORMOLIFE (LSHC-CT-2006-037733) is acknowledged.

New peptide drugs for pain and epilepsy: from conotoxins to galanin

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[c]Department of Pharmacology and Toxicology, University of Utah, Salt Lake City, Utah, USA

Neuroactive peptides comprise a group of venom-derived neurotoxins from marine cone snails (conotoxins), as well as endogenous neuropeptides. A majority of these peptides target ion channels or receptors with high potency and specificity, making them an attractive source of therapeutics for neurological diseases. However, to transform them into drugs, their pharmacological, pharmaceutical properties and bioavailability must be optimized. Our research efforts are focused on engineering conotoxins and anticonvulsant neuropeptides as drugs for pain and epilepsy. Discovery and structure/function studies on novel sodium channel blocking conotoxins, KIIIA and SIIIA, provided lead compounds for the treatment of pain. To test "cone snail drug-design strategy" on endogenous neuropeptides, we applied combinations of chemical modifications to galanin, an anticonvulsant neuropeptide that is naturally expressed in the brain. The modified galanin analogs penetrate blood-brain-barrier and exhibit potent antiepileptic and antinociceptive activities. We conclude that exploring the interface between biology and chemistry of neuroactive peptides may facilitate their transformation into future neurotherapeutics.

Effect of epitope modification on the secondary structure and antibody binding of a MUC2 epitope peptide

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[b]Noguchi Institute, Tokyo, Japan
[c]Department of Organic Chemistry, Institute of Chemistry, Eötvös L. University, Budapest, Hungary

MUC2 glycoprotein, produced by the epithelium of the colon and built up mainly of repeat units of ^{1}PTTTPITTTTTVTPTPTPTGTQT^{23}, can be underglycosylated in colon carcinoma. We have been studying the epitope structure of the MUC2 repeat unit with the mucin peptide specific MAb 996 monoclonal antibody. This antibody recognizes the ^{18}PTGTQ^{22} sequence as minimal, and ^{16}PTPTGTQ^{22} as optimal epitope [1]. Our interest lies in the modification of this epitope with maintained or enhanced specificity, and we aim to clarify the effect of different epitope modifications on MAb 996 antibody binding and their correlation with the secondary structure of the modified peptides: A) amino acid changes in the flanking region, B) glycosylation in the epitope core and in the flank. For this we have prepared A) X^{1}PTGTQX^{2} heptapeptides with X residues based on previous experiments with peptide libraries, B) the ^{16}PTPTGTQ^{22} peptide glycosylated with N-acetylgalactoseamine in position 17, 19, 21 or on all three threonines. The MAb 996 antibody binding properties of the peptides were determined in competitive ELISA experiments, and their solution secondary structure was studied by circular dichroism spectroscopy in water and in the ordered structure promoting trifluoroethanol. Our results show that A) although all amino acids in positions X^{1} and X^{2} resulted in antibody binding; in position X^{1} hydrophobic, in X^{2} aromatic residues provided stronger binding than that of the native peptide; B) glycosylation in position 19 (peptide ^{16}PTPT(GalNAc)GTQ^{22}) resulted in stronger antibody recognition and significantly altered secondary structure, while glycosylation in position 21 completely demolished the binding. These findings could be useful in determining the nature of antigen – antibody interaction, and perhaps designing synthetic peptide vaccines for tumour therapy.

Supported by: Hungarian Research Fund (OTKA F 034886, T037749), Ministry of Culture (FKFP 0153/2001)

[1] Uray K., Price M. R., Hudecz F., J. Pept. Sci., **4,** 319-326, 1998.

The effect of amyloidogenic peptides on the dimerization of human cystatin C

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Interactions between molecules are the basic tool used by all leaving cells to control and modulate all vital processes. Understanding the mutual influence of different compound is very important especially when this process can lead to any pathological consequences like e.g. formation of insoluble protein aggregates observed in the onset of diseases called amyloidoses. Growing evidence suggest that human cystatin C (hCC), which basic, physiological role is the regulation of the activity of the cysteine proteases is also engaged in the interactions with some amyloidogenic molecules like amyloid beta or serum amyloid A and is able to modulate the progress of amyloid formation [1,2]. On the other hand, cystatin C is an amyloidogenic protein itself, and a variant of hCC carrying a L68Q mutation causes hereditary cerebral hemorrhage with amyloidosis-Icelandic type (HCHWA-I). The question arises if the effect of molecules like Aβ or SAA on hCC is mutual and can also inhibit its fibrillization process.

We have addressed this question by studying the influence of peptides derived form the mentioned above molecules on the dimerization process of hCC. We have focused on this early stage of hCC aggregation because it was shown that prevention of this process also renders its further fibrillization [3]. The sequence of studied peptides was determined by us using the epitope excision approach [4]. The results of our experiments suggests that at the early stages of interactions amyloidogenic peptides seem to slightly increase the dimerization of hCC, but prolonged incubation leads rather to formation of insoluble aggregates than amyloid fibrils.

[1] Sastre M., Calero M., Pawlik M., Mathews P.M., Kumar A., Danilov V., Schmidt S.D., Nixon R.A., Frangione B., Levy E., *Neurobiol. Aging*, **25**, 1033-1043, 2004.
[2] Bokarewa M., Abrahamson M., Levshin N., Egesten A., Grubb A., Dahlberg L., Tarkowski A., *J. Rheumatol*., **34**, 1293-1301, 2007.
[3] Nilsson M., Wang X., Rodziewicz-Motowidło S., Janowski R., Lindstrom V., Onnerfjord P., Westermark G., Grzonka Z., Jaskolski M., Grubb A., *J. Biol. Chem*., **279**, 24236-24245, 2004.
[4] Juszczyk P., Paraschiv G., Szymanska A., Kolodziejczyk A.S., Rodziewicz-Motowidlo S., Grzonka Z., Przybylski M., *J. Med. Chem*., **52**, 2420-2428, 2009

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The influence of exogenous opioid peptides from human and cow's milk on proliferation of human peripheral blood T cells

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Milk proteins are precursors of biologically active peptides, including β-casomorphins (BCM) with agonistic effect and casoxins (CXN) with antagonistic effects on μ-opioid receptor. Those receptors are placed on the cells of nervous, immunologic, circulatory and digestive systems, hence numerous studies on the influence of exogenous opioid peptides on the human body. The influence of biologically active food peptides on functioning of the immunologic system seems to be particularly important. The research has shown BCMs to influence the proliferation of lamina propria lymphocytes [1] as well as human peripheral blood lymphocytes [2]. That suggests that exogenous opioid peptides participate in modulating the response of the human immunologic system.

The following paper aims at assessing the influence of peptides with agonistic and antagonistic effect on μ-opioid receptors on metabolism and proliferation of human peripheral blood lymphocytes.

The influence of the following compounds on proliferation of human peripheral blood lymphocytes isolated from healthy donors has been examined: bovine and human β-casomorphin-7, casoxin-6 and casoxin-D. The cells, isolated in the ficoll gradient, were counted and suspended in a growth medium (RPMI-10) that contained the examined peptide in concentrations of: 10^{-12} M, 10^{-9} M and 10^{-6}M. The culture of lymphocytes in the presence of the examined peptides was performed for 12 hours, after which metabolic activity of the cells was measured by the WST-1 colorimetric assay.

The influence of both μ-opioid receptor agonists and antagonists on proliferation of human peripheral blood lymphocytes has been demonstrated. In case of BCMs, the impact increased together with decreasing concentration of the peptide. A significant stimulation of proliferation in case of cells incubated in presence of μ-opioid receptor agonists - β-casomorphin-7 and morphine - has been observed. The obtained results suggest that exogenous opioid peptides supplied to the organism with a diet participate in regulation of functions performed by human peripheral blood T cells.

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A comparison of mass spectrometry techniques for the analysis of peptides

Podgórski John

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Design, synthesis and activity of α-aminoalkylphosphonate diaryl esters as serine proteinase inhibitors

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Proteolytic enzymes are implicated as pathological factors for many diseases due to local or genetic imbalance between them and endogenous inhibitors. Several pathogens including viruses and bacteria require proteolytic activity for their virulence and usually such activity is not inhibited by endogenous inhibitors also. Therefore design and synthesis of low molecular weight inhibitors for proteolytic enzymes are subject of extensive rational drug design for several diseases.

There are five classes of proteolytic enzyme including metalloproteinases, aspartyl, cysteine, serine and threonine (multicatalytic proteinese). The mechanism of catalysis is quite similar for cysteine, serine and threonine proteinases, where enzyme nucleophile (-SH or -OH of serine or threonine) attacks the hydrolyzed peptide bond. As a consequence most of the inhibitors designed for cysteine proteinases inhibit serine and threonine proteinase as well and *vice versa*. The lack of specificity concerning these three families of proteases are serious problem for rational drug design. Peptidyl derivatives of diaryl esters of 1-aminoalkylphosphonates are example of highly specific inhibitors for serine proteases. Moreover, it is possible to distinguish even two very close serine proteinases and obtain specific inhibitor for each of them. Literature background and recent development concerning this class of inhibitors will be presented.

Design, chemical synthesis and kinetic investigation of substrates of transmembrane serine proteases.

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Matriptase and HAT (human airway trypsin like protease) belong to the family of type II transmembrane serine proteases (TTSPs) [1]. These enzymes are very interesting objects for scientific investigation because of their important and mysterious physiological functions. Substrate libraries are the most convenient tool for characterization of these both enzymes.

The library consisting of fluorescence-quenched substrates was synthesized on the solid phase using portioning – mixing approach methods. The general formula of the peptide library synthesized is as follows:

ABZ-X_4-X_3-X_2-X_1-ANB-NH_2

ANB-NH_2 – amid of 5-amino-2-nitrobenzoic acid (quencher); ABZ – 2-aminobenzic acid (fluorophore); X_1 = Arg, Lys; X_2, X_3, X_4 - 19 proteinogenic amino acid residues excluding Cys;

The library was screened in solution against matriptase and HAT applying iterative approach. The two most active peptides sequences for both expetimental enzymes were selected and characterized by their kinetic parameters k_{cat}, K_M, k_{cat}/K_M. In the case of matriptase following substrates were obtained:

ABZ-Arg-Gln-Ser-Lys-ANB-NH_2 ($k_{cat}/K_M = 1532.78\times10^3$ $M^{-1}\times s^{-1}$)
ABZ-Arg-Gln-Ser-Arg-ANB-NH_2 ($k_{cat}/K_M = 1637.97\times10^3$ $M^{-1}\times s^{-1}$)

For HAT two the most active substrates were given:

ABZ-Arg-Gln-Asp-Lys-ANB-NH_2 ($k_{cat}/K_M = 195.21\times10^3$ $M^{-1}\times s^{-1}$)
ABZ-Arg-Gln-Asp-Arg-ANB-NH_2 ($k_{cat}/K_M = 454.65\times10^3$ $M^{-1}\times s^{-1}$)

To the best authors knowledge both substrate's pair displayed the highest specificity ever described in literature.

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Design and synthesis of new α-aminoalkylphosphonates as human neutrophil elastase inhibitors

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Human neutrophil elastase belongs to a serine protease superfamily. Stored in azurophilic granules and upon stimulation is released from leukocytes and participates in the early stages of the immune response. The main function of elastase is not only limited to degradation of elastin but elastase also degrades other extracellular matrix components such as laminin, fibronectin, proteoglycan or collagens [1,2]. In normal healthy organism the activity of elastase is precisely controlled by natural endogenous inhibitors (serpins) including α-1 PI, α_2-macroglobulin or secretory leukocyte peptidase inhibitor. Due to so called "oxidative burst" of neutrophils the balance between the enzyme and its inhibitors is disrupted which leads to development and progression of several diseases where chronic obstructive pulmonary disease (COPD), adult respiratory syndrome, cystic fibrosis or chronic bronchitis are only few examples [3].

Importantly, the character of how neutrophils circulate in blood, adhere and penetrate the vessels is similar to the mechanism of metastatic cancer cells migration. In both events elastase is one of the crucial proteolytic element. Indeed, overexpression of elastase was found in several human breast and lung cancers allowing the tumor to grow and spread within the body [4,5]. Thus, development of potent and selective inhibitors of neutrophil elastase is important in light of several human diseases.

We focused our attention on the design and the synthesis of α-aminophosphonate diaryl ester inhibitors of human neutrophil elastase. The major advantage of α-aminophosphonate inhibitors is the potency, selectivity and irreversible nature of their action, as only irreversible inhibitors are able to overcome the elastase/serpins imbalance [6]. We synthesized several low molecular weight Cbz-protected phosphonic analogues of Ala, Ile, Val, Leu, nVal, nLeu and Abu having various aromatic ester groups. For the most active inhibitors we prepared different *N*-derivatized compounds (such as peptidyl or simple *N*-acylated derivatives). We also applied combinatorial chemistry methods (e.g. Ugi reaction) for the α-aminophosphonate inhibitors of elastase development using α-isocyanoalkylphosphonate diphenyl esters as the starting substrates [7].

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Application of specific cell permeable cathepsin inhibitors in antigen processing of the MHC class II pathway

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Cathepsins of the cysteine, aspartyl, and serine classes are involved in antigen processing in the major histocompatibility complex (MHC) class II loading compartment. Investigation and manipulation of these proteases in living cells is difficult to perform due to the lack of highly specific cell permeable inhibitors. We tested several Cathepsin inhibitors for their ability to penetrate the cell membrane of peripheral blood mononuclear cells (PBMC). The commercially available reversible Cathepsin G-specific inhibitor I and the irreversible Suc-Val-Pro-Phe^{P} $(OPh)_2$ (Suc-VPF), for instance, are both cell permeable and specifically inhibit intracellular Cathepsin G in the PBMC. Furthermore, selective inhibition of Cathepsin G resulted in reduced tetanus toxin C-fragment (TTC) and hemagglutinin (HA) processing and presentation to $CD4^+$ T cells. An alternative strategy to modify immune reactions is the use of altered peptide ligands (APL). Therefore, we generated a glutamic acid decarboxylase 65 (GAD)-derived protease-resistant APL (prAPL) by cleavage site-directed modification. The resulting prAPL are resistant to lysosomal and serum proteases, bind with high-affinity to MHC class II molecules and have a prolonged half-life in the serum. These peptides significantly decreased the secretion of proinflammatory cytokines in peripheral blood lymphocytes from patients with type 1 diabetes mellitus (T1D). The strategy of designing specific immunomodulatory protease inhibitors and protease-resistant altered peptide ligands provides the basis for therapeutic intervention.

Design and synthesis of libraries of artificial isomerases

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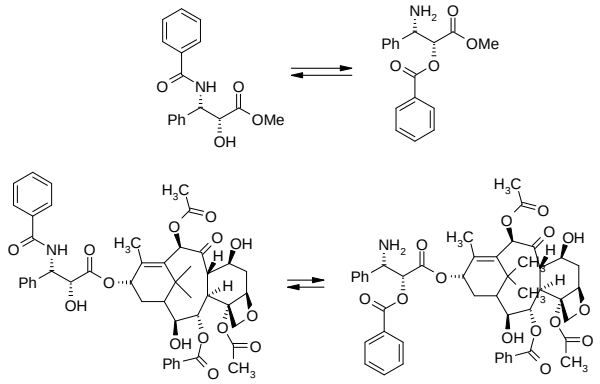
The design of the most of man-made catalysts is based on the concept of close contact of the substrates located in the outside of catalytically active core structure. Nature created much more efficient catalyst in accord with the opposite concept, based on the positioning of the substrate inside of the binding pocked of catalytically active moiety. In the consequence of the latter conceptual approach, the catalyzed processes may proceeds in the most suitable microenvironment, under milder conditions, much faster and with exceedingly high regio-, chemo-, and stereoselectivity.

In order to learn lesson from Nature, we attempted to designed catalysts by modification of the structure of our artificial receptors [1] formed by self organization of podands immobilized in the regular pattern on the solid support. The podands were prepared from N-lipidated oligopeptides tethered in the regular fashion to the cellulose *via* linker made from fenylenediamine and 2,4-dichloro-6-methoxy-1,3,5-triazine.

By incorporation into the peptide fragment residues of catalytic triade His, Ser, Asp (Glu) the library of highly potent artificial esterases were prepared and used as catalysts of hydrolysis and alcoholysis of p-nitrophenyl esters of N-protected di- and tripeptides [2]. For the assembly of the podands triazine coupling reagents were used [3] in all coupling steps.



Recently, following the general approach presented above, the library of artificial isomerases was designed, prepared, and used in catalysis of *O*→*N* (*N*→*O*) isomerisation of *N*-benzoyl-2-phenylisoserine, side chain of Paclitaxel and carboranes.

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HIV-1 Tat based peptides as allosteric inhibitors of proteasome activity

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Proteasome, involved in ubiquitin dependent turnover of cytoplasmic and nuclear proteins, plays a role in development of several diseases, including muscular dystrophy, stroke, inflammation and various cancers. Many small competitive inhibitors of the proteasome have been already developed but by blocking active centers of the enzyme, they indiscriminately halt cleavage of all proteasomal substrates, triggering apoptosis. Potential for more precise and substrate-specific regulation of the proteasome may be offered by inhibitors/activators which can interfere with the enzyme's gating mechanism and in an allosteric manner influence activity of its catalytic sites.

The aim of our work was to find small synthetic compounds which can bind to the core of the proteasome and allosterically enhance or suppress the performance of its active centers. As starting sequences we used fragments of proteins interacting with the proteasome outer ring, one of which is HIV-1 Tat protein. The designed two peptides – GRKKRRQRRRPS and RKKRRQRRDPI, comprising in their structure the basic domain of Tat protein, occurred to be very efficient in 20S proteasome inhibition. Both compounds were also interestingly selective blocking the housekeeping enzyme much more efficiently than its immuno counterpart. This result points out to the exciting possibility to differentiate between activities of the two forms of the enzyme and to independently cure diseases connected exclusively with misbehavior of housekeeping or immunoproteasome. The biological activity of the designed peptides will be related to their structure determined by means of CD, FT-IR, NMR and molecular dynamics simulation.

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Peptidomics- filling a gap between proteomics and metabolomics

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It is trivial to say here, for the audience of the Polish Peptide Symposium, that peptides and neuropeptides in particular play many important roles in our bodies. Many of their physiological functions were revealed but discovery process of such unique sequences always was a bottleneck in neurobiochemistry and neuropharmacology. Moreover, quantitation of short sequences was always ambiguous due to the lack of antibodies sufficiently specific for immunoassays. To link together peptides and our behavior, it is important to design new, high throughput strategies that will allow us to evaluate multiple pathways at the same time.

Strategies and concepts in peptidomics research underwent many changes during last decades, though total number of papers listed in the Medline under a key-word "peptidomics" barely reaches 30 publications. Besides measurement of changes in peptides and their precursors expression, novel strategies aim at direct application of this knowledge to clinical diagnosis and rational design of new drugs. So far, there is neither single, nor unified strategy to investigate and follow the onset of a given disease neither by proteomic nor peptidomics approaches, nor clear performance criteria for the entire methodology. Modern analytical tools, such as capillary HPLC and high resolution mass spectrometry are ideally suited to define peptide sequences and their modifications. There are, however, many limitations of the available procedures, including sample preparation and bioinformatics dealing with a vast number of data.

Lecture will provide a brief overview on trends and approaches in peptidomics, including own results.

Histidine-containing analogues of oxytocin and vasopressin analyzed by HR ESI-MS

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We have synthesized a series of new oxytocin (c(H-Cys-Tyr-Ile-Gln-Asn-Cys)-Pro-Leu-Gly-NH_2; OT) and Arg-vasopressin (c(H-Cys-Tyr-Phe-Gln-Asn-Cys)-Pro-Arg-Gly-NH_2; AVP) analogues in which one or both Cys residues were substituted by His. The series included also peptides with additional modifications: acetylation of N-terminal amino group and replacement of Tyr residue by Phe. The formation and fragmentation pattern of the complexes of the OT and AVP analogues with Cu^{2+} or Zn^{2+} ions was studied by high resolution electrospray mass spectrometry at pH 6-7.

Similary as in the case of the native peptides [1], their His-analogues are good ligands for the Cu ions. Complexes of a type $[peptide + M]^{2+}$ are visible in all recorded spectra, although the intensity of these peaks is rather low as compared to $[peptide + 2H]^{2+}$. During MS/MS fragmentation of the peptide-metal ion complex, a respective C-terminal tripeptide Pro-Aaa-Gly-NH_2 (Aaa = Arg or Leu) is easily cleaved, while the metal ion is retained by the N-terminal fragment. The ESI-MS/MS fragmentation pattern suggests that the Cu ion is bound to the imidazole nitrogens of both His residues, but His^1 seems to chelate metals more efficiently. Even in the case of the acetylated peptides we observe the N-terminal tripeptide fragments containing Cu (Zn) ions. The complexes with Zn ions are formed much less efficiently. The N-terminal acetylation results in a drastic deacrease in the affinity to Zn ions, which implicates an involvement of the amino group in the formation of these complexes.

Monohistidine analogues of OT and AVP easily undergo oxidation forming covalent dimers through intermolecular S-S bonds. This process is catalyzed in the presence of Cu ions and is most pronounced in the case of peptides with non-acetylated, N-terminal Cys residue. The oxidized peptides form complexes with single or two Cu ions, while the Zn ions prefer to bind to the monomeric, unoxidized peptides still present in the mixture. The acetylation of the monohistidine analogues as well as the position of the His residue in the peptide sequence has a significant influence on their ability to bind metal ions.

The results obtained by ESI-MS measurements are in very good agreement with those obtained by potentiometric techniques and NMR studies [2].

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The selective detection of glucose-peptide conjugates

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The products of non-enzymatic glycation of proteins are formed in chemical reaction between reducing sugar, and free amino groups located either at the N-terminal end of the polypeptide chains or on the lysine side chains. The glycated proteins as well as their fragments could be used as markers of diabetes mellitus, the aging process, and Alzheimer's disease which makes them the object of interest in clinical chemistry.

In our presentation the three new methods of selective detection of glucose-peptide conjugates in the mixture of compounds will be presented. The first of them is based on characteristic neutral losses in a sugar moiety. A convolution mapping analysis on mass spectra allows to reveal the signal originated from a peptide-derived Amadori product and to identify it. The second approach is based on the selective formation of borate complexes of peptide derived Amadori products. Formed adducts shows characteristic isotopic pattern, (Fig. 1) which simplifies the analysis of enzymatic digest. In addition to this, the complexation of borate ion stabilizes the sugar moiety and simplifies CID spectrum. The third method allows the identification of glycoconjugates in the mixture of peptides obtained by the enzymatic hydrolysis of proteins glycated with an equimolar mixture of glucose and [$^{13}C_6$]glucose. The obtained peptide-derived Amadori products may be identified on the basic of isotopic pattern. The proposed methods are complementary and can be combined to improve the sensitivity of detection of glycated peptides.

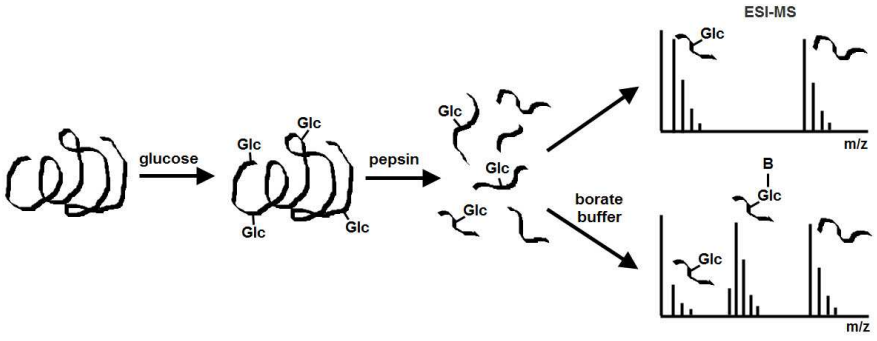

Fig. 1 The selective detection of peptide-derived Amadori products by ESI-MS based on the formation of borate complexes.

Human cystatin C binding sites to different proteins revealed by epitope-excision-extraction mass spectrometry

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Recent developments show that selective proteolytic excision combined with mass spectrometric peptide mapping (**Epitope-Excision-MS**) present high potential for the determination of epitopes for antigen-design and for the identification of antibody-paratope interactions. In this work we present an affinity method for protein-peptide interaction studies, that enabled the identification of interactions between human cystatin C (hCC) and amyloid beta peptide (A), serum amyloid A (SAA), monoclonal antibodies anti hCC (Cyst10 and Cyst13). Here we report the update of identification of the molecular interaction, and elucidation of epitope binding sites for all above mentioned cystatin C complexes. For the identification of the epitopes, proteolytic epitope extraction- and excision-MS methods were applied using different proteolytic enzymes. The eluted epitope peptide and protein fragments were analyzed by matrix-assisted laser desorption/ionization (MALDI) and ESI- mass spectrometry. These results might be of high importance for designing new inhibitors for aggregation processes of A, cystatin C and serum amyloid A.

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Latest developments in enhancing sensitivity and resolution in biomolecular NMR spectroscopy

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Bruker BioSpin has successfully energized and brought to its fullmagnetic field of 23.5 Tesla the world's first, standard-bore, high homogeneity 1 GHz NMR persistent magnet, enabling unique NMR applications. The 1 GHz NMR spectra demonstrate the enormous capabilities of this new high-end instrument, indicating a tremendous potential for all ultrahigh field high-resolution and solid-state NMR experiments. Dynamic Nuclear Polarization (DNP) experiments at 263 GHz/ 400 MHz provide a large gain in sensitivity and dramatically reduce signal averaging time. At present, signal enhancements from 20 to a factor of 80 are possible on a wide range of samples. This enhanced sensitivity enables researchers to study samples that otherwise would have been inaccessible to NMR, such as certain membrane proteins or samples which are available only in very limited amounts or dilute concentration. Unmodified NMR experiments benefit from continuous DNP-enhanced signal intensity through CW microwave irradiation. The SABRE (Signal Amplification By Reversible Exchange) hyperpolarisation technique is a new technology with non-hydrogenative para-Hydrogen induced polarization over a metal catalyst that leaves the molecule of interest unmodified. The technique is still under development and presently used for small organic molecules with accessible N-atom, such as pyridine, nicotineamide, etc. Signal enhancement of several 100fold can be achieved.

The role of increased resolution and scan speed of ion traps for top-down proteomics with ETD/PTR

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Innovative Aspect: Increasing resolution and scan speed of ion trap mass spectrometer enhances the sequence analysis capability of top-down proteomics with ETD/PTR
Introduction: Dedicated MS/MS-techniques for top-down proteomics are electron-induced fragmentation processes like electron capture or electron transfer dissociation. However, the analysis of ETD MS/MS data of highly charged proteins can be rather complicated, because a plethora of multiply charged and overlaid fragment ions can be expected. The complexity of the ETD MS/MS-data is significantly reduced when the initial ETD-step is followed by a subsequent proton transfer reaction (PTR) reducing the charge states of the multiple charge fragments. For common ion trap instruments, the charge reduction step is typically optimized leading to singly and doubly charged fragments. An increase in resolving power of the MS-instrument is strongly demanded if highly charged ETD-fragments ($z > 4$) have to be analyzed
Methods: All measurements were carried out on a Bruker amazon ion trap. An improved control of the non-linear ejection process and the development of the trap environment support faster scan modes as well as a higher mass resolution. The previously purified proteins were introduced into the ion trap with offline nanospray. ETD/PTR of the isolated protein is performed with reagent anions dedicated for either ETD or PTR. The formation of the different reagent anion is accomplished from only one neutral compound by altering the voltage of the negative chemical ionization source.
Results: We investigate the role of the increased resolution of a modified ion trap mass spectrometer on the top-down sequence analysis of larger peptides and proteins with ETD/PTR. For the sequence analysis of intact proteins with ETD/PTR MS/MS, the subsequent charge reduction step with PTR has to be tuned for generating mainly singly, doubly and triply charge fragments.One major drawback of the charge reduction step is the decrease of the total ion signal after the PTR-step. With each proton transfer the m/z of an ETD-fragment will be shifted towards higher masses. Any highly charged ETD-fragment will get out of scope if the final m/z of the ETD-fragment exceeds the maximum accessible m/z of the instrument.The higher accuracy of the resonant ejection process of the modified ion trap system allows a fivefold faster scan speed compared to the established maximum mode. With the resulting resolving power of the modified ion trap instrument ETD-fragments having five or even six charges are isotopically resolved (peak width ~ 0.1 Th). The scan speed above 4000 Th/sec and the high charge capacity of the modified ion trap system enables the fast identification of ETD-fragments within a seamless scan (scan range 100-3000).We will present the impact of the increased resolution on the sequence analysis of intact proteins (molecular weight up to 16 kDa). With the increased resolution we were able to sequence a purified biomarker protein which has been previously discovered via MALDI imaging of breast cancer tissues.

NrTP, a cell penetrating peptide exquisitely targetting the nucleolus of tumoral cells

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We have recently reported (J Med Chem, 2008, 51:7041-7044) the preferential nucleolar localization in live HeLa cells of designed peptides resulting from the structural dissection of crotamine, a rattlesnake toxin. Named NrTPs (nucleolar targeting peptides), these peptides are short, strongly cationic sequences with two repetitive KKG triads. NrTP1 (YKQCHKKGGKKGSG), a representative lead compound, and its retro version, rNrTP1 (GSGKKGGKKHCQKY), are both able to translocate the cell membrane and virtually exclusively home in the nucleoli of tumoral cell lines other than HeLa, e.g., human pancreatic adenocarcinoma (BxPC-3), human ductal mammary gland carcinoma (BT-474 or human colorectal adenocarcinoma (Caco2). Mouse neuroblastoma cells (N2a) also uptake NrTP1 quite efficiently at concentrations in the 6-50 µM range, as measured by flow cytometry. The identification of putative targets of NrTP1 in the nucleolus by proteomic methods is under way. Our findings are noteworthy for at least two reasons: (1) among scores of cell penetrating peptides, NrTPs are unique for their singular ability to target a specific subnuclear compartment, and (2) the nucleolus being the site of ribosome biogenesis and other vital cell functions, NrTPs may be useful for delivering drugs aimed at nucleolar proteins involved in cell cycle regulation, thus controlling the fate of tumor cells.

Neuropeptide analogues as prospective adjuvants of cancer pain treatment

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Cancer pain can be acute or chronic and visceral or somatic, depending on the type and stage of the disease. The acute pain source can be tumor encroachment or tumor metastases. Inflammatory and neuropathic pains can result from chemotherapy, radiotherapy and neural injury following surgical intervention [1].The progression of pain and fast development of tolerance to analgesics is common factor of cancer pain. This rationalizes development of new analgesics, more effective in time.

Recent studies indicated that overexpression of neuropeptide receptors (GPCRs) is common in various cancer cell membranes. Therefore, neuropeptides and their mimetics may modulate proliferation and migration of cancer cells. Development of proper new analgesics that may interact with cancer GPCRs and provide adjunct effect to main pharmacological cancer treatments may be a new tool in cancer treatment. We already have observed suppression of cancer proliferation by new peptidic opioid analgesic, biphalin [2]. Following results of structure activity relationships of biphalin we proposed new type of peptidic compounds especially designated for cancer pain treatment. The new series of compounds express broad spectrum of affinity to opioid mu, delta and kappa receptors. The peptides themselves suppress proliferation of melanoma human cell in vitro without any visible effect on normal fibroblasts or red blood cells.

Most of current anticancer drugs act via interaction with tubulines. Therefore, we conducted studies of colchicine in combination with newly synthesized peptide opioid analogues for evaluation of possible interactions between anticancer and antinociceptive compounds. Colchicine itself expressed high anticancer effect in in vitro test. Addition of opioid peptide analogues strongly and dose dependently enhanced antiproliferative properties of colchicine. Colchicine (similarly to other oncological drugs) expressed also strong effect on normal cells (eg fibroblasts). Therefore, it is worth to notice that opioid peptide analogues did not potentiated toxic effects of colchicine on fibroblasts. Summarized, we developed new generation of opioid analgesic compounds with significant adjunct anticancer properties. Further studies of these compounds in animal cancer pain models are in progress.

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Acknowledgements:
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Neuropeptide analogs as prospective selective carriers of platinum ion in anticancer therapy

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The most powerful and useful anticancer drugs belong to cisplatin derivatives. *Cis*-diaminedichloplatinum analogs are responsible for the inhibition of DNA transcription what leads to cell death. Cisplatin binds strongly to DNA in regions containing several guanine units, forming Pt-DNA links within strands. Through disrupting base-pairing guanine to cytosine cross-links lead to unwinding of the DNA. As a result cisplatin work against both types of cells, destroying cancer and normal type ones. Therefore more selective delivery system of platinum ion to cancer cells is still needed.
Based on the evidence that various neuropeptide receptors are present on different human cancer cell membranes in higher concentration, comparing to normal tissue, we proposed to use peptide ligands of such receptors as prospective selective carriers of platinum ions to cancer cells. We started with the opioid receptor system which is one of possible target of such directed chemotherapy. We designed hybride molecules which combine two fragments: one part of the molecule contains the opioid pharmacophore and the other fragment is designed to form a complex with platinum ion. The binding affinity at the opioid receptors and effect on the proliferation of the human glioblastoma cells of the synthesized compounds will be presented and discussed.

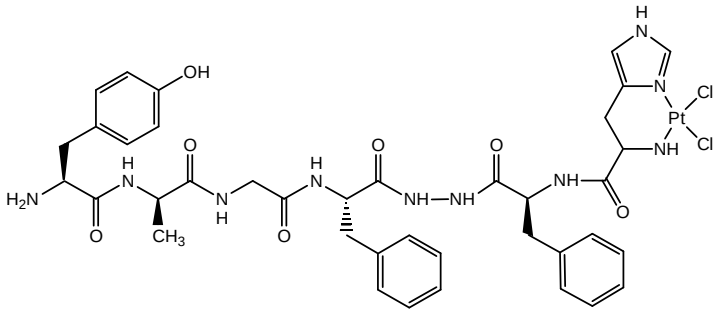

Fig. 1. Example of synthesized opioid platinum complex

Acknowledgement: Project supported by EU grant Normolife (LSHC-CT-2006-037733)

Strategies and limitations in synthetic immunodendrimer preparation. The influenza virus M2e epitope as a case study

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Dendrimeric immunogen presentations such as Multiple Antigen Peptides (MAPs) were introduced some 25 years ago [1] and are still regarded as one of the most efficacious approaches for antigenic presentation of peptides, including candidate vaccines. Since that time many studies have been undertaken to reproduce single epitopes to be used as a vaccines. MAPs are based on a small immunologically inert core of radially branching lysine dendrites onto which a number of peptide epitopes are attached. The result is a large macromolecule with a high molar ratio of peptide antigen to core molecule. MAPs were originally made available by standard stepwise solid phase peptide synthesis, but have later been produced also in solution through various forms of chemical ligation. As the ligation reactions usually involve the combination of pre-purified peptide modules, they can purportedly produce chemically more unambiguous materials than stepwise methods, where minute but cumulative synthetic errors (deletions, truncations, etc.) become amplified by multimerization and may predictably lead to relatively heterogeneous immunogens.

In this study two methods of MAP preparation have been evaluated: chemoselective ligation in solution and fully stepwise solid-phase peptide synthesis (SPPS). The pros and cons of both approaches have been investigated using a well-known epitope, the N-terminal ectodomain (M2e) of influenza type A virus matrix protein 2 (M2), as test model. M2 is one of the three types of transmembrane proteins expressed in the membrane of influenza type A virions and virus-infected cells, and plays an important role in the influenza virus life cycle. The M2 ectodomain is weakly immunogenic, and highly conserved amongst human influenza virus strains.

A series of different MAP constructs have been devised, synthesized and optimized by state-of-the art peptide synthesis methods. For this M2e peptide, the second approach, i.e., fully stepwise SPPS is advantageous. Introduction of flexibility-enhancing 6-aminohexanoic acid units appears to be also beneficial.

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The influence of selected opioids on the level of cancer markers in cancer cells

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Urokinase plasminogen activator (uPA) plays a key role in tumor-associated processes, increasing cancer cell invasion and metastasis, and therefore is used as a marker in cancer prognosis. In this study we have determined the effect of μ-opioid receptor (MOR) agonists and antagonists on the uPA secretion in MCF-7 cell line. It was shown that MOR agonists, such as morphine and endomorphins, greatly stimulate uPA secretion, while naloxone and MOR-selective antagonists elicit the opposite effect. The same tendency was observed also on the uPA mRNA level. However, neither agonists nor antagonists had any effect on proliferation of MCF-7 cells. The findings reported in this study may be useful in designing further experiments aimed at elucidating the role of the opioid system in cancer cells.

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Novel aromatic amino acids in peptides – recent advances and developments

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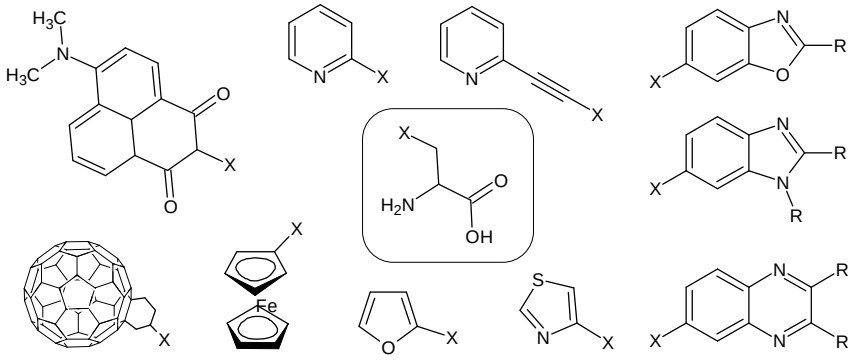
The advanced organic synthesis, including solid phase procedures, microwave assisted synthesis and broad range of protecting group, allows for synthesis of practically any combination of structural motifs, thus opening a whole range of possibilities for peptide chemistry. The search for peptides with desired properties increases the interest in novel amino acids, which may be used to determine structural features and change spectral characteristics of peptides, as well as lead to novel biological activity.

The molecular scaffolds formed by peptide chain could be structured by introduction of specific side-chain motifs, moreover, the shape shift may be evoked by UV light or redox reaction. Chelating elements in modified side chains of amino acids are responsible for binding metal ions, which results in different applications including imaging probes, biosensing, cytotoxicity, cellular localisation and increase of cellular uptake. Special amino acid residues are used as fluorescence markers and FRET components. The scope of the modifications is practically unlimited, including fullerene amino acid residues facilitating the uptake of peptides into cells.

In most cases the novel amino acids are synthesized in solution, and introduced into peptides as suitably protected derivatives. However, the on-resin post-assembly formation of special structures is also possible, making this a method of choice in the synthesis of combinatorial libraries of modified peptides.



Selected examples of novel aromatic amino acids

The recent developments, trends and perspectives in synthesis and applications of nonproteinaceous amino acids will be presented and discussed.

Synthesis and biological activity of 3-(2-benzoxazol-5-yl)alanine derivatives

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Benzoxazoles are a group of fluorescent heterocyclic compounds already widely used in chemistry, industry and medicine [1-3]. Taking these into account, benzoxazole moiety was introduced into alanine skeleton making a group of new fluorescent unnatural amino acids [4-7]. The synthetic route of these compounds both in solution [4,5] and solid-phase is based on the oxidative cyclization of the intermediate Schiff base obtained from 3-aminotyrosine and appropriate aldehyde. This quite easy modification of the benzoxazole ring enables to receive numerous derivatives with different potential applications according to their properties limitated mainly by a substituent in position 2 of the benzoxazole [4-7].

As benzoxazole moiety is present in naturally occurred as well as synthetic biologically active compounds, the activity of 3-(2-benzoxazol-5-yl)alanine derivatives as well as selected dipeptides with these amino acids was studied. The influence of substituent in position 2 and 5 of the benzoxazole ring on antimicrobial activity was established. Also, cytotoxicity of those compounds was studied using *in vitro* methods.

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This work was financially supported by the Polish Ministry of Science and Higher Education under grant 0212/H03/2007/33.

Novel benzimidazole-containing side chains in peptides

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Previously we developed a new solid-phase synthesis of benzimidazole-peptide conjugates, expecting these compounds to express novel biological properties. The peptide-heterocycle hybrids are obtained by the on-resin reaction between aldehydes and peptides containing β-(3,4-diaminophenyl)alanine residue, developed by us specifically for introducing such modifications into peptides.[1]

Here we present an efficient and straightforward solid phase synthesis method of substituted and/or fused benzimidazole scaffolds conjugated with peptides. The application of the increased concentration of aldehyde results in 1`,2`-disubstituted derivatives. The reaction with dialdehydes results in more extended conjugated ring systems, leading to novel amino acid residues containing oligocyclic [1,2-*a*]benzimidazole derivatives. The novel benzimidazole amino acids are formed as pairs of isomers, due to clockwise and counterclockwise ring closure. The isomers were separated by preparative HPLC and their structures were unambiguously assigned by the two-dimensional NMR spectra due to significant contacts generated by the CH_2 protons from the N-substituent in position 1 of benzimidazole skeleton and the specific chemical shift changes of the protons of the benzene ring.

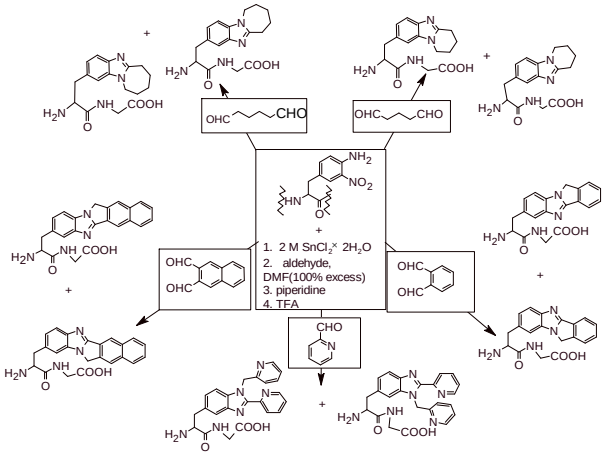

Figure 1. On-resin benzimidazole scaffold formation.

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Synthesis and biological evaluation of [Dmt[1]]endomorphin analogs with six-membered proline surrogates in position 2

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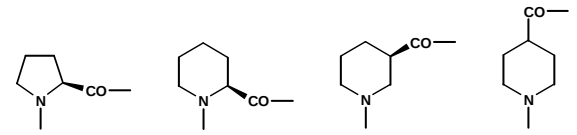
Endomorphin-1 (EM-1, Tyr-Pro-Trp-Phe-NH_2) and endomorphin-2 (EM-2, Tyr-Pro-Phe-Phe-NH_2), two endogenous opioid peptides, isolated from the mammalian brain, display strong analgesic effect [1]. They activate one of the three major opioid receptors, the μ-opioid receptor. Endomorphins are characterized by the presence of Pro^2 residue, which is a spacer connecting aromatic pharmacophoric residues [2]. In order to investigate structural requirements for position 2, we synthesized endomorphin analogs incorporating, instead of Pro, unnatural amino acids with six-membered heterocyclic rings, such as piperidine 2-, 3- or 4-carboxylic acids (Pip, Nip and Inp, respectively). (*R*)-Nip residue turned out to be favorable for improving μ-opioid receptor affinity. Introduction of 2',6'-dimethyltyrosine (Dmt) instead of Tyr^1 led to obtaining [Dmt^1, (*R*)-Nip^2]EM-2 which showed exceptional μ-opioid receptor affinity and high stability against enzymatic degradation in rat brain homogenate. In the *in vivo* hot-plate test in mice, this analog given intracerebroventicularly (i.c.v.), produced profound supraspinal analgesia, being much more potent than EM-2.

Tyr-X-Trp-Phe-NH_2

Tyr-X-Phe-Phe-NH_2

X= 

The structure of proline and unnatural amino acids used in the study.

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This work was supported by grants from Polish Ministry of Science No 125/N-POLONIUM/2008/0 and No NN 401 0064 35 and from the Medical University of Lodz No 503-1099-1.

Sulfonates of *N*-triazinylammonium salts as highly efficient, inexpensive and environmentally friendly coupling reagents for peptide synthesis in solution and SPPS

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Until now, the most popular coupling reagents are based on the chemistry of benzotriazole developed by Katricky. In conclusion, besides of many modifications, no particular one can be more efficient then common reactive intermediate i.e. active esters of *N*-hydroxybenzotriazole or *N*-hydroxy-8-azabenzotriazole. This important limitation of benzotriazole based reagents is particularly excruciating in difficult coupling, especially in coupling of sterically hindered substrates. In this cases significantly more reactive were found triazine based coupling reagents [1]. The other advantages of triazine coupling reagents are stability and modular structure [2] enabling multidirectional modification of the parent molecule towards congeners most sited to any given synthetic goal, synthetic strategy and reaction conditions.

Recently we attempted to develop the new family of triazine based coupling reagents derived from the salts of sulfonic acids.

Sulfonates of *N*-triazinylammonium salts were obtained by treatment of 2-chloro-4,6-dimethoxy-1,3,5-triazine with sulfonates of tertiary amines in the presence of sodium bicarbonate [3]. Taking advantage of the modular structure of triazine reagents allowing the fine tuning of their properties 4-(4,6-dimethoxy-[1,3,5]triazin-2-yl)-4-methylmorpholinium toluene-4-sulfonate and analogues were prepared by treatment of 2-chloro-4,6-dimethoxy-1,3,5-triazine with salts of N-methylmorpholine, N-methylpiperidine and N-methylpyrrolidine with toluene-4-sulfonic acid, methanesulfonic acid, trifluoromethanesulfonic acid and camphorosulfonic acid respectively.

We found all coupling reagents useful for activation of carboxylic components, with the activation rate depended mostly on the structure of tertiary amine constituent. The participation of triazine “superactive ester” as intermediate in the condensation has been proved in the model experiments. Utility of reagents was confirmed by peptide synthesis in solution and SPPS in the coupling involving Z-, Boc-, Fmoc- protecting groups affording di- pentapeptides in high yield in step by step approach and in fragment coupling. The efficacy of 4-(4,6-Dimethoxy-[1,3,5]triazin-2-yl)-4-methylmorpholinium toluene-4-sulfonate salt in SPPS was demonstrated by synthesis IAPP – amylin.

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Cyclolinopeptide a analogues containing 4-aminopyroglutamyl residue

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Cyclolinopeptide A (CLA), a cyclic nonapeptide with the sequence cyclo(-Pro[1]-Pro[2]-Phe[3]-Phe[4]-Leu[5]-Ile[6]-Ile[7]Leu[8]-Val[9]-) was isolated from linseeds and was shown to possess strong immunosuppressive activity in respect to both – humoral and cellular immune response. A peculiarity of CLA structure is the presence of cis-amide bond situated between both proline residues. Such structure of CLA was postulated by Siemion et al. in 1977 [1] and this hypothesis was confirmed by others on the base of X-ray, NMR and computational studies [2].
In the aim to answer the question, whether the *cis*-geometry of Pro-Pro amide bond is important for CLA biological activity and whether β-turn type VI is tolerated as well, we have synthesized analogues of CLA, in which Pro-Pro dipeptide segment has been replaced by all four stereochemical variants of 4-aminopyroglutamic acid residue. The effects exerted by our peptides in bioassays will be compared with those produced by natural CLA and CsA.

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Fast conventional synthesis of chemokine SDF-1α (1-68) on the symphony®

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Protein Technologies, Inc. 4675 S. Coach Dr. Tucson, AZ 85714. U.S.A. Tel:+1-520-629-9626, Fax:+1-520-629-9806, Email: info@peptideinstruments.com, Web: www.peptideinstruments.com

Human SDF-1α contains sixty-eight amino acids and is a member of the chemokine family of peptides. This long peptide was synthesized step-wise using our quality control conditions in 51 hours. The reaction times were then reduced to deprotection times of 2 x 2 min and coupling times of 2 x 2.5 min, resulting in a total synthesis time of 22 hours. The effect of different resins, resin substitutions and deprotection reagents on the crude peptide purities were compared. A small portion of crude peptide was purified using an *RP*-HPLC column and the mass of the final product was confirmed with MALDI-TOF mass spectrometry.

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Chemoenzymatic approach to the synthesis of bioactive tripeptide mimetics

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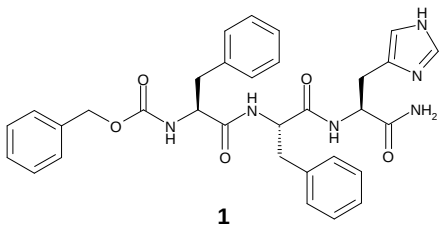
Biocatalysis is a methodology of growing importance among organic chemists aiming at the synthesis of bioactive compounds, resulting in the establishment of many successful synthetic protocols[1]. Main reason for this is the enormous selectivity of biocatalysts.



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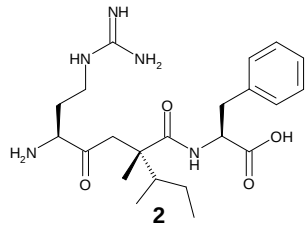

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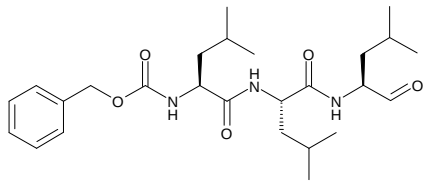

3 (MG-132)

Tripeptides and tripeptide mimetics are widely investigated due to their biological activity. Among them, an anti-inflammatory agent **1**, an antibiotic **2**, and human rhinovirus 3C protease inhibitors can be found.
Tripeptides with *C*-terminal aldehyde group are of special interest. Compound **3** (Mg-132) is a potent and selective inhibitor of 20S proteasome, which is often used as a reference in biomedical studies [2].
The results of our studies on the successful combination of mutlicomponent reactions with enzymatic transformations to the synthesis of bioactive tripeptide mimetics, will be presented [3,4,5].

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Glycosylation of peptides

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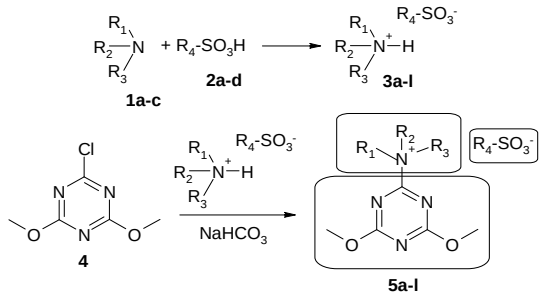
Posttranslational modifications (PTM) are covalent processing events that change the properties of a protein by the addition of a modifying group to one or more amino acids [1]. Growing evidences indicate that N-glucosylation is a PTM that may play a fundamental role in a large number of biological events [2]. The most well-known modifying groups in eukaryotic proteins are glycans. Protein glycans are classified into two groups: N-linked glycans attached to asparagine (Asn) residues and O-linked glycans attached to Ser or Thr residues. Currently, the synthesis of building blocks containing glycosyl moieties, linked to the carboxyl function of aspartic acid by an amide bond, typically requires glycosylamines and efficient coupling reagents.

In our previous studies [3], we examined triazine-based coupling reagent in synthesis of N-glycosylated-Asp derivative. A comparative study of the coupling reaction between glucosamine and aspartic acid was performed using DMT/NMM/BF_4, CDMT, HATU, TBTU, and BOP.

Recently we attempted to develop the new family of triazine based coupling reagents derived from the salts of sulfonic acids **3a-l**.



Herein, we present the studies on screening of family of N-triazinylammonium sulfonates **5a-l** in synthesis of building blocks containing glycosyl moieties and synthesis of fragment gp120 292-300 (NESVAINCT) [4] bearing two residue of N-glucosylated-Asp derivative in SPPS.

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Cyclic enkephalin-deltorphin hybrids containing a carbonyl bridge

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Six hybrid octapeptides in which the *N*-terminal cyclic structure related to enkephalin was elongated by the *C*-terminal portion of deltorphin have been synthesized. The *p*-Nitrophenyl carbamate of 1-Boc-1,2-diaminoethane was coupled to 4-methylbenzhydrylamine (MBHA) resin. The Boc group was removed by treatment with HCl/dioxane, and the peptide chain was assembled using Boc strategy. For deprotection of the amino function, HCl/dioxane was used. D-Lys and D-Orn were incorporated in position 2, and Lys, Orn, Dab, or Dap in position 5. The side chains of dibasic amino function were protected with Fmoc group. This protection was removed by treatment with 55% piperidine in DMF, and cyclization was achieved by treatment with bis-(4-nitrophenyl)carbonate. The *N*-ureidoethylamides of peptides were obtained by treatment of the peptide resin with 55%TFA in DCM. Using various combinations of dibasic amino acids, peptides containing 17-, 18-, 19- or 20-membered ring were obtained. The peptides were tested in the guinea-pig ileum (GPI) and mouse vas deferens (MVD) assays. Divers opioid activities were observed, depending on the size of the ring. Addition of the C-terminal portion of deltorphin resulted in a change of receptor selectivity in favor of the δ receptor. The conformational propensities of selected peptides were determined using the EDMC method in conjunction with NMR experiments carried out in water. This approach allowed the proper examination of the dynamical behavior of these small peptides. The results were compared with those obtained earlier with corresponding pentapeptide amides and *N*-(ureidoethyl)amides.

Microwave peptide synthesis

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The use of microwave energy sees dramatic increase in all fields of chemical synthesis applications. Since the first dedicated systems became available in the early 2000's, 1000's of peer reviewed papers have been published.
A new technique for microwave-assisted, solid-phase peptide synthesis is shown, that allows for coupling times per amino acid as short as five minutes, deprotection time as short as 3 minutes and cleavages within 15-30 minutes. In the LIBERTY solid phase peptide synthesizer, a single-mode microwave cavity is used to allow for a high microwave power density and a uniform field distribution, enabling totally automated synthesis of up to 12 peptides in a day with the fastest cycle times and greater product purity than conventional synthesis.
Microwave enhanced peptide synthesis produces cleaner peptides with higher yields. The specific interaction of a high energy microwave field with the highly polar peptide bond allows for faster molecular motion resulting in much shorter synthesis times per coupling step. Crude purities can be increased from 10% in traditional methods to 84% in microwave solid phase synthesis (e.g. Galanine synthesis).
Microwave energy allows for higher resin substitution and less excess reagents to be used, increasing scale-up potential when compared to traditional methods. Chain aggregation is lessened by microwave energy allowing for better reagent accessibility and the possibility of longer peptide sequences.

Simulations of protein folding thermodynamics using UNRES physics-based coarse-grained force field with replica exchange molecular dynamics

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Recently, we implemented a physics-based coarse-grained force field (UNRES) for mesoscopic molecular dynamics (MD) [1]. Initial results from UNRES MD simulations show that we are able to simulate folding events that take place in a microsecond or even a millisecond time scale. We compared three generalized-ensemble algorithms, namely, a replica exchange method (RE), a replica exchange multicanonical method (REMUCA), and a replica exchange multicanonical method with replica exchange (REMUCAREM), in both Monte Carlo and MD versions, to determine the thermodynamic characteristics of the UNRES force field [2]. Of those, the REMD method, especially in its multiplexed version (MREMD), turned out to be the most efficient. The multiplexed variant (MREMD) of the RE method, developed by Rhee and Pande [3], differs from the original REMD method in that several trajectories are run at a given temperature. Each set of trajectories run at a different temperature constitutes a layer. Exchanges are attempted not only within a single layer but also between layers. We present a detailed comparison of canonical MD, REMD, and MREMD simulations of protein folding with the UNRES force-field [4]. The RE method for umbrella sampling simulations has also been enhanced by applying a multidimensional extension in which pairs of replicas, not only at different temperatures, but also with different parameters of the potential energy are exchanged. Recently, we developed a fine-grained version of the UNRES code in which energy and force calculations have been split among a number of processors, thus introducing two-level (coarse-grained and fine-grained) parallelism. Each coarse-grained task handles a single MD trajectory in canonical MD or MREMD simulations and has a number of slave fine grain processors to compute energy and forces. Owing to fine-grained parallelism, we are now able to carry out ab initio simulations of large proteins in real time.

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ECD-MS investigation on deuterium distribution in the high pressure denatured ubiquitin

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Hydrogen deuterium exchange is an important method in studies of protein folding and conformation. The level of the protein deuteration may me determined by mass spectrometric measurement, since the exchange the single proton for deuteron increases the molecular mass of protein by 1 Da. In previous studies the spatial distribution of deuterium in the protein molecule was determined by combination of peptic hydrolysis and LC-MS. Recently spectacular development of HR-FT mass spectrometry creates the possibility of studying distribution deuterium in protein by "top down" approach, by direct gas phase fragmentation of intact, deuterated protein. The electron capture dissociation is a method of choice for such experiments, because provides many fragments covering the whole sequence of the protein with relatively low level of hydrogen scrambling – dislocation the deuterons along the protein sequence during the fragmentation process.

In our presentation we will describe research on determination the distribution the deuterium atoms in the ubiquitin molecule denatured under high pressure. The protein was exposed for high pressure causing the complete, reversible denaturation in a buffer containing D_2O. After decompression the protein was diluted with water. After 1 hour back exchange the ubiquitin sample was subjected for ECD experiments. The obtained fragments were identified and we calculated the content of deuterium in particular fragments to determine distribution of deuterium in ubiquitin molecule.

Conformational properties of *N*-acetyl-(*E)*-dehydrophenylalanine *N',N'*- dimethylamide

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The introduction of conformationally constrained amino acid residues into bioactive peptides is a useful and frequently applied modification method for studying resulting active conformation and to design new potent analogs. One group of such residues, found in naturally occurring peptides, is α,β-dehydroamino acids. The $C^{\alpha}=C^{\beta}$ double bond of dehydroamino acids makes the rotation of the side chain impossible and only the positions (*Z)* or (*E)* can be adopted. Receptor proteins frequently discriminate quite precisely between the (*Z*) and (*E*) disposition of the double bond $C^{\alpha}=C^{\beta}$ in their bioligands. Much more is known about the conformational preferences of (*Z*)-α,β-dehydropeptides than those of their (*E*)-analogues, because most of the preparative procedures yield exclusively or predominately the former isomers.

N-Alkylation of biologically active peptide is the next strategy which can also results in analogues with improved pharmacological properties, such as resistance to enzymatic degradation, receptor selectivity, enhanced potency and bioavailability. Our idea is to combine the conformational limitations of (*E*)-dehydrophenylalanine ((*E*)-ΔPhe) with the methylated C-terminal amide bond. The properties of Ac-(*E*)-ΔPhe-NMe_2 were studied by theoretical calculations, X-ray crystallography, FTIR and NMR spectroscopy. The theoretical and experimental results were compare with those of the related molecule Ac-(*Z*)-ΔPhe-NMe_2.

The Ramachandran maps of the Ac-(*Z,E*)-ΔPhe-NMe_2 were calculated by the MP2 and DFT methods *in vacuo* and in chloroform and aquerous solutions using polarizable continuum model (PCM). The minima observed on the surfaces were fully optimized at the MP2/6-31+G** and B3LYP/6-31+G** levels. The stability of the low-energy conformers were discussed in terms of the conventional N-H···O hydrogen bond as well as the weaker NH···N, C-H···O and N-H···π(phenyl) interactions. Also noncovalent C=O dipol attractions were analyzed. The lowest-enegy structure of Ac-*E*-ΔPhe-NMe_2 is the extended conformer E (ϕ, ψ = -174º, 129º) stabilised by the C_5 H-bond closing a five-membered ring and the lowest–energy conformer of Ac-*Z*-ΔPhe-NMe_2 is the conformer H (ϕ, ψ = -38º, 127º) stabilized by the N-H···π(phenyl) interactions.

The theoretically obtained results were compared to the experimental data of the studied molecule. The X-ray structural parameters, FTIR and spectroscopy results are in good accordance with theoretical findings.

Aneta Buczek is a recipient of a Ph.D. fellowship from a project funded by the European Social Fund.

NMR studies of cyclic dynorphin analogues

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Previously we have reported results of our studies of cyclic dermorphin and deltorphin analogues containing a carbonyl bridge [1-3]. In this presentation we report NMR studies of new biologically active opioid peptide analogues, which contains modified enkephalin sequence (*message*) [4] with residues Daa, Dxx: **1 –** D-Lys, Lys; **2 –** D-Lys, Orn; **3 –** D-Lys, Dap; **4 –** D-Orn, Lys; **5 –** D-Orn, Orn; **6 –** D-Orn, Dab; **7 –** D-Orn, Dap and C-terminal sequence of dynorphin-(1-13) (*address,* Arg-Arg-Ile-Arg-Pro-Lys-Leu-Lys-OH).

NMR spectra of peptides **1, 3, 4** and **7** were measured at 25 °C on a UNITY500plus (Varian) spectrometer. TOCSY and gHSQC spectra were measured for assignment of all proton signals. ROESY experiments were performed in order to obtain distance restraints.

The biological activity of peptides was measured with GPI and MVD tests and to have insight into relationship between this activity and conformation. The conformational space of each peptide wear explored using the EDMC method and NMR restraints.

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Phosphorylated quinines as novel polyfunctional chiral auxiliaries for NMR spectroscopy

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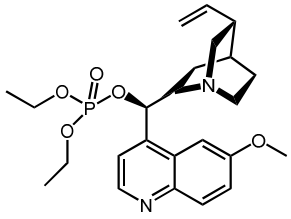
Quinine, the major component of *Cinchona* alkaloids, represents one of the most privileged molecule exploited in chiral recognition applications. Its versatility and efficiency is commonly attributed to the presence of several functional groups, which can be also modified in order to modulate their enantiodiscrimination potency. Thus, cinchona alkaloids and their derivatives have demonstrated widespread potentiality, being successfully employed as chiral resolving agents, chiral auxiliaries, catalysts in asymmetric processes and new chiral stationary phases for HPLC. Finally, they have been found to be chiral solvating agents (CSAs) of great application utility in NMR techniques, employed for determination of the enantiomeric composition of various analytes. Among modified quinine based CSAs considerable attention has been dedicated to carbamoylated derivatives.

In this work, we explore novel possibility of C9 hydroxyl derivatization and its influence on the enantiodiscrimination effect. The designed and performed functionalization led to the appropriately phosphorylated quinines (**1**, **2**) which could offer new interactions with analytes *via* oxygen atom rich moieties.



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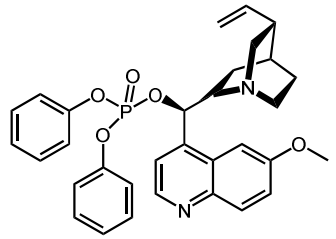

2

The modified compounds were subsequently validated as CSAs in ^{1}H NMR as well as ^{31}P NMR spectroscopy toward a collection of model chiral selectands. The introduction of a phosphoryl group was proved to enhance enantiodifferentiation effect, particularly for protected amino acids and their derivatives, in comparison to the parent molecule.

Peptidomimetics

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The term peptidomimetics covers a large and expanding field of research that has achieved profound successes. Peptidomimetic is a compound containing non-peptidic structural elements that is capable of mimicking or antagonizing the biological action(s) of a natural parent peptide and offering the advantages of increased bioavailability, biostability, bioefficiency, and bioselectivity.

The importance of these compounds is seen by introduction of term "peptidomimetics" into *Google*, what gives over 92,000 entries, whereas "peptidomimetic" yields around 90,000 files.

They typically arise from modification of an existing peptide in order to alter its properties. Examples of peptidomimetics have been isolated as natural products, synthesized as libraries from novel subunits, and designed on the basis of X-ray crystallographic studies and through an intricate knowledge of the biological mode of action of natural peptides. They offer challenging synthetic targets and are increasingly important medicinal agents and biological probes. As a consequence, peptidomimetics embrace much of what is modern medicinal and organic chemistry.

A variety of molecules have been designed to mimic the secondary structures of peptides, such as α-helices, β-turns, and β-sheets. In order to explore the structure-activity relationships of bioactive peptides, a number of strategies have been developed by incorporation of conformationally constrained amino acids, modification of the peptide backbone by amide bond isosteres, cyclizations, attachment of pharmacophores to a template or scaffold, and the synthesis of nonpeptide analogs. Thus, great strides have been made in the design of peptidomimetic drugs for the treatment of various pathological states.

α-Amino aldehydes as components for 1,2,3,4-tetrahydro-β-carbolines synthesis

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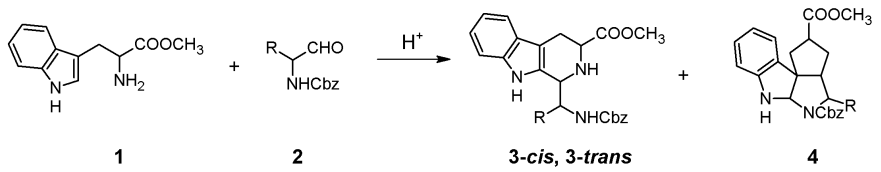
Tryptophan moiety is a structural motif of a large group of natural products (alkaloids, peptides). Cyclic analogues of tryptophan introduce local constraints and reduce the flexibility of the indol moiety. One of the possibilities to freeze indol moiety of tryptophan is the linkage of the indol ring with amine group by substituted methylene unit to form 1,3-disubstituted 1,2,3,4-tetrahydro-β-carbolines. Such compounds are synthesized by the Pictet-Spengler reaction.
We studied in detailes Pictet-Spengler reaction with the use of Trp-OMe as amine- and α-amino aldehydes as carbonyl-components. P-S reaction with amino aldehydes with NH-protection is very sensitive to conditions, particularly solvent and temperature. Amino aldehydes may epimerize and then small library of products with different stereochemistry is formed, especially in protic solvents.



Scheme 1. Possible products of the Pictet-Spengler reaction.

We report the influence of conditions on the ratio of libraries' components. We found that in aprotic apolar solvents compounds with additional 6-membered ring are formed. We also investigated conformation of the 6-membered ring in *cis* and *trans* isomers. Our results show that conformation of the newly created 6-membered ring depends on the substrates and the size of C-1 and C-3 substituents.

Selenopeptides and the integrated oxidative folding: technological advances in studying disulfide-rich peptides

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Many peptides from plants or animal venoms are an important source for novel biotherapeutics. Studying multiple cysteine-containing peptides requires optimization of their oxidative folding and the time-consuming determination of disulfide bonds connectivity. These limitations persuaded us to apply a selenocysteine approach to three disulfide bridged μ-conotoxin SIIIA that block sodium channels [1] and to a member of squash family, the *Ecballium elaterium* trypsin inhibitor II (EETI-II) [2]. Taking advantage of the isosteric nature of selenium and sulfur, we replaced a pair of cysteine residues (Cys) with selenocysteine residues (Sec) in each synthetic peptide. The more stable diselenide bond (E_0= -381mV) than the disulfide bond (E_0=-180mV) reduced the number of possible disulfide connectivities and improved the oxidative folding. We also demonstrate that all of the studied μ-selenoconotoxins block $Na_v1.2$ subtype of voltage-gated sodium channel, and all seleno-containing EETI-II analogues exhibited inhibitory activities toward trypsin. Next, we merged the selenopeptide technology with the NMR-based disulfide mapping by combining diselenide bridges with position-specific (^{13}C,^{15}N)-labeled disulfide bonds in a single peptide. Such "integrated oxidative folding" strategy allowed us to confirm the correctly folded selenoconotoxins by 2D NMR spectroscopy experiments.

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α, β-Hybrides of opioid peptides containing β^3-*homo*-amino acids

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The discovery of numerous endogenous neuropeptides that participate in the formation, transmission, and perception of pain offers numerous strategies for the development of new analgesics. Since the discovery of the endogenous opioid peptides, abundant analogues have been synthesized in attempts to develop an analgesic without the serious side effects.

Opioid peptides have long acted as model compound for the development of a new analgesic drug. A major problem with opioid peptides as drugs is their susceptibility to enzymatic hydrolysis when administrated *in vivo*. Several chemical approaches, such as the incorporation of D-amino acids, unnatural amino acids, α-α-disubstituted amino acids, cyclic moieties, or cyclization of peptides have resulted in obtaining more stable analogues. Among the numerous strategies of modification the substitution of proteinogenic amino acids with β-amino acids represents an interesting possibility.

In this communication we report the synthesis of new α,β-hybrides of opioid peptides containing β^3-*homo*-amino acids. The peptides were tested to explore their effect on opioid receptors binding. The β^3-*homo*-amino acids were obtained from respective α-amino acids by Arndt-Eistert homologation. α-β-Hybride of peptides were synthesized in solution using Boc-strategy.

Posters

All posters will be presented during the three poster sessions:

Poster Session I	Tuesday morning, 10:50-12:00
Poster Session II	Tuesday evening, 15:30-16:30
Poster Session III	Wednesday evening, 16:30-17:30

A roman numeral displayed by your poster number indicates a poster session e.g. P12/II corresponds to the poster carrying number 12 that will be presented in the evening on Thuesday (poster session II).

Tyrosine derivatives modified with diazonium salts – ESI-MS/MS and ESI-ECD studies

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The determination of amino acid residues localized on protein surface is an important method in the search for protein-protein interactions. The external tyrosine residues could be investigated using the coupling reaction. The reaction of phenols with diazotized aniline to form an azo dye is simple to conduct, relatively safe and efficient. Moreover, the colored products allow for easy monitoring of the reaction and distinguishing the respective fragments in tryptic digest. Collision induced electrospray dissotiation (ESI-MS/MS) is the method of choice in peptide and protein research and could efficiently be used to compare the modified and native proteins and their fragments. Electron capture dissociation (ECD) gives better sequence coverage with very little fragmentation of modified side chains. Therefore, ECD is method of choice for mapping of the chemical and post-translational modified proteins.

In this study we present the ESI MS/MS and ECD analysis of azo dyes consisting of tyrosine derivatives modified with series of diazotized anilines and the specific ions suitable for monitoring procedure.

In our experiments we selected the azo dye from p-bromo aniline because the specific isotope distribution of bromine (^{79}Br:^{81}Br ,1:1) creates characteristic isotopic pattern in MS spectrum, which could be used to unambiguously identify modified fragments.

To the best of our knowledge, neither CID nor ECD fragmentation patterns of the amino acid azo dye have been proposed yet.

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Antimicrobial activity of dipeptides with 3-[2-(2'-methoxy-4'-dimethylaminophenyl)benzoxazol-5-yl]alanine

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3-(2-benzoxazol-5-yl)alanine derivatives are unnatural amino acids with some antimicrobial [1] and cytotoxic [2] potential. According to literature, substituents in position 2 and 5 of the benzoxazole ring are the most important for the biological activity of the compound [3]. Among already studied derivatives substituted in position 2 of the benzoxazole, the most active one against bacteria and fungi is 3-[2-(2'-methoxy-4'-dimethylaminophenyl)benzoxazol-5-yl]alanine [1]. To determine an influence of modification in position 5 of the benzoxazole, the series of dipeptides with above-mentioned derivative was synthesized. All proteinogenic amino acids, except cysteine, were attached to C- and N-terminus of the benzoxazolylalanine, respectively. Dipeptides were synthesized in solution using Boc/Bzl tactics. The activity of all obtained peptides was screened against model Gram-positive (*Bacillus subtilis*) and Gram-negative (*Escherichia coli*) bacteria whereas antifungal activity was tested against yeast *Pichia pastoris*. All tests were performed using antibiogram method whereas the minimal inhibitory concentrations were determined using two-fold serial dilution technique. It was found that dipeptides studied had greater antibacterial than antifungal potential in contrast to the parent compound. Also, the activity depends on the structure of the compound. Additionally, the active derivatives were then tested against most important pathogens.

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Acknowledgement:
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Bcp^{1-} and $Dbcp^{1-}$ analogues of TIPP: potent and highly selective delta opioid agonists and antagonists

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The novel phenylalanine analogues 4'-[*N*-((4'-phenyl)-phenethyl)carboxamido]phenylalanine (Bcp) and 2',6'-dimethyl-4'-[*N*-((4'-phenyl)-phenethyl)carboxamido]phenylalanine (Dbcp) were synthesized and substituted for Tyr^1 in the δ opioid antagonist TIPP (H-Tyr-Tic-Phe-Phe-OH; Tic = tetrahydroisoquinoline-3-carboxylic acid) and in TIPP analogues. Unexpectedly, [Bcp^1]TIPP turned out to be a potent and selective δ opioid *agonist* in the mouse vas deferens assay, whereas [$Dbcp^1$]TIPP was a potent antagonist with similarly high δ receptor binding affinity. Docking studies using models of the δ opioid receptor in the inactive and activated state indicated that the two peptides had overall similar binding modes, except for the large, hydrophobic biphenylethyl moiety which occupied distinct receptor subsites with the agonist and the antagonist docked to the active and inactive form of the receptor, respectively. The different positioning of the biphenyl group of the two peptides appears to be due to additional hydrophobic interactions of the 2',6'-dimethyl groups of $Dbcp^1$ in the antagonist with aromatic receptor residues. The C-terminally truncated analogues H-Bcp-Tic-Phe-OH and H-Bcp-Tic-OH were δ antagonists with a weak δ partial agonist component, whereas the dipeptide H-Dbcp-Tic-OH was a δ antagonist with subnanomolar δ receptor binding affinity and very high δ receptor selectivity. Overall, the performed docking studies indicated that the δ receptor can accommodate the biphenylethyl moiety contained in the 1-position residue of TIPP-related peptides in a number of different binding modes that promote the interaction of the ligand with the receptor in either an activated or an inactive state.

Application of sustained off resonance irradiation (SORI) mass spectrometry for structural analysis of PEG-peptide hybrids

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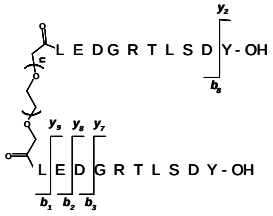
Sustained off resonance irradiation collision-induced dissociation (SORI-CID) is a general method of collisional activation of ions in Fourier transform mass spectrometric experiments. The fragmentation occurs by irradiating ions with a single off-resonant sine wave excitation pulse while the background gas pressure is raised to appr. 10^{-6} torr. The resulting large number of sequential low-energy collisions slowly heats the activated ions, therefore the dissociation mainly progresses through the lowest energy fragmentation channels. SORI-CID has, therefore, been extensively used for structural analysis of various biopolymers [1].

Our previous studies showed that the amino-terminal dimerization of peptides results in enhanced biological activity and the potency of the conjugates depends on the length of the linker. We decided to join fragments of bioactive proteins by a flexible bridge - poly(ethylene glycol). PEGylation increases solubility in physiological fluids as well as their plasma half-life and resistance to proteolytic cleavage [2,3,4].

We analyzed patterns of fragmentation of PEG-peptide conjugates using SORI-CID method and compared the results with that obtained by classical collision induced dissociation in MS/MS as well as electron capture dissociation (ECD). We performed a series of experiments using different collision energy and identified the fragments. The fragmentation of the PEG-bridges was observed for the pegylated amino acids only but not for PEG-peptide conjugates. This may suggests that the peptide part of the conjugate is more susceptible for both CID and ECD as compared to PEG chain.



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Investigation of surfactin composition in environmental strains of *Bacillus subtilis*

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Surfactin, cyclic lipopeptide produced by various strains of *Bacillus subtilis* is one of the most powerful biosurfactant ever discovered – a 20 µM solution decreases the surface tension of water from 72 to 27 mN m^{-1}. In addition to its strong surface activity, surfactin also exhibits strong antibacterial and antifungal properties. Those factors make it important for the biological control of plant pathogens [1]. Two strains of *B. subtilis*, previously isolated from soil, are able to control the phytopathogenic bacterium *Dickeya dianthicola* (former name *Erwinia chrysanthemi*), cause of blackleg in potato plants in the field and soft rot of tubers in storage. The work presented in this report was aimed at studying the surfactin production and composition of its isoforms in laboratory and natural isolates of *B. subtilis*. Detection of biosurfactant production was determined by oil spreading and drop collapse methods allowing screening and preliminary quantification of biosurfactant production [2]. Selected cultures are then more precisely investigated by the HPLC and LC-MS methods [3]. Natural isolate MB5 produces two times more surfactin as compared to the wild type strain *B. subtilis* 3610. Retention times of the isoforms peaks are the same, but areas are different. Analysis indicates, that *B. subtilis* laboratory strain 168 does not excrete such compound, which is consistent with the fact, that this strain is unable to produce lipopeptides by a frameshift mutation in the *sfp* gene. Surfactin is of great industrial interest, however it is not widely available because of low strain productivity and high recovery expenses. The enhanced production of surfactin by MB5 strain described here, connected with nutritional factors manipulation might result in more optimized yields of the lipopeptide.

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The impact of the cyclopeptide structure on the Cu(II) binding

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The peptides are an effective ligands for metal ions. A specific group of the peptides are peptides with the cycle structure of the peptide chain. The cyclopeptides play various roles in living organisms [1,2].

We present potentiometric as well as spectroscopic studies on the interaction of the copper(II) ions with three novel cyclohexapeptides: c(GHR**HHG**), c(GHR**HGH**) and c(GHR**GH**).

The insertion of the third His residue in the cyclopeptide sequence and increase of cyclopeptide ring size has not large impact on the Cu(II) binding as compared to the cyclopeptides with two His residue [3]. Additionally, three His residues in the peptide sequence does not force system to insert metal ion inside peptide cycle.

Independently from the size of the peptide cycle and numbers of the histydyl residues in the peptide sequence, the cyclopeptides prefer the $\{2xN_{Im}, 2xN_{amide}\}$ binding mode at the physiological pH and does not form complexes with four deprotonated amides.

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Effect of ε-aminocaproyl-S-benzyl-L-cysteine on amidolytic activity of saliva

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The ε-aminocaproyl derivatives of α-aminoacids with a bulky, hydrophobic side chain markedly inhibit the fibrinolytic activity of plasmin and the euglobulin fraction of plasma. Aminocaproyl-S-benzyl-L-cysteine (H-EACA-S-Bzl-L-Cys-OH) was the most active compound with selective antifibrinolytic activity. Its effect on fibrinolytic activity of plasma euglobulin fraction was lower than similar activity of clinically used antifibrinolytics: ε-aminocaproic acid (EACA) and t-aminomethylcyclohexanecarboxylic (AMCHA) [1, 2]. This compound shows only a slight effect on the amidolytic activity of tissue plasminogen activator (t-PA) and urokinase [3]. Recently, t-PA was shown to be to be physiologically active in human saliva [4]. In our examination of saliva fibrinolytic activity, we observed that H-EACA-S-Bzl-L-Cys-OH showed the higher antifibrinolytic activity in comparison with EACA and AMCHA. In order to explain this activity, we examined the effect of this dipeptide on amidolytic activity of human saliva with the use of synthetic substrates, specific for t-PA, plasmin and trypsin. H-EACA-S-Bzl-L-Cys-OH was the inhibitor of saliva amidolytic activity specific for tissue plasminogen activator.

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Dimerization of the immunosuppressory decapeptide fragmnet of ubiquitin. Synthesis and immunomodulatory activity

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Dimerization of the receptors is an essential step of various cellular signal transduction processes. Therefore, substances that are able to modulate the receptors dimarization may control such a process and are potential immunomodulators. Analogs of dimeric ligands may enhance interactions between two neighbouring receptors.

Previously, we designed the dimeric analogs able to mimic the dimeric nature of the nonapeptide VPRSGEVYT immunosuppressive fragment of HLA-DR superdimer molecule. We used the polyethylene glycol (PEG) to serve as a flexible linker as well as a group which improves the solubility of the dimeric analogs in water. N- and C-terminal dimerization of this immunosuppressive fragment of HLA-DR molecule resulted in the increase of the biological activity [1,2]. It proves our hypothesis that receptors of HLA-DR molecule fragment exist as dimers.

Our previous research showed that different fragments of ubiquitin exhibited very strong immunosuppressive activity, comparable to that of cyclosporine [3,4]. We believe that the ubiquitin fragments may also form dimeric forms and that this dimerization is responsible for their immunosuppressory activity. In order to check this suggestion we designed new dimeric analogs of ubiquitin fragments with two peptide chains connected in three different ways: head to head, tail to tail and head to tail.

The synthesized dimeric peptides were purified by preparative HPLC. The purity and identity of the products was analyzed by analytical HPLC, HR ESI-MS and HR ESI-MS/MS. Influence of such compounds on humoral immune response was checked. Effect of different method of dimerization on immunosuppressive potencyof the obtained products will be presented.

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Coordination abilities of the peptides with β–Asp residue in their sequence. The role of the α–COOH group in Cu(II) binding

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Peptides containing β-amino acid may form stable folded structures analogous to those encountered in naturally occurring proteins. Conformational stability of β–peptides is greater than the α–peptides [1]. The incorporation of β–amino acids has been successful in creating peptidomimetics that not only have potent biological activity, but are also resistant to proteolysis [2].

We choose to our research biological active peptides, *i.e*. fragments corresponding to the sequences of: the thymopoietins $^{32\text{-}36}$ [3], the fibrinopeptide A $^{1\text{-}4}$ [4] and the ubiquitin $^{50\text{-}59}$ [5]. All of those peptides have α-Asp in the sequence. To check the influence of β-Asp in coordination mode we decided to modify those peptides by replacing α-Asp with β-Asp: H-Arg-Lys-**βAsp**-Val-Tyr-OH (**TP**), H-Ala-**βAsp**-Ser-Gly-OH (**FP**) and H-Leu-Glu-**βAsp**-Gly-Arg-Thr-Leu-Ser-Asp-Tyr-OH (**UQ**).

The group of peptides containing β–Asp were investigated towards copper (II) ions. For each peptide-copper(II) system a detail spectroscopic (EPR, CD, UV-VIS) and potentiometric analysis have been done.

All studied peptides form stable complexes with Cu(II) ions (from 1N to 4N coordination mode with amine group and amide nitrogen). The obtained results were compared with α–aspartic acid analog peptides.

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Voltamperometric study of the lysine peptide chain containing anthraquinone moiety

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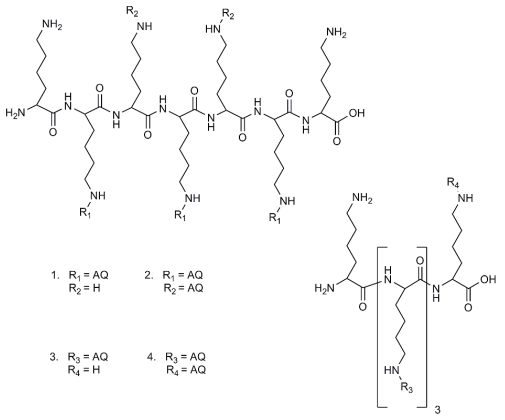
Anthraquinones are very interesting compounds for the investigations in electrochemical chemistry due to the fact that they contain electrons, the reducible p-quinone system and are electroactive [1,2]. The electrochemistry of quinone species differ in aqueous and non-aqueous environments. In aqueous solution the reduction process shows a single, two-electron reduction peak, while in aprotic solvent quinones undergo two, one-electron reduction processes.The design of aminoacid attached to a redox unit has been an important subject to develop a practical electrochemical method to determine electron transfer per molecule and to determine the diffusion coefficient of the primary substrate of quinone moiety [3,4]. The peptides (Scheme 1) were synthesized manually by a solid-phase method using the Fmoc/Boc strategy [5] using L-N^{ε}-(9,10-dioxo-9,10-dihydroanthracen-1-yl)-lysine [6].



Scheme 1

The quinone is attached with the lysine peptide chain and then can act as the transduction agent. Discussion of an influence of increasing number of anthraquinone species on the electron transferred during the electrode reaction will be presented.

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Voltamperometric studies of the behavior of aminoanthraquinone in Lys - Ala peptide chain

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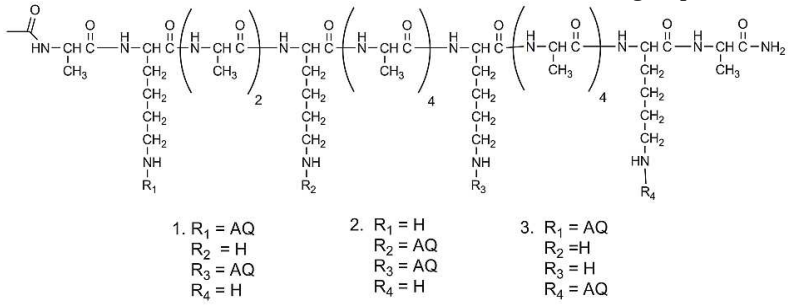
The anthraquinone derivatives constitute an important class of antitumor drugs, such as anthracycline, anthrapyrazoles which mainly can act by intercalating the base pairs of DNA of the cells of tumor tissues [1]. Anthraquinone posses planar ring system which connected with polypeptide has a significant biological and redox properties [2].

Electrochemical studies of peptides derivatives with quinone system can provide new mean of analysis and characterization. The location size and shape of voltammograms depends upon the extent of interaction of the substituent groups on the aminoacid chain and the rate of electron transfer between these groups.



1. R_1 = AQ, R_2 = H, R_3 = AQ, R_4 = H
2. R_1 = H, R_2 = AQ, R_3 = AQ, R_4 = H
3. R_1 = AQ, R_2 = H, R_3 = H, R_4 = AQ

Scheme 1.

The electrochemical behavior of Lys – Ala peptide chain was investigated in water (pH=7) and dimethylosulfoxide (DMSO) solutions by cyclic voltammetry and chronoamperometry at a glassy carbon and platinum electrode. The results are consistent with reversible reduction of the polymer in an n_p electron reaction, where n_p is near or equal to the number of substituent electroactive 1-aminoanthraquinone groups per molecule. The shape of the voltammetric response suggests no interaction between these electroactive centers. The peptides (Scheme 1) were synthesized manually by a solid-phase method using the Fmoc/Boc strategy using L-N^{ε}-(9,10-dioxo-9,10-dihydroanthracen-1-yl)-lysine. Presented results are the explanation of electro-conducting amino acid chain of lysine-alanine containing two redox moieties in peptide chain.

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Synthesis of pseudodipeptides containing aminomethylenephosphinic bond and their activity towards leucine aminopeptidases

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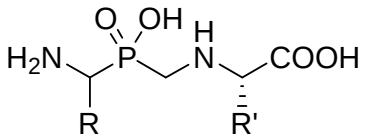
Title compounds were synthesized in a Mannich-type, three component condensation using amino acids, formaldehyde and N-protected phosphinic acids, followed by the deprotection of the adduct. Application of the amino acids containing the secondary amino group (for example *N*-benzyl) allowed to obtained the products with good yield and purity. Contrarily, the presence of the non-substituted N-termini caused a range of side reaction. Their products, including various cyclic compound, were separated and identified.



Final pseudodipeptides of novel modified backbone, bearing selected P1 and P1' structural fragments, were tested for their inhibitory activity towards cytosolic (LAP, E.C.3.4.11.1) and microsomal leucine aminopeptidase (APN, E.C.3.4.11.2). They appeared moderate competitive inhibitors of both peptidases, exhibiting higher affinity to APN.

Fast conventional synthesis of parathyroid hormone (1-84) on the Symphony®

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Email: info@peptideinstruments.com,
Web: www.peptideinstruments.com

Human parathyroid hormone (1-84) (PTH) is produced by the parathyroid glands and regulates calcium and phosphate metabolism. PTH acts on PTHR1 receptors to stimulate bone formation and is used as a treatment for osteoporosis [1]. This long peptide was synthesized step-wise using classical conditions in 144 hours (6 days). The reaction times were then reduced to deprotection times of 2 x 1 min and coupling times of 2 x 2.5 min, resulting in a total synthesis time of 28.3 hours. The effect of different resins and coupling reagents on the crude peptide purities were compared. A small portion of crude peptide was purified using an *RP*-HPLC column and the mass of the final product was confirmed with MALDI-TOF mass spectrometry. All syntheses were performed on a Protein Technologies, Inc. Symphony® or Prelude™ peptide synthesizer.

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Design and synthesis of new cathepsin L inhibitors with potential anticancer properties

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Natural isothiocyanates inhibit lysosomal cathepsins. Lysosomal cathepsins belong to a cysteine proteinases family and are well known to be involved in cancer growth and progression. The primary role of cathepsin B, which is responsible for extracellular matrix destruction and MMP's activation, is well established, whereas the cathepsin L function is still not fully understood. However its essential role in heparanase activation was proved [1].

Isothiocyanates are natural compounds with strong anticancer properties. Although the mechanism of action is not yet fully known their role in tubulin degradation [2,3] and cancer prevention through the inhibition of phase I enzymes [4,5,6] as well as phase II enzymes activation [7,8] was shown.

Inhibition of papain by some natural isothiocyantes was discovered 30 years ago [9]. We have found that natural isothiocyanates, including 2-phenylethyl isothiocyanate, 4-methoxybenzyl isothiocyanate and allyl isothiocyanate are highly potent inhibitors of cathepsin L in nanomolar range. The mode of inhibition as well as an influence on cathepsin B activity is under investigation. It needs to be established if such antiproteolytic activity *in vitro* will show a relationship with potential anticancer properties *in vivo*.

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α-Hydroxy and α-amino phosphonates – quinine derivatives

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Quinine molecule represents one of the most attractive natural chiral structure. It is commonly used to construct stereoselective catalysts applied in asymmetric synthesis of organic compounds. Quinine and its derivatives are effective enantiodiscrimination agents used in various separation and analytical techniques. For example, stationary phases modified with *O*-carbamoylated quinines are chiral solid supports in chromatography of amino acids [1]. The same compounds, acting as chiral chemical shift reagents in NMR techniques, help to determine the enantiomeric excess of analyzed substances [2]. Phosphoroorganic derivatives of quinine could be used in related application, but their analysis would employ faster and simpler for interpretation ^{31}P NMR spectra. However, there is a need for synthesis of such compounds stereomerically pure and then their evaluation for enantiodiscrimination properties.

In this work we present the results of our study on synthesis of these kinds of derivatives. The designed modifications were achieved by transformation of vinyl group to the carbonyl one, then obtained aldehydes were converted to α-hydroxyandhttp://www.organic-chemistry.org/synthesis/C1P/phosphonates/ hydroxy-phosphonates.shtm α-amino phosphonates. The phosphorylation of the hydroxyl group with chlorophosphates was also performed.

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Novel phosphonic and phosphinic inhibitors of ureases

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Sustainable nitrogen management is a must in modern agriculture regulated by growing number of legislation directives defining obligations which regard crop fertilizing and management of livestock manure. Urea constitutes over 50% global N-fertilizer usage which is accompanied by growing application as animal feed additive. Regardless the source of urea deposited in soil it undergoes hydrolysis catalyzed by urease (urea amidohydrolase, E.C.3.5.1.5). Urease product ammonium serves as the primary substrate in the two-step nitrification process carried out by autotrophic nitrifying bacteria. Enhanced ureolysis and nitrification in urea fertilized soils results in 50 % N-losses due to ammonia volatilization and NO3- leaching. Strategies to prolong maintenance of urea derived nitrogen in the NH^{4+} form which can be utilized by plants or incorporated into soil organic matter by simultaneous use of nitrification and ureolysis inhibitors are of high agronomic and environmental impact. The most active urease inhibitors phosphordiamidates and thiophosphordiamidates lack stability due to rapid P-N bond hydrolysis. Novel group of urease inhibitors based on P-C bond system were designed and obtained. The compounds represent expanded transition state analogs of urease reaction derived from *P*-methyl-aminomethanephosphonic acid (structural analog of phosphoric acid diamide). Urease was purified from *Bacillus pasteurii* CCM 2056^{T} using Q-Sepharose and Cellufine phosphate and urease microtitre assay based on Berthelot reaction was developed to evaluate inhibitory activity. All studied compounds showed competitive reversible type of inhibition against urease with inhibition constants in nanomolar range for the the most active N,N-(dimethyl)-aminomethanephosphonic acid and N,N-(dimethyl)-aminomethanephosphinic acid.

Physicochemical and antimicrobial properties of short cationic lipopeptides

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Most of natural occurring host defense antimicrobial peptides (AMPs) have positive net charge. Cationic charge and amphipathicity of peptide determine antibacterial and antifungal activity of this kind of molecule.

The purpose of this study was to synthesize a group of short cationic lipopeptide similar in structure to AMPs, but instead of well defined amino acid hydrophobic region, containing palmitic or miristic fatty acid. The lipopeptides were synthesized by the solid-phase procedure using 9-fluorenylmetoxycarbonyl (Fmoc) metodology [1] and purified by solid phase extraction (SPE) with octadecyl RP phase [2]. The lipopeptides were subjected to microbiological tests: MIC (Minimal Inhibitory Concentration) and MBC (Minimal Bactericidal Concentration) on reference strains of *Staphylococcus aureus* ATCC 6538, *Bacillus subtilis* ATCC 9372, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 9027, *Klebsiella pneumoniae* ATCC 700603, *Proteus vulgaris* ATCC 6038, according to the procedures outlined by the National Committee for Clinical Laboratory Standards (NCCLS).

Synthetic lipopeptides with net charge from +2 to +4 containing palmitic acid at N-terminus (Pal-KK-NH_2, Pal-KKK-NH_2, Pal- KKKK-NH_2) were most active against Gram positive and less active against Gram negative bacteria among all tested compounds. All lipopeptides were found to have no activity against *Proteus vulgaris*.

Due to that short synthetic lipopeptides have surfactants' structure (surfactant = **surf**ace **act**ive **a**g**ent**) such as hydrophobic tail and hydrophilic head, we investigated their surface active properties. For this measurements we used automatic tensiometer. The surface tension was measured at different concentrations of lipopeptides in distilled water with a du Noüy ring method at 20˚C. The surface tension vs. concentration plots were used to determine critical micelle concentration (CMC).

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β-Methyl-α,β-dehydro-α-amino acids – synthesis and conformation

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Dehydroamino acids play an important role in the study concerning relationship between structure and biological activity of peptides. The sp^2 hybridization of the carbon atoms in the position α and β as well as a possibility of π-electron coupling between the $C^{\alpha}=C^{\beta}$ double bond and the neighboring amide bonds have considerable impact on the conformational properties of dehydroamino acid residues. In a consequence, the values of the torsion angles ϕ, ψ and the kind of the stabilizing forces involved do not fit to those found in the conformers of saturated analogues. These features make them an attractive tool both in conformational research and modification of biologically active peptides [1].

In last decade some new dehydropeptides and their modification occurring in nature have been discovered. Amongst them, dehydrobutyrine with C-terminal ester bond has been found. This structural element constitutes phomalide – cyclic pentadepsipeptide produced by fungi *Phoma lingan* [2]. Interestingly, the position of the β-methyl group in the side chain decides biological activity [3]. Fitotoxicity is demonstrated only by phomalide, in which the β-methyl group is in the *E* configuration. Izophomalide, in which β-methyl group is in the *Z* configuration does not show toxicity. In contrast, in majority of naturally occurring dehydropeptides the Z configuration can be found. A plausible explanation of this phenomenon is a difference in the conformational properties of the dehydrobutyrine Z/E isomers. However, the literature data are very limited [4].

We can expect, than esters of dehydroamino acids are interesting building unit of unique and unknown properties. The aim of this work is investigation into the conformational preferences of dehydroamino acids esters. In this study, a systematic conformational analysis of the dehydrobutyrine and dehydrovaline models (Ac-ΔAbu-NHMe, Ac-ΔVal-NHMe) as well as their analogues having C-terminal ester bond (Ac-ΔAbu-OMe, Ac-ΔVal-OMe) is performed. The obtained results concerning synthesis, X-ray and FTIR analysis as well as theoretical studies involving analysis of the potential energy surfaces of the studied compounds will be presented.

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* *is a recipient of a Ph.D. fellowship from a project funded by the European Social Fund*

Theoretical studies on the PrkC complexes formation as a tool for investigation of the kinase autophosphorylation

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Our study consisted of the construction of four PrkC dimers and two trimers using Z-DOCK program. PrkC is a kinase encoded by *Bacillus subtilis* genome; it is a member of Hanks super-family of the eukaryotic-like receptor protein kinases [1]. All created configurations of six complexes were refined using RDOCK procedure [2]. The highest rank configurations of each complex were optimized in Amber 8.0 force field and subjected to the 5 ns molecular dynamics simulation runs in order to study the possible flexibility of the complexes in the native-like environment. This procedure enabled us further relaxation of created models. Detailed analysis of protein-protein docking results and subsequent 5ns MD simulations suggest a few modes of interaction of protein molecules within the PrkC complexes. The discussion of our results with comparison to recently published experimental studies [3] on the dimerization and its influence on activation of the homological to the PrkC kinase (PknB) were carried out. We used PrkC/PrkC complexes as models for study of the possible characteristics of interface between the PrkC molecule and it's, still unknown, native substrate.

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Acknowledgements:
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Modification of the position 5 of 3-(2-benzoxazol-5-yl)alanine derivatives and its influence on their antimicrobial activity

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Searching for new drugs is still a challenge for science, mainly because of civilizing development and globalization which cause that diseases, especially infectious ones, spread out very quickly. As a result there is a need for new structures of potential drugs. One of them could be a benzoxazole moiety, a basic skeleton of a group of fluorescent unnatural amino acids, 3-(2-benzoxazol-5-yl)alanines, among which are also biologically active compounds [1,2]. Basing on our previous studies [1,2], three derivatives were selected (3-[2-(2'-methoxy-4'-dimethylaminophenyl)benzoxazol-5-yl]alanine, 3-[2-(2-quinolinyl)benzoxazol-5-yl]alanine, 3-[2-(4-boronophenyl)benzoxazol-5-yl]alanine) and their dipeptides with alanine, tyrosine, histidine and aspartic acid on C- and N-terminus, respectively, were synthesized. Dipeptides were synthesized in solution using Boc/Bzl tactics. The activity of all obtained peptides was screened against model Gram-positive (*Bacillus subtilis*) and Gram-negative (*Escherichia coli*) bacteria whereas antifungal activity was tested against yeast *Pichia pastoris*. All tests were performed using antibiogram method whereas the minimal inhibitory concentrations were determined using two-fold serial dilution technique.

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Acknowledgement:
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Solid-phase synthesis of 3-(2-benzoxazol-5-yl)alanine derivatives

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3-(2-Benzoxazol-5-yl)alanine derivatives are a group of fluorescent unnatural amino acids. Because of quite easy modification of the position 2 of the benzoxazole there are many different applications of these compounds such as fluorescent probes, pH and/or metal ion or sugar sensors as well as antimicrobial agents [1-3]. The synthesis of these compounds in solution is quite efficient and it was optimized previously [4]. However, in order to make the direct incorporation of benzoxazolylalanines into the peptide chain easier enabling application of these compounds in solid-phase combinatorial chemistry, the optimization of their solid phase synthesis was performed. Wang resin was used as a solid support and Fmoc-3-nitrotyrosine as a substrate. Four different aldehydes (2-methoxy-4-dimethylaminobenzaldehyde, 4-formylboronic acid, quinoline-2-carboxaldehyde, quinoline-8-carboxaldehyde) were used to obtain the intermediate Schiff base which underwent oxidative cyclization to the heterocyclic compound in the presence of four different agents (air or Mitsunobu reaction or NBS or DDQ), selected basing on the literature [5-7]. The products were identified by means of LC-MS analysis.

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This work was financially supported by the Polish Ministry of Science and Higher Education under grant 0212/H03/2007/33.

Simplified oxidative folding of disulfide-depleted selenoconopeptides

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Despite the therapeutic promise of disulfide-rich, peptidic natural products, their discovery and structure/function studies have been hampered by inefficient oxidative folding methods for their synthesis. Here we report that converting a three disulfide-bridged conopeptide KIIIA into a one-disulfide selenoconopeptide dramatically simplified its oxidative folding while preserving bioactivity. Selenoconopeptides were synthesized using the Fmoc chemistry. Side chain of selenocysteines was protected with p-methoxybenzyl group. One-step oxidation yielded a single folding species that maintained its ability to potently block a neuronal subtype of sodium channels. Next, we applied this technology for a rapid positional scanning of Lys7 in disulfide-depleted selenoconopeptide analogs of KIIIA, with a goal to improve the sodium channel subtype selectivity of KIIIA. One substitution, K7L, significantly improved the selectivity profile. The disulfide-depleted selenopeptide strategy offers the advantage of a regioselective folding method that is compatible with high-throughput synthesis and promises to accelerate the progress of using disulfide-rich peptides as research tools and drugs.

Basic dendrimeric peptides derivatized with hydrazinonicotinamide (HYNIC) and bis-picolylamide (BPA) – synthesis, structure and properties

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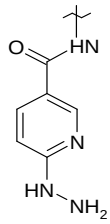
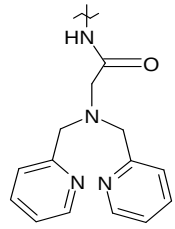
There is currently a great deal of interest in the development of receptor-targeted delivery of radioligands for medical diagnostics or drug delivery. In such an approach molecule carriers two functions – part responsible for receptor recognition and part with radioligand chelating properties. Numerous pathogenic processes (like cancerogenesis) are associated with inflammation caused by bacterial infections. Medical diagnostics in such a case might require compounds which selectively attach to membranes of various bacteria. Recently, we developed several classes of amphiphilic dendrimeric peptides with high antimicrobial potency against Gram(+), Gram(-) bacteria and fungi from C. albicans family [1,2]. Their mechanism of action is due to selectivity and high affinity towards membranes of procariotic organisms. Here we present synthesis and structure of two isomeric groups of dendrimeric compounds which contain the above mentioned dendrimeric peptides functionalized with hydrazinonicotinamide (HYNIC) or bispicolylamine (BPA) residues capable to coordinate Tc-99m or other cations. These bifunctional peptide dendrimers can be used for medical diagnostics.



HYNIC



BPA

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Radiolabeled [Lys^{40}(Ahx-HYNIC)NH_2]Exendin-4, a new agent for the detection of insulinomas

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Insulinomas are neuroendocrine tumors derived from pancreatic β-cells. The receptors of glucagon-like peptide 1 (GLP-1) are highly overexpressed in human insulinomas. In insulinomas, the dentisity of the GLP-1 receptor is higher than any of other peptide receptors, including somatostatin receptors[1,2]. GLP-1, the natural ligand of the GLP-IR, is rapidly degraded in vivo. A more stable agonist of GLP-1 is Exendin-4. Exendin-4 was modified C-terminally with Lys^{40}-NH_2, where the lysine side chain was conjugated with Ahx-HYNIC (Ahx is aminohexanoic acid).

Materials and Method

[Lys^{40}(Ahx-HYNIC)NH_2]Exendin-4 was custom-synthesized by Peptide Specialty Laboratories. Peptide purity checked by HPLC was 80%. 20 µg [Lys^{40}(Ahx-HYNIC)NH_2]Exendin-4 was dissolved in 200µl water. After added 50 mg tricine in 500µl water, 5 mg EDDA (ethylenediamine-N,N'-diacetic acid) and 40 µg $SnCl_2$ in 100 µl 0.1N HCl to peptide solution, radiolabelling was carried out by the addition 0.5-1 ml of generator eluate (10-20mCi radioactivity) followed by 30 min incubation at 80^{o}C. Radiochemical purity of [Lys^{40}(Ahx-HYNIC-^{99m}Tc)NH2]Exendin-4 controlled by TLC and HPLC showed over 90% radiochemical yield and percentage of non-bound ^{99m}Tc-pertechnetate as well as colloidal forms of ^{99m}Tc was in the range of 5%. Wet ^{99m}Tc-labelling of [Lys^{40}(Ahx-HYNIC)NH_2]Exendin-4 was performed to optimize the amount and concentration of reagents, temperature and reaction time which was then transferred to [Lys^{40}(Ahx-HYNIC)NH_2]Exendin-4 dry kit formulation.

Results and Conclusion

[Lys^{40}(Ahx-HYNIC)NH_2]Exendin-4 was successfully labeled with technetium-99m with radiochemical yields over 90%. The main peak in HPLC radiochromatogram indicated two radiolabeled species. Further characterization of these species is planned as well as the in vitro and in vivo evaluation of [Lys^{40}(Ahx-HYNIC-^{99m}Tc)NH2]Exendin-4 to confirm its diagnostic potential.

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Cyclolinopeptide a analogues containing 4-aminopyroglutamyl residue

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Cyclolinopeptide A (CLA), a cyclic nonapeptide with the sequence cyclo(-Pro[1]-Pro[2]-Phe[3]-Phe[4]-Leu[5]-Ile[6]-Ile[7]Leu[8]-Val[9]-) was isolated from linseeds and was shown to possess strong immunosuppressive activity in respect to both – humoral and cellular immune response. A peculiarity of CLA structure is the presence of cis-amide bond situated between both proline residues. Such structure of CLA was postulated by Siemion et al. in 1977 [1] and this hypothesis was confirmed by others on the base of X-ray, NMR and computational studies [2].
In the aim to answer the question, whether the *cis*-geometry of Pro-Pro amide bond is important for CLA biological activity and whether β-turn type VI is tolerated as well, we have synthesized analogues of CLA, in which Pro-Pro dipeptide segment has been replaced by all four stereochemical variants of 4-aminopyroglutamic acid residue. The effects exerted by our peptides in bioassays will be compared with those produced by natural CLA and CsA.

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Short nociceptin analogues containing 4-aminopyroglutamyl residue

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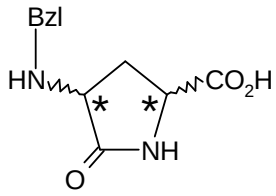
Nociceptin, a 17-amino-acid neuropeptide, is a natural ligand NOP receptor, which is related to the opioid receptor family. However, classical opioids have poor affinity for NOP receptor and nociceptin displays no affinity towards opioid receptors. Nociceptin has been implicated in many behavioral processes and inhibits same effects of opioids therefore it has been described as being functional anti-opioid. In this communication we present the synthesis and preliminary biological activity data of four new nociceptin (1-6) analogues modified in positions 1-2 with all four stereochemical variants of N-benzylated 4-aminopyroglutamic acid residue.



Synthesis and conformational analysis of salivary proline-rich protein P-B

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P-B is a 57-amino acid residue peptide present in human saliva. This peptide is not a degradation product of a larger protein, but a mature protein itself. Its biological role reemains still undefined. P-B is usually included into the basic proline–rich protein family.

In this report we describe chemical synthesis and conformational studies of this peptide using CD spectroscopy and Fourier-Transform Infrared Spectroscopy (FTIR).

The P-B peptide was synthesized manually by the solid phase method using Fmoc chemistry. The peptide was purified by HPLC and characterized by the MALDI-TOF technique.

The results showed that the CD spectra were particularly independent of the pH in the range of 2-12. Similarly, heating of the sample from 0 to 60ºC did not influence the P-B conformation dramatically. Quantitative spectral analysis revealed that P-B exists in solution mainly as a mixture of either the statistical coil and β-sheet or extended conformation. The percentage of polyproline II helix amounts only to about 10%. In turn, the fraction of the PPII motif calculated on the basis of deconvoluted FTIR spectra in the hydrated film constitutes even less than 10%.

This work was supported by the University of Gdańsk (DS 8452-4-0135-9, DS 8360-4-0133-9 and BW 8000-5-0254-9).

In vitro antimicrobial activity of a lactoferricin fragment 1-11 and its analogues against human pathogens

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Human lactoferricin (hLfcin) is a cationic peptide that is released upon proteolitic cleavage of the N-terminus of human lactoferrin (hLf). hLf has been shown to have, amongst numerous properties, some antimicrobial activity but hLfcin is still more active than its precursor.

The purpose of this study was the synthesis and in vitro study of antimicrobial activity of **a** human lactoferricin fragment 1-11 (GRRRRSVQWCA, hLfcin 1-11) and its analogues modified at the N-terminal or C-terminal part.

The peptides were synthesized using the solid-phase method and purified by high performance liquid chromatography.

The peptides were subjected to microbial tests on selected Gram-positive and Gram-negative bacteria and yeasts using two kinds of medium, the Muller-Hinton Broth II and Bacto Peptone 1% broth.

All the peptides were assayed according to the methods outlined by NCCLS. Minimal inhibitory concentration (MIC) of each peptide was determined against the *S. aureus, S .epidermidis, B. subtilis, E. coli, P. areuginosa, P. vulgaris* and *C. albicans* strains.

Our results have shown that the 1-11 fragment of hLfcin was effective against Gram positive bacteria, especially *S. epidermidis* and *B. subtilis*. It was less active against Gram negative bacteria and yeasts. It was ineffective against *P. vulgaris*. Our results show also that the peptides are more active in the 1% Bacto Peptone broth, where the concentration of cations is low. More details will be released during the presentation.

This work was supported by the University of Gdańsk (DS 8452-4-0135-9 and BW 8000-5-0254-9).

The stability of synthetic opiorphin in human saliva

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Opiorphin is secreted into human saliva and inhibits two enkephalin – catabolizing ectoenzymes, the human neutral ecto-endopeptidase (hNEP) and human ecto-aminopeptidase (hAP-N). This peptide inhibits chemically- and mechanically-induced pain behaviour by activating endogenous opioid-dependent pathways. Human opiorphin behaves as efficiently as morphine in the in vivo rat pain model.

The aim of this study was to investigate the stability of opiorphin in aqueous solutions and in the whole non-stimulated human saliva over different pH ranges.

The peptide was synthesized manually by the solid phase method using 9-fluorenylmethoxycarbonyl (Fmoc) chemistry. The crude peptide was purified by reversed phase high performance liquid chromatography (RP HPLC) and analyzed by the matrix-assisted laser desorption/ionization-time-of-flight mass spectrometry (MALDI-TOF) technique.

Samples of the whole non-stimulated saliva were collected from some volunteers between 9.00 a.m. and 10 a.m. For 2 h prior to saliva collection they were refrained from eating, drinking, smoking, and oral hygiene. Saliva from each individual was collected over a 5 min period by spitting into chilled disposable polypropylene tubes.

The synthetic opiorphin was dissolved in water up to a concentration of 5mg/ml (standard solution). The samples of saliva were mixed and divided into three parts (samples A, B and C). Sample A was acidified with acetic acid (Sample A1) and 0.1% trifluoroacetic acid in methanol (Sample A2). Sample C was treated by sodium hydroxide (Sample C1) and 10mM Tris HCl of pH 8.1 (Sample C2). After 1 hour of storage of the samples at room temperature, the standard opiorphin solution was added to all samples. The presence of opiorphin was checked by the MALDI-TOF MS technique immediately after sample preparation, and after 4 hours, 24 hours, 3 days, a week, two weeks, three weeks and a month. In some cases, additional HPLC analyses were run.

In this presentation, difficulties related to maintaining the stability of the synthetic opiorphin in the samples will be discussed.

This work was supported by the University of Gdańsk (DS 8452-4-0135-9 and BW 8000-5-0254-9).

The studies on the mechanism of the gas phase fragmentation of Amadori products

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Nonenzymatic glycation is one of the most common nonenzymatic posttranslational modifications. According to the Maillard hypothesis the formation of protein-sugar conjugates is considered as an important cause of the diabetes associated complications. The gas phase fragmentation of peptide-derived Amadori products was investigated using synthetic compounds regioselectively deuterated at aminofructose moiety. The eliminated molecule CH_2O contains exclusively protons attached to carbon C6 of the aminofructose moiety. The hydrogen atoms connected with carbon C1 of aminofructose moiety are partially eliminated as a component of water molecules during the dehydration process and partially dislocated within the fragmented peptide molecule. On the basis of these observations we proposed the hypothetical mechanism of Amadori products' fragmentation. The fragmentation of peptide-derived Amadori products is initiated by elimination the protonated OH group attached to the carbon C1. The first stage of collision induced dissociation of Amadori products is similar to the dehydration of fructose in acid water solution.

A functional genomics approach to characterize a novel germination pathway dependent on the protein phosphatase PrpE in *Bacillus subtilis*

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Upon nutrient starvation a Gram–positive soil bacterium *Bacillus subtilis* is capable of metamorphosing into metabolically dormant, extremely resistant endospore (spore). When conditions are again favorable for growth, the spore returns to vegetative growth through a process of germination. Protein phosphatase PrpE has been recently shown to be essential for nutrient-induced spore germination in *B. subtilis*. It seems plausible that the phosphatase is the first identified component of a novel regulatory phosphorelay pathway controlling germination [1]. However, the cognate kinase or transcription regulators involved in the pathway remain elusive. Our approach to identify such proteins is to isolate and map mutations that reverse the defective germination phenotype of spores produced by the strain devoid of PrpE phosphatase. To fulfill this aim the strain lacking PrpE was subjected to random transposon mutagenesis. Screening of the mutant library resulted in the identification of 12 mutations that restored the ability of spores to germinate normally. Mutations were mapped to 8 different open reading frames, some located within putative operons, in most cases encoding proteins of unknown function. The identified genes or operons encode potential candidates for components of the PrpE-dependent regulatory pathway controlling spore germination in *B. subtilis*.

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Cyclolinopeptide A modiefied with (*R*)-4-methylpseudoproline – synthesis and biology

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Pseudoprolines (ψpro), the name of 1,3-oxazolidines and 1,3 –thiazolidines derived from Ser, Thr Cys, have been originally introduced by Mutter and coworkers and they have been extended as a versatile tool in a structure-activity relationship [1-3]. In general ψpro-containing peptides exibit enhanced rate constant for *cis-trans* isomerisation compared to the native proline analogues depending on stereochemistry and the degree of substitution at the C-2 position of the cyclic ring.

α-Alkyl-α-hydroxymethyl amino acids, which belong to the family of nonproteinogenous, α,α-disubstituted amino acids with a hydrophilic side-chain [4] like serine and threonine can be transformed into suitable pseudoproline (oxazolidine) unit named 4-alkyl-pseudoproline [5].

4-Alkyl-pseudoprolines, due to the presence of C-4 substituent on the cyclic ring, have chemical and conformational properties different from pseudoprolines derived from serine and threonine. Previously we report the chemical synthesis and conformational analysis of the short peptides containing (*R*)- and (*S*)-4-methylpseudoproline derived from α-methylserine which is the simplest and natural occuring amino acid. On the basis of the NOESY experiment we suggested that the geometry of 4-methyl-pseudoproline peptide bond with the preceding amino acid residue is *trans.*

The goal of this communication focus on the synthesis and biological study of the new analogues of cyclolinopeptide A which is known as a natural immunosuppressant possess activity comparable with cyclosporine A. It is known that immunosuppressive activity of CLA is connected with the presence of the tetrapeptide Pro-Pro-Phe-Phe sequence containing Pro-Pro cis amide bond [6]. That's why modification of this unit can help in the better understanding of the CLA's mechanism of a biological action.

We present CLA analogues containing proline residues replaced by (*R*)-4-methyl-pseudoproline in position 6 or 7 respectively. The linear nonapeptides were prepared manually using standard solid-phase procedure "step by step" on Wang resin, using Fmoc group for N-amino protection and TBTU as a coupling reagent or BTC for a difficult coupling. The cyclization of linear precursors has been made in solution using EDC/HOBt as a coupling reagents. The biological activity of the newly synthesized compounds will be evaluated.

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Synthesis and biological studies of 4-aminophenylalanine modyfication of Cyclolinopeptide A

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Cyclolineopeptide A (CLA) (**1**) has well documented immunosuppressive activity[1], unfortunately its physical proprieties precluded its use for immune response and immune modulated therapy. The most undesirable physical property of CLA is its very low solubility in water.

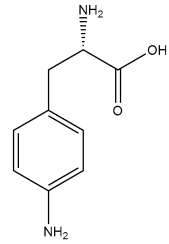
CLA(**1**): cyclo(Leu^1-Ile^2-Ile^3-Leu^4-Val^5-Pro^6-Pro^7-Phe^8-Phe^9)

We are presenting now a synthesis of 4-aminophenylalanine (p-NH_2)Phe (**2**)derivative of CLA which will represented better solubility in water. It is postulated, that tetrapeptide fragment of CLA, responsible for immunosuppressive activity consist of Pro-Pro-Phe-Phe. That particular tetrapeptide fragment preserve characteristic features: *cis* amide bound between Pro-Pro units, and "edge-to-face" interaction of two aromatic rings [2, 3]. The latest can be influenced by additional amino group in (p-NH_2)Phe.

In this communicate we present two new analogues of CLA **3** and **4** modified by 4-aminophenylalanine. The synthetic strategy and biological activity will be discussed.

(**3**) cyclo(Leu^1-Ile^2-Ile^3-Leu^4-Val^5-Pro^6-Pro^7-(p-NH_2)Phe^8-Phe^9)

(**4**) cyclo(Leu^1-Ile^2-Ile^3-Leu^4-Val^5-Pro^6-Pro^7-Phe^8-(p-NH_2)Phe^9)



2

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Synthesis and biological studies of 4-nitrophenylalanine modification of Cyclolinopeptide A

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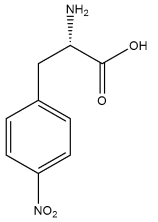
Immune response suppressors are used in the medical praxis to prevent graft rejection after organ transplantation and in the therapy of some autoimmune diseases. Cyclolinopeptide A (CLA) (**1**), a cyclic, hydrophobic nonapeptide isolated from linseed, possesses strong immunosuppressive and antimalarial activity[1]. It is postulated that both the Pro–Pro *cis*-amide bond[2] and an 'edge-to-face' interaction between the aromatic rings of two adjacent Phe residues[3] are important for biological activity. CLA(**1**): c(Leu^1-Ile^2-Ile^3-Leu^4-Val^5-Pro^6-Pro^7-Phe^8-Phe^9)

In this communicate we present three new analogues of CLA modified by 4-nitrophenylalanine (p-NO_2)Phe (**2**) in positions 8 or 9 and both 8 and 9 (**6**-**8**). Linear peptides **3**-**5** were synthesized by the manual solid-phase peptide synthesis (Merrifield resin) strategy and were cyclized with the 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide/1-hydroxy-benzotriazole (EDC/HOBt) reagent to cyclic CLA analogues **6**-**8**. The synthetic strategy and biological activity will be discussed as well as conformational analysis.



2

(**3**) H-Leu^1-Ile^2-Ile^3-Leu^4-Val^5-Pro^6-Pro^7-(p-NO_2)Phe^8-Phe^9-OH
(**4**) H-Leu^1-Ile^2-Ile^3-Leu^4-Val^5-Pro^6-Pro^7-Phe^8-(p-NO_2)Phe^9-OH
(**5**) H-(Leu^1-Ile^2-Ile^3-Leu^4-Val^5-Pro^6-Pro^7-(p-NO_2)Phe^8-(p-NO_2)Phe^9-OH
(**6**) c(Leu^1-Ile^2-Ile^3-Leu^4-Val^5-Pro^6-Pro^7-(p-NO_2)Phe^8-Phe^9)
(**7**)c(Leu^1-Ile^2-Ile^3-Leu^4-Val^5-Pro^6-Pro^7-Phe^8-(p-NO_2)Phe^9)
(**8**) c(Leu^1-Ile^2-Ile^3-Leu^4-Val^5-Pro^6-Pro^7-(p-NO_2)Phe^8-(p-NO_2)Phe^9)

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Intrathecal administration of novel opioid-neurotensin hybrid peptides results in long-standing antinociception in tail-flick test in rats

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Opioid analgesics are frequently used for the long-term management of different pain states, including chronic pain. However, prolonged exposure to opioids results in dramatic effects, such as sedation, respiratory depression, strong dependence and tolerance. This fact induced us to create novel chimeric compounds displaying additive or synergistic antinociception, resulting in an improved safety profile with lower side-effects.

Neurotensin (NT) is a tridecapeptide which has been demonstrated to be involved in the control of various physiological activities in both the central nervous system and in the periphery [1, 5]. This endogenous peptide is well known to act as a pain modulator, and the analgesic effect induced by it arise irrespective of the opioid system. Due to its implication in pain transmission [2, 3, 5] as well as in the central integration of pain responses, it is promising to achieve a potential drug for relief of pain resistant to conventional treatment by combining two highly active nociceptive substances.

In previous study [4] we presented the synthesis (according to structure-activity relationship) and binding affinity to mu opioid receptor of selected opioid-neurotensin peptide analogues. These hybrid peptides injected intrathecally into rats show the antinociceptive potency. The antinociceptive effects elicited by two selected compounds are dose-dependent; moreover, the analgesia is observed even in very small doses and is higher than for morphine at a dose 3 nmol/rat.

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Analgesic activity of opioid peptides in mice bred for stress induced analgesia

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In 1983 prof. Bogdan Sadowski from Institute of Genetic and Animal Breeding in Jastrzębiec developed genetic mouse lines selectively bred for high (HA) and low (LA) swim- stress induced analgesia. Selected animals differ in sensitivity to both, pain or stress responses.

It is well known that when the stressors act on animal, exogenous opioids (endorphins, enkephalins) are released on the periphery. The presence of blood-brain barrier limits the distribution of peptides to the central nervous system.

Our previously studies displayed that opioid peptides are more potent in HA mice than in LA mice after peripherally application in the tail flick test and stress potentiated analgesic activity of given peptides but only in HA mice. Different analgesic potency of opioid peptides and alkaloid (morphine) in selected mice was a premise to conduct an ultramicroscopic study of their blood – brain barrier. The results of in vitro studies displayed pathological changes in morphology of BBB in HA mice. It can be suggested that these structures were transformed into the pathological forms during long lasting selective breeding program.

These results indicate that leaking BBB in HA mice is more permeable for exogenous opioids which are released on the periphery after stressful stimuli and a higher level of analgesia can be observed. Opioids peptides given to stressed animals are more potent than in non-stressed animals. Interactions between exogenous and endogenous opioids produce additive analgesic effect which is observed as a potentiated analgesia in HA mice.

Presented studies have been supported with EU grant Normolife.

Synthesis, structure and immunogenity studies on fragments of human metallothionein-3 (MT-3)

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Metallothioneins (MTs) belong to a special class of proteins, composed of between sixty-one and sixty-eight amino acids, including 20 cysteine residues.
The MT family is composed of four primary types: MT-1, MT-2, MT-3, MT-4 [1].

Metallothioneins are induced by *in vivo* exposure to heavy metals and a great variety of hormones [2]. They are involved in transport and managing of metal ions in every single cell in our body. MTs also regulate blood levels of trace metals, assist in neuronal development and have a radical scavenging property [1,2].

Defective functioning of metallothionein-3 is a distinctive feature of autism [3]. Physiological roles for that isoform include mainly zinc metabolism in neurons, response to neural damage and repair [4]. Abnormality of mentioned protein results in impaired brain development (especially in the first 30 months of life), which could result in incomplete maturation of the G.I. tract and brain [1]. Another important consequence of MT malfunction is loss of metallothioneins protective detoxification (e.g. heavy metals) [2].This disorder is often unnoticed in infancy and early childhood.

Early diagnosis of that disorder is very important. That is why I decided to create a test, which could help in a early diagnosis (especially in infants). I chose three fragments of metallothionein-3, with a potential antigenicity properties (antigenic determinants). They consist of a 12, 13 and 15 amino acids. The three regions in metallothionein appear to be important in the interaction of the molecule with the antisera [5].
Synthesis was performed on a solid phase using Fmoc strategy.

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Interaction of metal ions with fragments of human metallothionein-3 (MT-3)

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Metallothionein are low molecular weight proteins, which strongly bind to heavy metals, such as Cd(II), Zn(II) and Cu(I). They are characterized by a high cysteine content (approximately 30% of all amino acid residues). Most of them have two metal clusters, containing three and four bivalent metal ions respectively [1,2]. A single MT protein can bind a total of 13 copper ions in +1 valence [2].

Copper is an essential metal in living organisms – acts as a cofactor for many enzymes, in which is bound to specific amino acid residues in an active site [3].
Cu is generally found in bivalent state, bun when this metal is in monovalent form, it is able to transfer one electron and generate reactive oxygen species, e.g. hydroxyl radical (responsible for lipid peroxidation in membranes and protein oxidation) [4]. Therefore copper homeostasis must be regulated very tightly [3,4].

Metal binding ability of the whole MT-3 protein is well known, but we decided to synthesize three long fragments of it and check their binding ability and interaction with copper ions.

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This work was supported by University of Gdańsk (DS/8452-4-0135-9).

New tachykinin-opioid chimeric analogues as potential new analgesics

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Many endogenous components play important roles in the formation, transmission and modulation of pain. The endogenous opioid system is therefore a major target in pain management. Substance P (SP) a Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2 undecapeptide is widely distributed throughout the central nervous system and highly expressed in brain areas critical for the regulation of pain, and for affective and stress behaviors. Endogenous SP produces both hyperalgesia and, at low doses, naloxone-sensitive analgesia. For years numerous research studies have been conducted on the development of new compounds effectively targeting both the opioid and tachykinin systems and thus modulating pain transmission. Unfortunately, using a combination of drugs each acting specifically on one type of receptor has disadvantages, including different pharmacodynamic and pharmacokinetic profiles of each of the component drug. Thus, combining multiple active pharmacophores into one molecule is a promising approach. It is known that the C-terminal sequence of SP is essential for activation of the neurokinin-1 (NK-1) receptor. This fragment injected alone into the periaqueductal gray matter (PAG) produces an anxiogenic effect. The N-terminal fragment such as SP(1-7) induces antinociception and desensitization of SP-induced behaviors. SP(1-7) has been suggested to modulate the inducement of opiate tolerance and withdrawal behaviors in rodents. Therefore, goals for new chimeric compound synthesis containing both the NH2- and COOH- terminal domains include producing compounds with higher enzymatic stability, better blood-brain barried permeability and higher receptor selectivity. This presentation shows the way from an idea through synthesis to the application of a new opioid-tachykinin chimeric compound in pain therapy.

Presented studies have been supported with UE grant Normolife.

Enkephalin derivative, cyclo[N^{ε}, N^{β}-carbonyl-D-Lys^2, Dap^5] enkephalinamide (cUENK6), induces a highly potent antinociception in rats

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The aim of the study was to evaluate whether the newly synthesized analog of enkephalin, cyclo[N^{ε}, N^{β}-carbonyl-D-Lys^2, Dap^5] enkephalinamide (cUENK6), a **highly potent μ- (guinea pig ileum assay) and δ-receptors (mouse vas deferens assay) ligand,** induces an antinociceptive effect in the hot-plate test and tail-immersion test after intracerebroventricular administration. Our study indicated that this peptide at the dose of 0.25 nmol produced comparable but at the dose of 0.5 nmol stronger than morphine (13 nmol), antinociceptive effect in both tests. Furthermore, rats with developed tolerance to morphine indicated cross-tolerance to antinociceptive effects of cUENK6. The antinociceptive effects of cUENK6 and morphine were inhibited by non-selective opioid receptor antagonist - naloxone. More detailed study indicated that the δ-opioid receptor antagonist – naltrindole very strongly and, to the lower extent, μ-opioid antagonist - β-funaltrexamine (β-FNA), inhibited antinociceptive effect of cUENK6 in the tail-immersion test. Nor-binaltorphimine (nor-BNI), a κ opioid receptor antagonist, did not influence this effect. These data suggest the dominant role of δ-opioid receptors as compared with μ-receptors in mediation antinociceptive effect of cUENK6. Furthermore, we found that cUENK6 is much more effective in inhibiting pain in the hot-plate (ED_{50} =0.0792 nmol) than in the tail-immersion (ED_{50}= 0.3526 nmol) test. However, cUENK6 at the antinociceptive doses induced hypolocomotion, and although this effect is observed after administration of opioid agonists in rats as a one phase of their biphasic action (inhibition followed by activation), in our study it was not naloxone-reversible. Therefore, our study suggests that not only opioid receptors may be involved in behavioral effects of cUENK6.

Transportan and its analogues as a new tool for delivery of macromolecules

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Plasma membrane has a property of an effective barrier to hydrophilic compounds. The need to deliver biologically active agents like drugs into cells has encouraged researchers to develop various delivery vectors. Cell-penetrating peptides (CPPs) have the ability to translocate through cell membranes with high efficiency. When covalently linked to a larger cargo such as polypeptides, oligonucleotides or proteins, CPPs still retain their translocation properties what results in fast transfer of cargo molecules into cells. Transportan (TP) is a 27 amino acid long peptide which contains 12 functional amino acids of the neuropeptide galanin at the amino terminus and mastoparan at the carboxyl terminus, connected via lysine. Transportan 10 (TP10) was developed as an analogue to transportan by deleting the first six N-terminal amino acids in order to reduce its toxicity. The cationic nature of CPPs is crucial for their ability to bind and traverse the anionic cellular membrane.

The aim of this study was to evaluate the effect of transportan and its analogues on growth and cell cycle kinetics of HCT116 and HT29 cell lines.

HCT116, human colorectal carcinoma cell line and HT29, human colorectal adenocarcinoma cell line were used in this study. Transportan, transportan 10 and biotynylated transportan 10 were synthesized in the Faculty of Chemistry, University of Gdańsk. The cytotoxic effect of TP and its analogues was determined using MTT assay. Characteristics of the cell cycle distribution was evaluated by flow cytometry after PI staining. The efficiency of cellular delivery of proteins was assessed by the use of streptavidin-FITC as a test cargo that was analyzed by fluorescent microscopy.

In preliminary experiments the influence of various concentrations of TP and its analogues on the viability of HCT116 and HT29 cells was evaluated. TP and TP10 did not affect cell proliferation at concentration up to 10μM. Biotynylated TP10 is more cytotoxic than TP and TP10. HCT 116 was more sensitive to toxic effects of TP, TP10, biotynylated TP10 than HT29. We did not observed any significant alterations in cell cycle distribution after treatment of HCT116 and HT29 cells with transportan and its analogues at concentrations 0,1μM and 5μM. It was found that biotynylated TP10 is able to deliver streptavidin-FITC into HCT 116 and HT 29 cell line cells.

Cell penetrating peptides have low toxicity and a high yield of delivery and might become a widely used tool for delivery of drugs and in the field of gene regulation.

Metal ion binding by Opiorphin and Sialorphin

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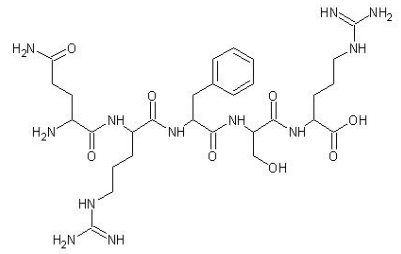
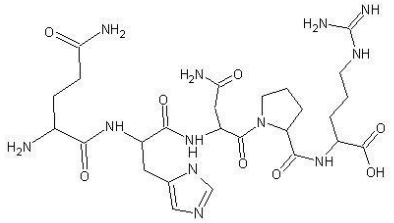
Opiorphin H-Gln-Arg-Phe-Ser-Arg-OH (Scheme 1a) is the peptide isolated from human salivia [1]. It show similar biological activity to morphin but six times stronger [2]. Sialorphin H-Gln-His-Asn-Pro-Arg-OH (Scheme1b) is a hormonal mediator [2].



Scheme 1a



Scheme 1b

Based on the potentiometric and spectroscopic studies, we present the coordination abilities both peptides towards Cu(II) ions. The analysis of the obtained results shows that the sialorphin binds copper(II) more effectively than opiorphin. It forms stable complex with three nitrogen boud to the metal ion in pH range 3.8÷9. However above pH 9 both peptides form bind copper(II) by four nitrogens.

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ESI-MS studies on high pressure denaturation of ubiquitin

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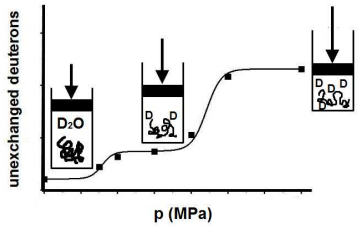
The studies on high pressure denaturation of proteins can provide information concerning their folding and function. High pressure can change the protein's conformation and properties by the rearrangement or destruction of noncovalent bonds, which normally stabilize the tertiary structure of proteins [1]. Till now high-pressure experiments were integrated with the analytical methods such as FTIR, NMR, small-angle X-ray scattering, densitometry, fluorescence, and UV absorbance spectra [2,3] However, they need a special, complicated and expensive equipment, what may limit the availability of those techniques. We present a new convenient method for studying high-pressure denaturation of proteins by electrospray mass spectrometry (ESI-MS) combined with deuterium-hydrogen exchange (DHX).

The treatment proteins with various pressures resulted in different degree of the protein unfolding. Consequently, a different number of protons are available for exchange with deuterons. Decompression causes refolding of the protein molecule, in which a certain number of deuterons are trapped inside the hydrophobic core. Redissolving the deuterated protein in an aqueous buffer causes the deuterium-hydrogen exchange of the deuterium atoms located on the protein surface only. It allows monitoring the deuterium content in the protein molecule by electrospray mass spectrometry under atmospheric pressure. Depending on the degree of deuteration after high-pressure treatment, the DHX kinetics are different and gives information how many amide protons were exchanged to deuterons during the proteins refolding. The dependence of this number on pressure value provide information on the denaturation of protein under high pressure. The obtained results are in a good agreement with data presented in the literature. The high pressure denaturation of lysozyme is a biphasic process.



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More environmental -friendly analysis and separation of peptides using RP-HPLC

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The rapid development of pharmaceutical industry enforced by escalating needs of the present civilization, apart from increasing high standards of living, results in unfavourable influence on natural environment. The increased demand for pharmaceutical resources and search for new technologies is connected with needs of using huge amounts of chemicals, which show harmful activities, difficulties in regeneration and utilization, problems with storage and the danger of release into the environment. Recycling costs usually exceed capabilities of research labs and production plants. The same reason causes also finding cheaper and more accessible chemicals, without respect to their toxicity.

Our efforts are directed to develop procedures enabling elimination of hazardous materials generated by chemical laboratories, and to replace common harmful solvents with more environmental-friendly substitutes. Currently we have concentrated our efforts on an important process which is the purification of new compounds using High Performance Liquid Chromatography. Because of its performance and formerly a good price, the most common organic solvent used in HPLC is acetonitrile. Due to the world-wide unstable situation in the manufacture of acetonitrile, its availability and price may change dramatically. We have experimentally proved that using some methods and conditions, we can successively use cheaper, less toxic and more easily recyclable solvents. Our greatest hopes are in ethanol, an organic solvent with low toxicity to the environment, which during the separation process of peptides gives much better results than the commonly preferred acetonitrile. We are also developing a rapid and efficient method of solvent purification for HPLC analysis and separation .

Alloferon, *any*-GS and their new analogues. synthesis and anti-herpes activity

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One of the most common viral infections in humans is caused by *Herpes Simplex Virus* (HSV). Development of new antiviral products is very much required. Recently, several of new peptides with antiviral or antitumor activity have been isolated from insects [1].

The subject of our study was the synthesis and search for new biological properties among selected insect peptides with antiviral or antitumor activity, such as:

- alloferon (**I**) isolated from the blow fly *Calliphora vicina* [2] and its four analogues ([des-His^1]-alloferon (**II**), [Lys^1]-alloferon (**III**), [Arg^1]- (**IV**), [Ala^1]-alloferon (**V**));
- oligopeptide Any-GS (**VI**) isolated from the wild silkmoth *Antheraea yamamai* [3] and its shortened analogues ([2-5]-Any-GS (**VII**), [3-5]-Any-GS (**VIII**), [1-4]-Any-GS (**IX**)) as well as five analogues modified at position 1 ([Asn^1]-(**X**), [Arg^1]-(**XI**), [Gln^1]-(**XII**), [Gly^1]-(**XIII**), [Ala^1]-Any-GS (**XIV**)).

Peptides were synthesized by the standard solid phase method. The biological properties of the compounds were tested *in vitro*: 1/ their antiviral activity was evaluated in respect to *the Herpes Simplex Virus* type 1 McIntrie (HSV-1MC) in Vero cells line, infected with HSV-1MC 1TCID50/cell; 2/ their cytotoxic activity was evaluated in Vero cell line by the MTT (3-[4,5 dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) method. In preliminary investigations we found that all these peptides strongly inhibited the replication of HSV-1 in Vero cells. Moreover, these compounds did not show any cytotoxic activity against the Vero cells.

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Application of α-alkylserines β-lactones in organic synthesis. Ring opening with pyrrolidine, piperidine and morpholine

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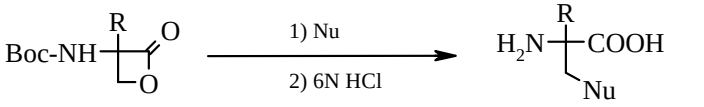
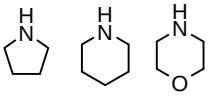
N-Protected β-lactones of serine, threonine and cysteine are useful and versatile intermediates in the enantioselective synthesis of β-substituted α-amino acid *via* ring opening with various nucleophiles. A large variety of carbon, nitrogen, oxygen, sulfur, and halogen nucleophiles were used to attack chiral, *N*-protected serine β-lactones at the β-carbon yielding optically pure *N*-protected β-substituted alanines [1].

On the other hand, building blocks such as α,α-disubstituted glycines have become important in medicinal chemistry and biochemistry. α,α-Disubstituted amino acids have been successfully used to force peptides into their biologically active conformations, often resulting in peptidomimetics with remarkable resistance to enzymatic degradation [2].

An easy access to α-hydroxymethylamino acids provided by general method developed in our laboratory [3] encouraged us to explore the possibility of utilizing these derivatives as starting materials for the synthesis of multifunctional α,α-disubstituted amino acids [4]. Herein we present the ring-opening reaction of α-alkylserine β-lactones with pyrrolidine, piperidine and morpholine:



R = -CH_3, -$CH_2CH(CH_3)_2$, -$CH(CH_3)_2$, -CH_2Ph Nu = 

Synthesis of β-pyrrolidine-(piperidine, morpholine)-α-alkylalanines.

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GHK analogues – synthesis, biological activity and application in cosmetics

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GHK (glycyl-histidyl-lysine, Gly-His-Lys) is an endogenous tripeptide isolated from human plasma. Peptide offers a wide range of biological activities such as stimulation of hair growth, collagen synthesis, superoxide dismutase-like activity, wound healing, tissue repair and angiogenesis [1,2]. Tripeptide also possesses a high affinity for Cu^{2+} ions and spontaneously builds a complex (GHK-Cu). GHK-Cu also plays a key role in mammalian organism because it directly participates ***in wound*** healing and tissue repair [2-4].

In our preliminary studies we decided to check the antimicrobial activity of GHK analogues and their permeability through the model membrane of a skin barrier. Therefore, we synthesized several GHK analogues: GGHK, AHK, BHK, GFK and their *N*-terminus modified derivatives (where G = Gly, H = His, B = β-Ala, K = Lys, F = Phe, A = Ala) using a standard Fmoc procedure. Peptides were purified by solid-phase extraction (SPE) and characterized by MS, amino acid analysis, elemental analysis and RP-HPLC analysis.

Design and chemical synthesis of other GHK derivatives improves their biological and physicochemical properties. The transport of compounds through the stratum corneum plays a beneficial role in dermatology and pharmacology [3,4]. The synthetic peptides will be further investigated in respect of potential application in new cosmetics.

In this work we synthesized some GHK derivatives: GGHK, BHK, GFK (where G = Gly, H = His, B = beta-Ala, K = Lys, F = Phe) using a standard Fmoc procedure. Peptides were purified by solid-phase extraction (SPE) and characterized by MS and RP-HPLC analysis. Next we examined these GHK analogues for their antimicrobial activity and their permeability through the model membrane of a skin barrier using a Franz diffusion cell. Our preliminary results suggest that GHK analogues are able to migrate through the liposome membrane.

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Synthesis and biological activity of tuftsin-acridine conjugates

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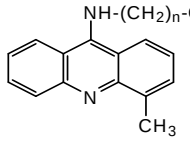
Acridine derivatives which are known as antimicrobial, antiviral and anticancer agents have been in the center of interest of scientists. The heterocyclic molecules demonstrate a noteworthy series of compounds which interact with different biological targets such as topoisomerase I, II, telomerase and protein kinases [1].

Therefore, we proposed a new series of tuftsin-acridine conjugates. Tuftsin as a natural peptide indicates not only immunological stimulating factor but also antibacterial, antifungal, antiviral and antineoplastic properties. In spite of its wide range of activity, tuftsin is unstable in plasma and it has become the aim of new analogues formation that are more resistant to proteolytic degradation [2,3].

Continuing our studies of the synthetic therapeutic agents, we synthesized some tuftsin-acridine conjugates **1a-d** (Fig. 1) that were prepared using a Fmoc solid phase strategy. Tuftsin analogues were modified at the ε-amino group of lysine via the introduction of the glycine residue to obtain an isopeptide bond. Peptides were linked to the acridine molecule via flexible linkers. The carboxylic group of linker was connected to *N*-terminal group of peptide-resin also using standard SPPS method.
Final products were characterized by elemental analysis, MS and ^{1}H-NMR spectroscopy. The obtained conjugates were sent to assay their biological activity and the results will be described as a structure-activity relationship.



1a-d

R = H-Thr-Lys-Pro-Arg-OH (**1a**)
H-Thr-Lys(Gly)-Pro-Arg-OH (**1b**)
H-Arg-Pro-Lys-Thr-OH (**1c**)
H-Arg-Pro-Lys(Gly)-Thr-OH (**1d**)

n = 2, 3

Fig. 1. The conjugates of tuftsin analogues with acridine derivatives

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Bioactive peptides from spinal cord

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The spinal cord from animals is a meet production waste that can be a source of bioactive proteins/peptides. In our department we have used hydrolysis to obtain a mixture of peptides from pig spinal cord. The hydrolisate of pig spinal cord proteins was tested in the properties of induction of oral tolerance in animal model of sclerosis multiplex – experimental allergic encephalomyelitis /EAE/. The fermentation is an alternative method for obtaining a mixture of peptides or it can be also used for purification of hydrolisates.

The aim of the present study was to use fermentation protein proteolysis associated to obtain small proteins/peptides from the spinal cord. Initial conditions for fermentation of spinal cord were optimized based on the studies of whey fermentation. Preparation fresch spinal cord or pre - digested was fermented at different medium by using yeast (Saccharomyces cerevisiae).

These proteins/peptides in the present study may have commercial value as a natural preparation of natural product with applications as an addition in foods and cosmetics.

The synthesis and activation of 3-benzyloxycarbonylamine-1*H*-pyrazole-5-carboxylic acid

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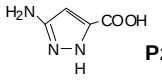
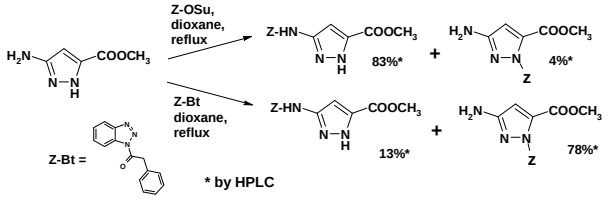
The 3-amine-1*H*-pyrazole-5-carboxylic acid (Pz) is an unnatural heterocyclic amino acid, which can be a potentially important building block of peptide hybrids, applied as drugs against Alzheimer's disease [1].



Pz

Ac-Pz-Pz-Lys-Val-Phe-OMe,
Glycol-Pz-Lys-Lys-Pz-OMe,
Glycol-Pz-Lys-Val-Pz-OMe.

Pz with a donor-acceptor-donor (DAD) hydrogen bond pattern fits to the fragment of the β-amyloid protein. Thus, the formation of the insoluble aggregates, causing Alzheimer's disease is efficiently prevented. The Pz amino acid contains not only exocyclic amino group, but also acidic NH group in the heteroaromatic ring. In a consequence, the choice of reagents and reaction conditions to acylate selectively the exocyclic amine group is limited [2]. We have been found that Z-OSu is the best reagent to the protect the exocyclic the amino group by Z-group.



The synthesis of the peptide hybrids requires incorporation of the pyrazole derivatives to the proteinogenic amino acids as well as to the residue of pyrazole amino acid. Unfortunately, insolubility of the 3-N-acyl-1*H*- pyrazole-5-carboxylic acid in the organic solvents and the acidic character of the NH group limit the choice of the activation of the carboxylic group. We have found that the method azides is the activation by choice [3]. This method failed, however, when the 3-amine-1*H*-pyrazole-5-carboxylic methyl ester is used as an amino component, due to lower of nucleophility of the exocyclic amino group. We have found that DCC, DMTMM and acid chloride are the effective activations in this synthesis.

Method	Solvent	Time	Temperature	Yield by HPLC
DCC	DMF	24	rt	58%
Acid Chloride	Toluen/DMF	48	reflux/rt	55%
DMTMM	DMF	48	rt	69%

Synthesis of Z-Pz-Pz-OMe

The structure of Z-Pz-Pz-OMe was proved by ^{1}H NMR: (DMSO-d_6): δ [ppm] 13.476 (2H 2xNH-ring, br) 11.203 (1H, NH-Z, brs) 10.299 (1H NH-amide, brs).

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[3] data not published

N-(ureidoethyl)amides of cyclic enkephalin analogs

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Novel N-(ureidoethyl)amides of cyclic enkephalin analogs have been synthesized [1]. The p-nitrophenyl carbamate of 1-Boc-1,2-diaminoethane was coupled with 4-methylbenzhydrylamine (MBHA) resin. The Boc group was removed by treatment with HCl/dioxane, and the peptide chain was assembled using Boc strategy. For deprotection of amino function, HCl/dioxane was used. D-Lys or D-Orn were incorporated in position 2, and the side chains of Lys, Orn, Dab, or Dap in position 5 were protected with Fmoc group. Side chain protection was removed by treatment with 55% piperidine in DMF, and cyclization was achieved by treatment with bis-(4-nitrophenyl)carbonate to form a urea bridge. The peptide was cleaved from the resin by treatment with 45% TFA in DCM. The peptides were tested in the guinea-pig ileum (GPI) and mouse vas deferens (MVD) assays. Divers opioid activities were observed, depending on the size of the ring. In comparison with [Leu5]enkephalin, all peptides were more active in the GPI assay (between 125 and 12 times), and some of them were also more potent in the MVD assay. The conformational propensities of each peptide were determined using the EDMC method in conjunction with NMR experiments. This approach allows treating the dynamical behavior of small peptides properly. The results were compared with those obtained previously [2] for corresponding non-substituted amides and are in agreement with the biologically active conformation proposed by us earlier.

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Novel analogues of arginine vasopressin modified in the *N*-terminal part of the molecule with L-indoline-2-carboxylic acid

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Since the original synthesis of arginine vasopressin (AVP) in 1954, thousands of analogues of this hormone have been synthesized in the course of extensive investigation of structure – activity relationships. Despite huge efforts in many laboratories, design of analogues, which are very active either as agonists or antagonists and truly selective for individual receptors, still remains an area of great interest.

Bearing this in mind, we decided to learn how the substitution of position 2 with a sterically restricted amino acid derivative, L-indoline-2-carboxylic acid (Ica), would affect biological potency of the analogues. We designed the following peptides: [Ical^2]AVP (**I**), [Mpa^1,Ica^2]AVP (**II**), [Ica^2,D-Arg^8]AVP (**III**), [Mpa^1,Ica^2,D-Arg^8]AVP (**IV**) [Ica^2,Val^4,D-Arg^8]AVP (**V**), and [Mpa^1,Ica^2,Val^4,D-Arg^8]AVP (**VI**). During the synthesis of the analogues we faced some difficulties in the coupling reaction between Ica and either the Fmoc-Cys(Trt) or Mpa(Trt) derivatives. The well-known coupling reagents (TBTU, DIC, HATU, PyBOP) were not efficient to obtain a good product. To overcome the problem, we applied a new commercially available activator DMTMM (4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride). Using this approach, we were able to obtain in good yield three peptides with Mpa in position 1. Both the weak basicity and the effect of steric restriction of the modifications are responsible for the difficulties in the synthesis.

The obtained peptides were tested for their pressor, antidiuretic, and in vitro uterotonic activities. We also determined the binding affinity of these compounds to human OT receptor. The Ica^2 substitution resulted in a significant change of the pharmacological profile of the peptides. The new analogues (**IV**, **VI**) were moderate oxytocin antagonists (pA_2 ~7.09 or 7.50) with the exception of peptide **I** which turned out to be a very weak agonist (0.02 IU/mg). It is worth emphasizing that these new peptides were exceptionally selective as they virtually did not interact with the V_{1a} and V_2 receptors.

Acknowledgements:
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Design, synthesis and biological effectiveness of new analogues of AVP with (S)-1-adamantyl-glycine in position 2

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The neurohypophyseal peptide, arginine vasopressin (AVP), mediates a wide variety of peripheral and central physiological and behavioral effects by acting on four different G-protein coupled receptors, termed V1a (vascular), V1b (pituitary), V2 (renal), and OT (uterine).

To obtain more potent and selective AVP analogues, we decided to replace the Tyr^2 with a bulky unnatural non-proteinogenic α-amino acid, (S)-1-adamantyl-glycine (Adg). It should be noticed that the modification, apart from reducing the flexibility, also changed the character of the molecule from aromatic to aliphatic. We designed the following analogues: [Adg^2]AVP (**I**), [Mpa^1,Adg^2]AVP (**II**), [Adg^2,D-Arg^8]AVP (**III**), [Mpa^1,Adg^2,D-Arg^8]AVP (**IV**), and [Adg^2,Val^4]AVP (**V**). The peptides were synthesized manually using Fmoc-chemistry. The purity and identity of each peptide were determined by HPLC and MALDI TOF mass spectrometry.

All the peptides were tested for pressor, antidiuretic, and in vitro uterotonic activities. We also determined the binding affinity of these compounds to human OT receptor. None of the analogues displayed significant biological activity. A low level of antiuterotonic activity was found in the case of one peptide, [Mpa^1,Adg^2]AVP (**II**), with pA_2~ 6.64, but its affinity to the oxytocin receptor was very low (affinity constant 1530 nM). The remaining compounds showed a slight agonist activity in uterotonic tests (values ranging from 0.20 to 1.9 IU). With respect to antidiuretic activity, only [Mpa^1,Adg^2,D-Arg^8]AVP (**IV**), showed a weak agonism (about 10% of that of AVP). Regarding the pressor activity, all the analogues were inactive over the concentration range tested.

It is hypothesized that the bulky planar side chain of chosen modifications and its aliphatic character might have contributed to an almost complete loss of activity of the analogues. On the basis of these results, we are undertaking further SAR studies using NMR and theoretical molecular modeling methodology.

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Partial funding for this work was provided by Polish State Committee for Scientific Research under the grant No. grant 0230/H03/2008/35 and 2385/B/H03/2008/34.

New arginine vasopressin analogues acylated on the *N*-terminal part of the molecule

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Arginine vasopressin (AVP), a neurohypophyseal hormone and neuromodulator, is a cyclic nonapeptide with a disulfide bridge between Cys residues at positions 1 and 6. Numerous studies have been carried out on the structure – activity relationship of arginine vasopressin during the last three decades, trying to cast light on the mechanism of its action. It has been demonstrated that conformation of the N-terminal part of AVP analogues is crucial for their pharmacological activity.

In an effort to enhance the potency or pharmacological properties of arginine vasopressin analogues, we decided to learn how acylation of the N-terminal part of the molecule with bulky acyl groups would affect biological potency. The [cis–Apc^2, Val^4]AVP peptide was chosen as a reference compound because it showed very interesting biological activity in all tests. This analogue turned out to be a potent oxytocin antagonist (pA_2 = 8.22±0.11), weak pressor antagonists (pA_2 = 6.85), while its antidiuretic potency was lower than that of AVP but with significantly prolonged action. The new analogues were obtained by acylation of the N-terminus of the peptide with 1-adamantanecarboxylic acid (Aca), 4-tert-butylbenzoic acid (t-Bba), 4-hydroxybenzoic (Hba), 4-aminobenzoic acid (Aba). We obtained the following peptides: Aca[cis–Apc^2, Val^4]AVP (**I**), t-Bba[cis–Apc^2, Val^4]AVP (**II**), Hba[cis–Apc^2, Val^4]AVP (**III**), Aba[cis–Apc^2, and Val^4]AVP (**IV**). The peptides were tested for their pressor, antidiuretic, and in vitro uterotonic activities. The proposed modification resulted in suppression of all biological activities. All the new peptides did not virtually interact with the V_2 receptor. The acylation of the N-terminal part of molecule eliminated the effect on blood pressure with the exception of analogue **III** which remained a weak antagonist (pA_2~7.0). Regarding the oxytocic activity, three of the analogues (**I**, **III**, **IV**) were weak antagonists (pA_2 values ranging from 5.70 to 7.61). Surprisingly enough, acylation of the reference compound with 4-tert-butylbenzoic acid transformed its antiuterotonic activity in agonistic one (10.1 ± 3.8 IU/mg). On the other hand, all the peptides turned out to be highly selective antagonists of oxytocin.

Acknowledgements:
Partial funding for this work was provided by Polish State Committee for Scientific Research under the grant No. grant 0230/H03/2008/35 and 2385/B/HO3/2008/34.

Complexes of hybrid tripeptide peptidomimetics containing dehydroamino- and aminophosphonic acid in the chain with Cu^{2+} and Zn^{2+} ions

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Dehydropeptides, the compounds which inclusive one or more dehydroamino acid residues, are very interesting as an object of conformational studies. Presence of double bond between C^{α} and C^{β} and two neighboring peptide bonds lead to coupling of π electrons, which not only influence on side chain but also on all peptide conformation [1-5]. Additionally, introduction of dehydroamino acid residue into the short peptide chain changing considerably the binding abilities of peptide ligands towards copper(II) ions [6]. In order to find out if this effect would be also observed in the case of phosphonopeptides, we have undertaken the synthesis of hybrid tripeptides containing Δ^{Z}Phe in position 2 and phosphonic residue in position 3 in the peptide chain. Their binding abilities towards Zn^{2+} and Cu^{2+} ions have been examined by use of potentiometric and spectroscopic methods.

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Synthesis and application of libraries of artificial receptors

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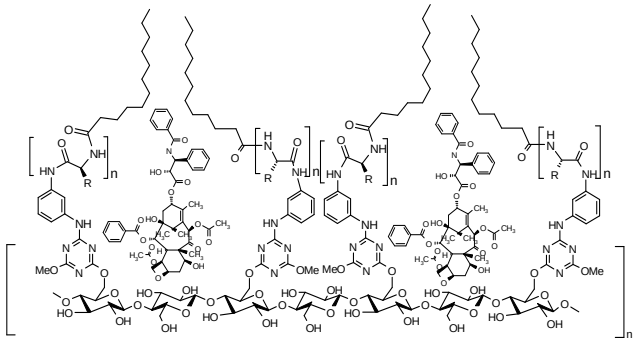
Recently, we found that supramolecular structures formed from N-acylated peptides attached to the CH_2OH groups on the surface of cellulose support *via* aminophenylamino-1,3,5-triazine recognize small guests molecules, resembling the artificial receptors. In the contrary to the rigid structure of the most of artificial receptor described in the literature [1] (especially those, prepared by imprinting in the polymer matrix), the supramolecular structures formed by N-lipidated peptides are highly flexible. Thus, it is expected, that the host structures adjust their shape to fit the guests molecules most efficiently. Therefore, the nature of the host-guest interactions is complex, and the binding process depends on the structure of amino-acids as well as lipidic fragment of the receptor and analyte structure. However, we found that the combinatorial approach successful for designing the receptor structure specific for the given structural motifs of the guest molecules [2]..



Recently, the library of receptors were designed and prepared in order to study binding pattern of biologically active compounds with different cytostatic activity: Paclitaxel®, and group of 2-chloroethylamino derivative of triazine [3].

The aim of this studies is to verify the hypothesis, that binding pattern of artificial receptors could be correlated with pharmaceutical activity, and subsequently, that library of artificial receptors could be applied as the new tool for preliminary screening pharmaceutically active compounds.

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New chromogenic substrates of Proteinase 3

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Proteinase 3 (PR3) along with cathepsin G (CatG) and neutrofil elatase (HNE) is a member of neutrofil serine proteases (NSP). Those three enzymes are synthesized and stored in active form in azurofile granules of neutrofiles. PR3 is main antigen in Wegeners granulomatosis [1], chronic inflammatory disorder of unknown etiology. It is characterized clinically by respiratory tract and renal disease, in patient serum there is observed the high prevalence of antineutrophil cytoplasmic antibody (ANCA) mostly against proteinase 3. The aim of this study was to obtained chromogenic and fluorogenic substrate that will could utilized for determination of PR3 in biological fluids. Previously selected PR3 substrate [1] was used as a starting structure.

The general formula of synthesized peptides is as follows: ABZ-Tyr-Tyr-Abu-ANB-X-NH_2, where ANB (stated for 5-amino-2-nitrobenzoic acid) served as served as chromofore and acceptor of fluorescence, ABZ (aminobenzoic acid) is donor of fluorescence in these FRET peptides and X is proteinogenic amino acid (except Cys). The introduced modifications influenced substrate activity of the peptides synthesized. The highest value of specificity constant for PR3 was obtained for peptide with Gln in discussed position (k_{cat}/K_M = 284,000 $M^{-1}\times s^{-1}$) which was twice as active as the reference compound (with lack of substituent in X position). In addition, for the introduced modification more efficient energy transfer was observed mainly due the batochromic effect.

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Acknowledgments:
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Assessment of analgesic potency of an opioid peptide analogue biphalin in a mouse model of neuropathic pain

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Neuropathic pain arises from a lesion or disfunction of the peripheral or central nervous system. Among peripheral mononeuropathies sciatic nerve compression or damage is a fairly common disorder. Patients afflicted with neuropathic syndromes experience abnormal chronic pain characterized by allodynia and hyperalgesia. Neuropathic pain serves a challenge for clinicians worldwide due to its complexity and resistance to treatment. There is much doubt and controversy around the use of opioid therapy in neuropathic pain since this type of pain is thought to be refractory to opioids. Nevertheless, promising results employing various animal pain models encourage further research on the effectiveness of opioid analgesia in human subjects. The discovery of a dimeric enkephalin analogue-biphalin opens new possibilities in neuropathic pain treatment arising from a lesion of the peripheral nerve. After intravenous administration biphalin produces profound analgesia comparable to an equipotent dosage of morphine as proven in earlier pharmacological studies in animal acute and chronic pain models. Simultaneously biphalin lacks the shortcomings typical for other opioid drugs such as morphine and additionally shows good permeability through the blood-brain barrier.

We sought to investigate the analgesic effects of biphalin in a murine model of peripheral nerve injury, which mimics mononeuropathic pain conditions seen in human patients.

Acknowledgements:
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Conformational studies of arginine vasopressin analogues modified at position 2 with D-α-2-indanylglycine

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Vasopressin (AVP), also known as arginine vasopressin or antidiuretic hormone, is a circulating endogenous nonapeptide hormone with potent vasoconstrictor and antidiuretic properties. It is used to treat diabetes insipidus and acute oesophageal variceal haemorrhage[1].

The conformation of the N-terminal part of vasopressin analogues is crucial for their pharmacological activity. Proper orientation of the Tyr^2 side chain is necessary for activity. In this project, we study the effect of the substitution of D-α-2-indanylglycine in position 2 of AVP analogues on their conformation. The two following analogues are subject of this study: [D-Igl^2]AVP (I) and [Mpa^1,D-Igl^2]AVP (II) (Mpa = 3-mercaptopropionic acid). These peptides are strong antioxytocic agents and exhibit only negligible antidiuretic activity (likewise [L-Igl^2]AVP and [Mpa^1,L-Igl^2]AVP). Regarding pressor activity, the new analogues are weak antagonists of AVP (in contrast to [L-Igl^2]AVP and [Mpa^1,L-Igl^2]AVP, devoid of any activity in the concentration range tested). Analogue (II) due to substitution of Mpa^1 has lower affinity to human oxytocin receptor then analogue (I). This relationship can also be observed for [L-Igl^2]AVP and [Mpa^1,L-Igl^2]AVP (unpublished data).

In the interaction of peptide hormones with their membrane receptors a significant role plays lipid bilayer, therefore we have carried out our studies of new vasopressin analogues in a sodium dodecylsulfate (SDS) micelle using 2D nuclear magnetic resonance (NMR) and theoretical methods. Conformation-activity considerations well be attempted.

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Acknowledgements:
This work was supported by the Polish Scientific Research Committee Grant No. N N204 181736 grant. The calculations were carried out in the Academic Computer Centre (TASK) in Gdańsk, Poland

The role of disulfide bridge in SFTI-1 trypsin inhibitor in the inhibition of serine proteinases

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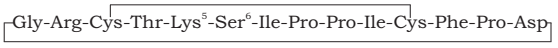
The trypsin inhibitor SFTI-1 isolated in 1999 from the sunflower seeds by Luckett *et al*. [1] is currently the smallest naturally occurring peptidic proteinase inhibitor.
Its primary structure is shown bellow:



One of the first questions addressed to the SFTI-1 was about the role of cyclic elements (disulfide bride and head-to-tail cyclisation). In our first paper on SFTI-1 [2] we showed that monocyclic SFTI-1, with disulfide bridge only, inhibited bovine β-trypsin with the same strength as the wild inhibitor. Korsinczky *el al.* [3] proved that a disulfide bridge is essential for maintaining the structure of such analogues and that the presence of disulfide bridge in the sequence of SFTI-1 increased its proteolytic stability. This finding is again in a good agreement with our previous [2] and more recent results describing kinetic studies of peptomeric analogues of this inhibitor [4,5].
Based on these very interesting results, we decided to synthesize a series of SFTI-1 monocyclic analogues in which disulfide bridge was formed by combination of Cys, Hcy, Pen and Nhcy (*N*-sulfanylethylglycine)) introduced in positions 3 and/or 11. In analogues synthesized in substrate specificity position P_1 we introduced Lys or Phe or peptoid monomers *N*-(4-aminobutyl)glycine (Nlys) or *N*-benzylglycine (Nphe) that mimicking these proteinogenic amino acids.
The peptides were synthesized manually by solid-phase method using Fmoc chemistry. *N*-Substituted glycine derivatives were introduced into the peptide chain by the submonomeric approach. In this report, the inhibitory activity of synthesized peptomers in relation to their proteolytic susceptibility will be discussed.

[1] Luckett S., Santiago Garcia R., Barker J.J., Konarev A.V., Shewery P.R., Clarke A.R., Brady R.L., J. Mol. Biol., **290**, 525-533, 1999.
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This work was supported by Ministry of Science and Higher Education (grant no. 2889/H03/2008/34).

Analogues of trypsin inhibitors SFTI-1 modified in absolutely conservative P_3' position

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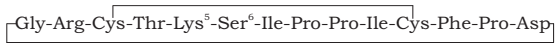
In 1999 Lucket e*t al*. [1] isolated from the sunflower seeds the low-molecular trypsin inhibitor SFTI-1, which belongs to the Bowman-Birk inhibitors (BBI) family. Its primary structure is shown bellow:



The P_1-P_1' reactive site of this circular inhibitor is located at the Lys^5-Ser^6. A common secondary structure element among BBIs is a 9 residues loop that comprises two Cys residues with VIb β-turn located in the centre [2]and entirely conserved element, Pro residue in P_3' position (Pro8 in SFTI-1) formed with proceeding Ile7 *cis*-peptide bond [3]. Although the role of L-proline in position P_3' of BBIs was extensively studied, surprisingly, only analogues containing in this position Ala residue were described.
Here we have designed and synthesized five new monocyclic (with disulfide bridge only) SFTI-1 analogues modified in discussed region by peptidomimetic moiety:
[Hyp^8]SFTI-1 (**1**), [$^7\psi^8(CN_4)Ala^8$]SFTI-1 (**2**), [$^9\psi^{10}(PEG)_1$]SFTI-1 (**3**),
[$^8\psi^{10}(PEG)_1$]SFTI-1 (**4**), [$^7\psi^{10}(PEG)_2$]SFTI-1 (**5**), where Hyp – hydroxy-L-proline, PEG – poly(ethyleneglycol) chain**,** CN_4 – 1,5-disubstituted terazole ring.
The peptides were synthesized manually by solid-phase method using Fmoc chemistry. Tripeptide building block, containing tetrazole moiety Boc-Ser-Ile-ψ[CN_4]-Ala-OH, was synthesized separately in solution, and was coupled to the peptidyl-resin.
To evaluate the influence of introduced modifications on the inhibitor–enzyme interaction, we determined equilibrium association constants (K_a) of the synthesized inhibitors with bovine β-trypsin.
The obtained results indicated that only two analogues (**1** and **2**) were able to inhibit the experimental enzyme. Three analogues in which fragments Pro^9-Ile^{10}, Pro^8-Pro-Ile^{10} and Ile^7-Pro^8-Pro-Ile^{10} were substituted by amino-PEG-acid spacers appeared to be completely inactive. It shows, that the conformational freedom of PEG abolishes bended structure that is stabilized in SFTI-1 by *cis* peptide bond.

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This work was supported by the University of Gdańsk (BW/8000-5-0256-9).

Conformation-activity relationships of 8 delto-/dermorphin *N*-terminal tetrapeptides cyclized(1-4) by a urea unit

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[b]Peptide Laboratory, Department of Chemistry, Warsaw University, 02-093 Warsaw, Poland
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Given new conformational data gathered by us, we revisit [1] structure-activity analysis for 8 cyclo(N^{ω},$N^{\omega'}$-carbonyl-D-Daa^2,Daa^4)-dermorphin-(1-4)-NH_2 analogues, derived from combinations of D-Lys, D-Orn, D-Dab and D-Dap in position 2 with their L-counterparts in position 4, thus giving tetrapeptides restrained by 14-16 membered rings. The in vitro activities determined in the GPI and MVD assays indicate that the most active peptides are those restrained by 15- and 16-membered rings with Dab in position 4. The novelty relies on analyzing their NMR spectra using time-averaged molecular dynamics with restraints [2] in complement to the former weighed fits of low-energy conformations. With major conformational constraints imposed by the 14-16 membered rings, they show well-defined conformations of the backbone with more conformational freedom manifested by the exocylic Tyr^1 and Phe^3 side chains. We try to identify which structural fragments are responsible for high activity towards opioid μ and δ receptors.

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The peptomeric analogues of SFTI-1, modified in P_1 and P'_1 position by *N*-substituted glycine residues

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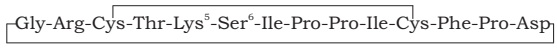
Serine proteinases are widely distributed in nature and are responsible for many physiological processes. Their uncontrolled activity may be dangerous to the organism and evoke a series of critical pathological conditions. Therefore, serine proteinase inhibitors, that control activity of these enzymes are a promising class of therapeutic agents. In 1999 Luckett *et al.* [1] isolated from sunflower seeds a trypsin inhibitor SFTI-1, the smallest one among the most potent inhibitors of the Bowman-Birk family. Its primary structure is shown bellow:



The reactive site P_1-P'_1 of the SFTI-1 inhibitor is located between residues Lys^5-Ser^6.
In our previous work [2] we have shown that *N*-substituted glycine derivatives (peptoid monomers) in P_1 position are recognized by the enzyme and do not affect the inhibitory activity. Our studies [3] have also proved that the linear analogue of SFTI-1 with *N*-benzylglycine (Nphe) in P_1 position inhibits α-chymotrypsin, but peptide bonds formed by this derivative are not proteolytical-resistant. The rate of this cleavage is lower than for the "regular" peptide bond. High chymotrypsin inhibitory activity of the linear peptomeric analogue of SFTI-1 indicates that this compound can be a good model for our studies.
Continuing our investigation on peptomeric analogues of SFTI-1 we have designed and synthesized series of linear and monocyclic analogues of SFTI-1 with *N*-substituted glycine in position P_1 or/and P'_1 peptoid monomers (Nphe, Nhse) that mimic proteinogenic amino acids. We expected that introduction into the peptide chain such derivatives would result in the increased resistance to proteolysis of the obtained *pep*tide-pep*to*id hybrid poly*mers* (peptomers) and in consequence yield effective proteinase inhibitors.
The compounds were synthesized manually by solid-phase method using Fmoc chemistry. *N*-Substituted glycine derivatives were introduced into the peptide chain by the submonomeric approach. It this report, the inhibitory activity of synthesized peptomers in relation to their proteolytic susceptibility will be discussed.

[1] Luckett S., Santiago Garcia R., Barker J.J., Konarev A.V., Shewery P.R., Clarke A.R., Brady R.L., J. Mol. Biol., **290**, 525-533, 1999.
[2] Stawikowski M., Stawikowska R., Jaśkiewicz A., Zabłotna E., Rolka K., ChemBioChem, **6**, 1057-1061, 2005.
[3] Łęgowska A., Bulak E., Wysocka M., Jaśkiewicz A., Lesner A., Dębowski D., Rolka K., Bioorg. Med. Chem., **16**, 5644-5652 2008.

This work was supported by Ministry of Science and Higher Education (grant no. 2889/H03/2008/34).

N-Methyl-α,β-dehydroamino acids – synthesis and conformation

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Dehydroamino acids with a methylated N-terminal tertiary amide bond occur in natural small cyclic peptide toxins. *N*-Methyldehydrophenylalanine constitutes tentoxin, product of several phytopathogenic fungi of the genus *Alternaria*. Tentoxin induces chlorosis in many dicotyledoneae plants. *N*-Methyldehydrobutyrine was found in nodularins, cyclic pentapeptide hepatotoxins produced by cyanobacterium *Nodularia spumigena*. *N*-Methyldehydroalanine and *N*-methyldehydrobutyrine are structural motif of microcystins, a group of cyclic heptapeptides produced by a number of cyanobacterial genera, *Anabena*, *Nostoc* and *Oscillatoria*, the most notable of which is the widespread *Microcystis* from which the toxins take their name. Nodularins and microcystins show strong inhibitory activity to protein phosphatases 1 and 2A and have been reported to be tumor promoters.

To investigate their conformational preferences, a systematic analysis was performed on *N*'-methylamides of *N*-acetyl-*N*-methyldehydroamino acids (Ac-ΔMeXaa-NHMe, where Xaa = Ala, (Z)-Abu, (E)-Abu, (Z)-Phe, and (E)-Phe). The compounds were synthesised. X-Ray analysis, spectral methods as well as theoretical studies at DFT level involving analysis of the potential energy surfaces were applied.

The main feature of the studied *N*-methyldehydroamino acids is their considerable tendency to adopt the configuration *cis* for the N-terminal tertiary amide bond [1-4]. Therefore, it can potentially be new promoters of *trans-cis* isomerisation of the amide bond useful in peptide design.

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Tripeptides as potential inhibitors of urokinase

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Plasminogen activators are highly specific serine proteases capable of activating plasminogen to plasmin. Studies have indicated that urokinase has an ability to degrade extracellular matrix, and is a key mediator in cellular invasion, growth and the metastasis of tumor [1, 2]. Elevated levels of uPA in cancer cells usually indicate a poor prognosis for patient survival [3, 4]. Consequently, a selective inhibitor for u-PA may be therapeutically useful in cancer treatment. It is desirable that a synthetic u-PA inhibitor has adequate potency and selectivity for u-PA relative to t-PA, plasmin, thrombin and trypsin to avoid the possibility of antifibrinolytic side effects.

We present the synthesis and the investigation of effect peptides of general formula X-SO_2-D-Ser-Ala-Arg-OH, where X = CH_3, Ph, Ph-CH_2, 4-CH_3-Ph, 4-CH_3-PhCH_2, 1-naphtyl, 2-naphtyl, 4-Cl-Ph, 4-Br-Ph, 2,4,6-trimethyl-Ph, 2,4,6-triisopropyl-Ph, 4-acetamido-Ph on amidolytic activity of urokinase, thrombin, plasmin, trypsin, t-PA and kallikrein. We expected that the use of specific tripeptide sequence to urokinase would cause high urokinase selectivity. The peptides were synthesized on the solid phase manually using standard Fmoc-based strategy.

The examined compound did not influence the enzymatic activity of kallikrein.

According to the obtained results compound 2,4,6-triisopropyl-Ph-SO_2-D-Ser-Ala-Arg-OH was the most selective compound toward urokinase. Compound CH_3-SO_2-D-Ser-Ala-Arg-OH was selective inhibitor of trypsin and Ph-SO_2-D-Ser-Ala-Arg-OH of thrombin. The obtained values of K_i are higher than K_i of the earlier described inhibitors [5, 6]. However, 2-phenethyl-SO_2-D-Ser-Ala-Arg-al (Tamura et. al.) is the alkylating agents and irreversibly inhibit urokinase by forming a covalent adduct with an active site of the enzyme. Previously analysis for the effect of H-D-Ser-Ala-Arg-NH-$(CH_2)_8$-NH_2 (Markowska et al.) on the activity of urokinase showed that a derivatives with these kinds of the sequence competitively inhibits urokinase [7].

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Structure of the *C*-terminal fragment (87-105) of human serum amyloid A

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Human serum amyloid A (SAA) is a highly conserved apolipoprotein associated with high-density lipoproteins (HDL) in plasma. Predominantly produced by the liver, it takes part in several activities: HDL metabolism, pathogen defense and cholesterol transport, it also plays major role in host defense during the acute phase of inflammation. SAA has been involved in several pathological conditions including atherosclerosis, rheumatoid arthritis, Alzheimer's disease and cancer [1].

Recent *in vitro* studies on interactions betweeen SAA and human cystatin C (hCC) found direct interactions between the C-terminal 19-amino acid fragment (87-105) of SAA and the C-terminal 28-amino acid sequence (93-120) of hCC (Czaplewska et al, unpublished). This project intends consecutive structural studies of these two peptides: SAA(87-105) and hCC(93-120) in solution using 1-D and 2-D NMR At the moment, we have done a set of NMR spectra and some structure-solving preliminaries for SAA(87-105). Subsequently, having completed these tasks for the both peptides we hope that structural data will help elucidate some biologically relevant subtleties of interactions between SAA and hCC.

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Short peptides containing L-lysine and ε-aminocaproic acid as potential plasmin inhibitors

Midura-Nowaczek Krystyna[a], Purwin Maciej[b], Bruzgo Irena[a], Markowska Agnieszka[a]

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[b]Pharmacy Super-Pharm, Galeria Biala, Bialystok, Poland

The derivatives of lysine have been widely examined as potential synthetic substrates and inhibitors of plasmin. During our earlier investigations on the active-centre directed plasmin inhibitors, a series of lysine amides and dipeptide derivatives with C-terminal lysine cyclohexyl, benzyl and hexyl amides were examined. The compounds with cadaverine residue connected with the lysine were also tested [1-3].

In the search for new low molecular plasmin inhibitors with a simple and easy to synthesize structure, we obtained eight short peptides containing L-lysine and ε-aminocaproic acid. Every synthesized dipeptide or tripeptide was transformed into methyl ester or unsubstituted amide. Some of the compounds also have protected amino groups. The effect of the obtained short peptides on the amidolytic activities of plasmin, thrombin and trypsin was determined as the IC_{50} values.

According to the obtained results, two ε-aminocaproic acid residues and one residue of lysine are necessary in the plasmin inhibitor structure. The dipeptides are practically inactive. The amide of tripeptide with Boc-substituted N-terminal amino group of EACA was the most selective inhibitor of the amidolytic activity of plasmin. The derivative with a C-terminal amide residue and the unsubstituted N-terminal amino group of EACA was a weak inhibitor of plasmin and thrombin. Practically no difference in the plasmin activity inhibition was observed in the methyl esters of tripeptides with Boc substituted and unsubstituted N-terminal amino groups of EACA. However, the compound with Boc group seems to be a more selective inhibitor. The substitution of N^{ε}-amino group of lysine in tripeptides results in the disappearance of the inhibitory activity or its drastic decrease. Our results suggest that the simple derivatives of lysine containing EACA may be efficient and selective active-centre directed inhibitors of plasmin.

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[2] Midura-Nowaczek K., Lepietuszko I., Bruzgo I., Acta Polon. Pharm. 63, 33-37, 2006.
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Conformational studies of peptides corresponding to the *C*-terminal fragment of domains A and B bacterial protein G

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Domains A nad B of bacterial protein G posses different three dimensional structure (domain A is all α-helical, domain B is α/β protein) and does not share too much of sequence identity. In both proteins the C-terminal fragment in the native structure forms α-helix and β-hairpin in domain A and B respectively. Recently Alexander and coworkers [1] using molecular biology methods obtained series of domain A and B sequences which are very similar (some pairs of domain A and B variants share 88% of identical amino acid residues in corresponding positions) in amino acid sequences but retains three-dimensional structure of wild-type protein. Interestingly earlier studies shows that peptides corresponding to the C-terminal fragments of the domains A and B bacterial protein G reveals that such peptides form meta-stable three-dimensional structures in solution [2,3].

The aim of this work is to determined structures and conformational dynamics series of 18-residues peptides which share large number of identical residues. Sequences of peptides used in our study are based on C-terminal fragments of variants of domains A and B of bacterial protein G obtained by Alexander and coworkers [1]. According to limited literature data peptides based on sequence of the C-terminal fragment of domain A and B should form structures similar to α-helix and β-hairpin respectively. The three dimensional structure of all peptides will be determined by using data from NMR experiments such as COSY, ROE and TOCSY, supported by time-averaged MD simulations.

[1] Aleksander P.A. et al., PNAS, **104**, 11963-11968, 2007
[2] Bai Y., et al., Protein Sci., **6**, 1449-1457
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Acknowedgement:
This work is financially supported by University of Gdańsk grant BW/B50B-5-0296-9.

The studies on application of Ugi reaction to the peptoids synthesis

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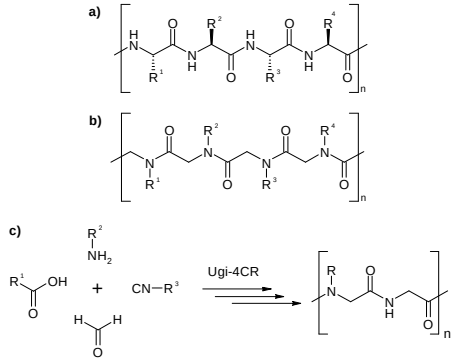
Peptoids are a specific group of polymeric compounds which mimic a structure and properties of peptides. Generally, skeleton of peptoids is composed of *N*-substituted glycines (Scheme 1). Fundamental improvements of the peptoids properties comparing to the corresponding in peptides are: (a) enhancing of bioavailability by increasing of lipophility; (b) resistant on hydrolytic action of enzymes; (c) loss of chirality and simplification of the synthesis involving no risk of racemization due to lack of stereogenic centers.

Peptoids are also known as HIV protease inhibitors [1] and compounds that mimic the structure, function, and mechanism of helical antimicrobial peptides. [2] Peptoids have been also studied as 19S proteasome inhibitors. [3]

A new approach for the peptoid synthesis takes advantage of multicomponent Ugi reaction (Ugi-4CR). A series of peptoids have been synthesized by Ugi reaction with the application of formaldehyde as a formyl component. Different forms of formaldehyde have been examined. Results of the studies on application of Ugi reaction for the synthesis of oligopeptoids will be presented.(Scheme 1).



Scheme 1. a) Structure of peptides; b) Structure of *N*-substituted glycines (peptoids); c) General approach for the application of Ugi reaction for the peptoids synthesis.

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Conjugation of systemin with AZT by employing click chemistry

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The 1,3-dipolar cycloaddition also known as the Huisgen cycloaddition is a chemical reaction belonging to the larger class of cycloadditions. The cycloaddition reaction between the azide and alkyne appended substrates allows the synthesis of the desired conjugates in high purity and yields irrespective of the sequence and functional groups on either of the two substrates [1]. The Huisgen cycloaddition known also as a 'click-reaction' or "click chemistry" was employed for the synthesis of systemin–AZT conjugate. Using a plant peptide (18-aa) hormone systemin modified at N-terminus with propiolic group and 3'-azido-2',3'-dideoksythymidine (AZT) by employing a click chemistry, a conjugate of systemin-AZT has been obtained. The click reaction was catalyzed by Cu(I) in water/Bu^{t}OH mixture. In water, 2-fold excess of AZT drives the reaction to completion in a few minutes with no side products. The reaction was easily monitored by capillary electrophoresis (CE). Circular dichroism (CD) study show that systemin does not change its secondary structure after conjugation with AZT and adopts a random coil conformation in aqueous solution.

[1] Meldal M, Tornøe C.W., Chem. Rev., **108**, 2952-3015, 2008.

Acknowledgement:
This work was supported by the University of Gdansk grant no. DS 8291-4-0130-9.

Mechanism of formation of the *C*-terminal β-hairpin of the B3 domain of the immunoglobulin binding protein G from *Streptococcus*

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Proteins fold in different ways but a folding pathway always proceeds through sequential events, which lead to the native conformation. One of the methods, which helps to deduce the folding mechanism, involves a conformational study of protein fragments corresponding to regular secondary structure. Short fragments of proteins enable one to study local interactions isolated from the protein context and, therefore, indicate the importance of these interactions in determining protein secondary-structure elements. Thus, these fragments may play important roles as nucleation centers in initiating protein folding through local interactions, and provide knowledge about the earliest events of protein folding.

6-, 8-, 12-, 14-, 16-, and 20-residue peptides corresponding to the C-terminal β-hairpin of the B3 domain of the immunoglobulin binding protein G from *Streptoccocus* were studied by using circular dichroism (CD) and nuclear magnetic resonance (NMR) spectroscopy at various temperatures, and by differential scanning calorimetry (DSC). The common feature of each of the investigated fragment was the preservation of the turn region (Asp51 - Thr56) as in the native protein, regardless of the temperature. Thus, it is concluded, that the turn region in the C-terminal β-hairpin, stabilized by the flexible mostly non-native hydrogen bonds, is the first folding initiation center on which the rest of the polypeptide chain could find the favorable environment to fold. The investigations also showed that, the hydrophobic interaction between Tyr50 – Phe57 (so-called "1st pair" of hydrophobic residues) and between Trp48 – Val59 (so-called "2nd pair" of hydrophobic residues) play a role as a hairpin zippers in the next folding events. Additionally, the study showed that the folding and unfolding processes for the C-terminal β-hairpin might not be identical, what was suggested previously [1].

[1] Munoz V, et al. Nature **390**,196-199,1997

Point mutation V57N opens the way to monomeric human cystatin C

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Human cystatin C (hCC) is a low molecular mass protein (120 amino acid residues, 13,343 Da). At physiological conditions hCC wild type is a monomeric protein, but under crystallization conditions forms a domain swapped dimer. Loop 1 is the only part of hCC which undergoes significant structural change during the dimerization process. Experimental and theoretical studies revealed that this region of cystatin fold is conformationally unstable and the main destabilization is connected with Val residue (Val57 for hCC) located on the tip of the loop. In order to assess the influence of the unfavorable Val residue on the dimerization of the hCC, the mutants in which this residue was substituted by residues favored in this position of β-turns, like Asn, was obtained. The experimental structure of monomer hCC was not defined so far. One can deduce the shape of the protein from the structure the of dimeric hCC or the structural similarity of hCC to monomeric chicken cystatin. We resolved the experimental structure of monomer of the stable mutant hCC V57N (Figure 1) using X-ray method with resolution 2.04Å.

Figure 1.Structure of monomeric V57N hCCAcknowledgements:

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Chemical and enzymatic synthesis of L-DOPA derivatives

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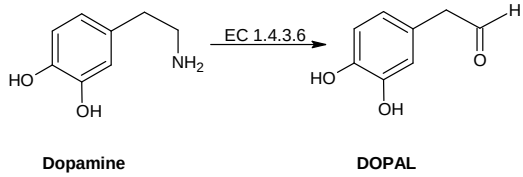
L-DOPA (3',4'-dihydroxy-L-phenylalanine) and dopamine (3',4'-dihydroxy-phenylethylamine), DA, are involved in many very important physiological processes. DA is a neurotransmitter in the nervous system of mammals and disturbance in production of DA leads to many pathologies such as Alzheimer's and Parkinson's diseases (PD), or schizophrenia.

PD is connected with progressive apoptosis of the cells producing DA, and this leads to the lack of DA in the brain. Although PD has been known since 1817, there is no explicit reason why dopaminergic cells die. Also the recent investigations *in vitro* and *in vivo* strongly indicate that the elevated concentration of 3',4'-dihydroxyphenylacetaldehyde (dopaldehyde, DOPAL), one of metabolites of DA, causes the death of neuron cells observed in PD.

In this study we have elaborated the synthesis of DOPAL using chemical and enzymatic methods. Chemical synthesis was done by modification of pinacol-pinacolone rearrangement of adrenaline, described by J. H. Fellman [1]. The second method was based on enzymatic oxidation of dopamine, catalyzed by diaminooxidase (EC 1.4.3.6), *Scheme 1*.



Dopamine **DOPAL**

Scheme 1. Enzymatic synthesis of DOPAL

The purpose of this study is to develop a useful methods for the synthesis of tritium and deuterium labeled DOPAL needed to investigate the mechanism of enzymatic reduction of dopaldehyde to related alcohol using KIE (*Kinetic Isotope Effect)* technique.

[1] Fellman J.H., Nature, **182**, 311-312, 1958

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Study of the interaction of PAMAM G4 dendrimer with selected amino acids and 5-fluorouracil in water solutions

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Dendrimers have recently been studied very intensively because of their possible applications, especially as nano-containers. These exceptional polymers have globular architecture but detailed on numeral environmental conditions. Polyamidoamine denrimers (PAMAM G-4) with ethylenediamine core are known as potential carriers for medicaments. One of them is 5-fluorouracil– an oncological drug, which is used as the chemotherapy agent.

The first aim of investigations was to evaluate the amount of 5-fluorouracil molecules associated with PAMAM G4 dendrimer based on ethylenediamine core. Polyamidoamine dendrimers possess cationic amino groups on the structure of molecule and it can be an excellent platform for attachment modifiers. It is also possible that dendrimers associate some ligands and among them also amino acids. Therefore the another aim of study was to find the interaction of dendrimer and the glycine (Gly) and serine (Ser) molecules. Glycine was selected as representative of the simplest amino acid with a hydrogen atom as substituent, while serine possesses hydroxyl group in its substituent. The interactions PAMAM G4 dendrimer with glycine, serine and 5-fluorouracil were studied using isothermal titration calorimeter VP-ITC MicroCal. The calorimetric measurements were carried at the temperature of 25°C. About 250 amino acids molecules may interact with PAMAM molecule. Curves describing heat effects of glycine and serine interactions with G4 PAMAM revealed linear course up to the molar ration amino acid to G4 PAMAM equal 250 : 1. It is likely to be in proportion to a consequence of the binding amino acid molecules with 64 superficial $-NH_2$ groups plus 124 internal amido groups and 62 tertiary amine groups of G4 dendrimer molecule. Circa sixty 5-fluorouracil molecules interact at first with PAMAM G4 molecules, mainly with the terminal amino groups of dendrimer.
Further amino acids and 5-fluorouracil molecules interacting with PAMAM G4 molecule create dispersion layer.

Investigated interaction of several L-α-amino acids with NaCl in aqueous solutions at 298.15 K

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There is an invariable pool of free L-α-amino acids in the biological fluids which an essential for proper functioning of organisms. These fluids contain also a specified quantity of ions, especially sodium, potassium and chloride ions, indispensable for the metabolic processes of living organism to proceed.
Thermodynamics parameter that characterize the interactions of zwitterions of amino acids with dissociated NaCl in water solutions is the enthalpic pair interaction coefficients, derived from McMillan-Mayer [1] modified theory.
These coefficients ($h_{A\text{-}NaCl}$) describe the sum of interactions between zwitterions of amino acids and dissociated sodium chloride with the competitive participation of water molecules. To determined these parameters, solution enthalpies of L-α-cysteine, L-α-proline, L-α-asparagine and L-α-glutamine in water and aqueous solutions of NaCl were measured by calorimetry at 298.15 K.
The obtained enthalpic coefficients ($h_{A\text{-}NaCl}$) compared with the hydrophobicity parameter of amino acid side chains based on the values of enthalpic heterogeneous pair interaction coefficients between zwitterions of amino acids and urea molecules [2].

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[2] Palecz. B., J. Am. Chem. Soc., **127,** 17768-17771, 2005.

Study of interactions between *N*–acetyl–*N'*–methyl–L–α–amino acid amides and urea molecule in water at 298.15 K

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The data of thermodynamics investigations of a simple organic substance containing functional groups analogous to those of protein molecule can be helpful in understanding behaviour of protein in constitutional fluids of organism. By this reason small peptides are regarded as a useful model comoounds for the study of this type of interactions.

It has been measured the enthalpies of solution of *N*-acetyl-*N'*-methyl-L-α-threoninamide, *N*-acetyl-*N'*-methyl-L-α-tyrosinamide, *N*-acetyl-*N'*-methyl-L-α-tryptophanamide, *N*-acetyl-*N'*-methyl-L-α-histidinamide in water and in aqueous urea solutions at 298.15 K, using the "isoperibil" type calorimeter. The standard solution enthalpies of amides in water and aqueous urea solutions were determined. These data were used to calculate the heterogeneous enthalpic pair interaction coefficients based on McMillan-Mayer's theory [1].

The enthalpic heterogeneous pair interaction coefficients are a measure of the energetic effects of interactions between amides and urea molecule taking place with the competitive participation of water molecules. The calculated coefficients were compared with the values of enthalpic pair interaction coefficients between amino acids and urea molecule [2].

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[2] Palecz. B., J. Am. Chem. Soc., **127,** 17768-17771, 2005.

Problem of ivDde removal during Fmoc-based solid phase peptide synthesis

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The amino-side-chain group of lysine, ornithine, diaminobutyric acid and diaminopropionic acid is very useful for the introduction to peptides variety of modifications as well as for the synthesis of branched and cyclic peptides. There are special orthogonal protecting groups for Fmoc SPPS syntheses in which on-resin derivatization is desired. Among them are used: expensive **Aloc** protecting group – cleaved by nucleophiles in the presence of Pd catalyst, **Adpoc** or **Mtt** that are more acid labile than Boc and are cleaved by repeated treatment with 1-2% TFA in DCM, and the most commonly used **ivDde** protecting group.

Fmoc-N^{ε}-1-(4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)-3-methylbutyl-L-lysine Fmoc-Lys(ivDde)-OH, is a valuable tool for the preparation of atypical peptides using Fmoc based SPPS. The ivDde group is stable to the commonly used 20% piperidine in DMF solution, but can be easily removed from the side chain by the treatment with 2% hydrazine monohydrate in DFM solution (5+7 min) leaving all other side-chain protecting groups intact. ivDde group is also stable to the common reagents employed for Boc cleavage (TFA or 50% TFA in DCM solution) and DBU at the normal concentration (2% in DMF solution) used for Fmoc removal.

In our recent studies several peptide analogues, *e.g.* 27-amino-acids residue transportan (GWTLNSAGYLLG**K**INLKALAALAKKIL-NH_2), 21-amino-acids residue transportan10 (AGYLLG**K**INLKALAALAKKIL-NH_2), and 15-amino-acids residue N-terminal galanin fragment (GWTLNSAGYLLGP**K**A-NH_2), modified with Lys(ivDde) were synthesized using standard Fmoc/Bu^t protocol. Analysis of products of syntheses, after treatment with 2% hydrazine monohydrate in DMF and cleavage of peptides from resins, has still shown the presence of ivDde-protected peptides in a large amount (about 65% in case of transportan and about 50% in case of transportan10).

In this study we have used different ivDde-deprotection conditions to investigate the susceptibility of the ivDde group for its selective "on resin" removal from ε-amino group of Lys residue within transportan and transportan10 structure. Crude peptides, were analysed by reverse phase high performance liquid chromatography and identified by MALDI-TOF mass spectrometry. Relative yields of synthesis products were determined by HPLC peak-area integration.

This work was supported by the University of Gdańsk grant BW 8000-5-0363-9.

Synthesis and biological properties of new transportan analogues containing adenine or benzimidazole derivatives

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Transportan (Tp) is a 27-amino-acid residue peptide with following primary structure GWTLNSAGYLLG**K**[13]INLKALAALAKKIL-NH_2. Tp is a chimeric cell-penetrating peptide containing 12 functional amino acids from the amino-terminus of the neuropeptide galanin and wasp venom peptide toxin, mastoparan in the carboxyl terminus, connected *via* a lysine residue. Mastoparan, possesses a variety of biological activities such as inhibition of the growth of Gram-positive bacteria, activation G-proteins. The other component of transportan, the amino-terminal fragment 1-12 is the smallest highly active galanin receptor ligand with agonist properties. Galanin is a neuroendocrine peptide widely distributed and has several biological functions in the endocrine systems as well as in the central and peripheral nervous system, such as effects on hormones release – inhibition of insulin secretion, affects memory, learning, neurons degeneration and feeding. In the gastrointestinal tract galanin modulates gastric smooth muscles activity.

In this study we have synthesized several new transportan analogues carrying different adenine derivatives or different benzimidazole acetic acid derivatives attached to the ε-amino-side group of L-Lys[13] residue. Next we characterized the biological properties of these new chimeric galanin analogues, investigating their action on rat isolated gastric smooth muscles. Studies have shown that all peptides contacted longitudinal rat gastric funds strips in a concentration-dependant manner. We observed that all synthesized analogues of transportan contracted rat gastric fundus strips stronger than non-modified peptide. The most active analogues were peptides containing adenine acetic acid and benzimidazole acetic acid.

This work was supported by the University of Gdańsk grant BW 8000-5-0363-9.

Immunosuppressory activity of ubiquitin fragments

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Ubiquitin is an 76-amino acid polypeptide present in all eukaryotic cells and highly conserved in evolution. Its major intracellular actions, such as targeting proteins to proteasome, are well described, although the extracellular function remains unclear. It was shown that ubiquitin itself as well as some of its fragments demonstrate a significant immunosuppressory activity for both cellular and humoral immunological response[1,2].

Recently we showed that the ubiquitin hydrolysate obtained after digestion with pepsin exhibits a very high effect on the humoral immune response of mice in SRBC test, even at low doses[3]. This high immunosuppresory activity could be the effect of either the presence of superpotent peptide fragments or kind of a synergystic or additive effect.

To examine the contribution of particular cryptides[4] (functional peptide products of protein degradation) to immunosuppressive activity of the peptic hydrolysate, we identified the peptidic products of digestion using ESI-MS, ESI-MS/MS and LCMS (Fig.1) and synthesized analogues of the most abundant fragments. The peptide corresponding to the ubiquitin[5-15] sequence (VKTLTGKTITL) exhibited the most significant, but interestingly inversely dose-dependent, immunosuppressory activity.

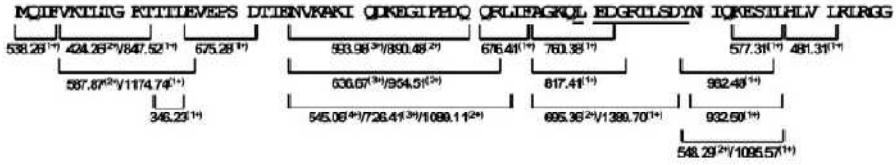

Fig.1. Most abundant peptic fragments of ubiquitin with the corresponding m/z values

The extraordinary immunosuppressive activity of the ubiquitin peptic hydrolysate and its origin is the major concern of our presentation.

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Synthesis and biological activity of 1-aminoalkylphosphonate diaryl esters as inhibitors of Spl A protease isolated from *S. aureus*

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Staphylococcus aureus is dangerous, life treating human pathogen which antibiotic resistance is still increasing. It reveals a great ability to overcome host defense mechanisms and colonize diverse organs. This pathogen is responsible for several toxinoses such as staphylococcal scalded skin syndrome (SSSS), toxic shock syndrome (TSS) and food poisoning; but the most serious *S. aureus* infection is septicemia. Screening of staphylococcal proteins library with antiserum from patients with *S. aureus* endocarditits resulted in new serine proteases discovery which are expressed during the infection [1]. One of these protease, Spl C is a product of gen belonging to the operon encoding up to six homologous Spl proteases including Spl A [2]. Spl A is a serine protease which can be very important pathogenic factor during bacterial infection [3].

In this report we describe the series of new derivatives substituted at phenyl ester rings 1-aminoalkylphosphonates – analogues of phenylalanine and leucine – as inhibitors of Spl A protease. 1- aminoalkylphosphonates diaryl esters are covalent, selective and irreversible inhibitors of serine proteases [4]. The chemical nature and position of examined substituent clearly demonstrates a strong structure-activity relationship. The IC_{5o} values toward Spl A proteases for all new inhibitors will be reported.

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Studies of interactions between Cystapep 1 analogs and phospholipids bilayer membranes using isothermal titration calorimetry (ITC)

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Mechanism of action of most of the antimicrobial peptides is disruption of microorganisms cell membrane. Efforts to understand interactions between plasmalemma and peptides antibiotics are increasing in importance. Isothermal titration calorimetry (ITC) is a great tool to study interactions between various lipid bilayer model membranes and peptides active against pathogens. Application of this technique include the measurement of binding affinity and its thermodynamic parameters.

We were using ITC to investigate interaction between Cystapep 1 analogs and model of procariotic or eucariotic cell membrane. Cystapep 1 is a peptidomimetic compound structurally based upon the *N*-terminal part of the inhibitory site of human cystatin C and active against several clinically important Gram-positive bacteria [1, 2]. In ITC research we have studied binding for both active and inactive analogs of Cystapep 1. Large unilamellar vesicles composed of zwitterionic (in the case of *Eucaryota*) and anionic (in the case of *Procaryota*) phospholipids were used as model membrane system. Our research indicate that Cystapep 1 analogs interact only with model of bacterial cell membrane. None of investigated compounds interact with model of eucariotic plasmalemma. Interaction between procariotic cell membrane model and Cystapep 1 analogs is not strongly depend on antibacterial activity of studied peptidomimetics. We have found that some of inactive compounds binding with liposomes as well as active peptidomimetics. This fact may indicate that mechanism of action of Cystapep 1 analogs is probably not related with interruption of bacterial cell membrane system.

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Acknowledgements:
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Biologically active osteocollagen type I and the process of its synthesis

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Collagen type I is present in the skin and skeleton of the vertebrates. It constitutes 95% of the organic components of the bones, with non-collagen proteins accounting for the remaining 5%. Structural components of the bones also include hydrated calcium and phosphate salts, known as hydroxylapatite.

The present period of civilization "explosion" shows a significant and accelerated increase in the number of incidences and the expansion of civilization diseases. One of those diseases is osteoporosis.

Therefore, it has been considered important for health reasons to obtain a substance that mimics the natural structure of the bones, with their compact and spongious layers, as well as periosteum. Such a substance that constitutes a combination of biologically active collagen type I and hydroxylapatite, meets the criteria of immunotolerance. The formula of the substance as well as its purpose are best reflected in its name of OSTEOCOLLAGEN.

Biologically active osteocollagen type I is a composite substance containing 20-40% of biologically active collagen type I and 60-80% hydroxylapatite of the summative formula $[Ca_{10}(PO_4)_6(OH)_2]$, blended according to the following formula:

Biologically active collagen type I
+
Hydroxylapatite
↓
Biologically active OSTEOCOLLAGEN type I

Monitoring dimerization and oligomerization process of human cystatin c using chemical and photochemical cross – linking

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Human cystatin C (hCC) is a low molecular mass, single chain protein (120 amino acids) belonging to the group of reversible inhibitors of cysteine proteases [1]. Naturally occurring mutant L68Q is found in patients with hereditary cerebral hemorrhage with amyloidosis [1]. Amyloidoses comprise a class of diseases characterized pathologically by the presence of deposits of fibrillar, aberrantly folded proteins, known as amyloid. Recent studies suggest that soluble protein oligomers, not fibrils, are the main pathogenic agent. Understanding the mechanism of formation this oligomers and their conversion to larger assemblies is necessary for prevention of many neurodegenerative diseases. Oligomerization is a dynamic process, in which many different species may exist in the mixture at a certain time point. This fact renders application of some, commonly used biochemical tools like electrophoresis or chromatography for studies of the oligomerization process. We have investigated the oligomerization of human cystatin C using a cross-linking approach. We have used less specific, but more effective methods like photochemical (PICUP) and chemical, glutaraldehyde-mediated methods, a well as more directed, thiol-specific cross-linking reagents with varying linker length. To the last purpose single cysteine mutants of hCC were obtained. According to our results the oligomerization of cystatin C is the most likely mediated by association of pre-formed dimers.

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Structure-activity relationship for antibacterial peptidyl derivatives based upon the binding site of human cystatin C

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The Cystapep 1 (Cpv) is a peptidomimetic compound structurally based upon the N-terminal part of the inhibitory site of human cystatin C and active against several clinically important Gram-positive bacteria. In this work the conformational studies, antibacterial activity and cytotoxicity against eucaryotic cells of new analogs of Cpv are shown. In contrast to Cpv peptidomimetic, the new two compounds are inactive against the Gram-positive pathogens, whereas two of them are more active than Cpv. The *in vitro* cytotoxicity studies show that none of tested compounds are toxic to mammalian cells. Structural studies comprising NMR and molecular dynamic calculations demonstrate that only Cpv is a stabile molecule with one major and very tight conformation, whereas rest of the studied compounds are flexible. We suggest that the characteristic side-chain orientation and the length of the amino-acid side-chains of the compounds are the reason for distinct antibacterial properties.

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Search for inhibitors or activators of human proteasome

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Cell metabolism is strictly depended on number and activity of many different proteins, which are regulated by transcription, translation and post-translative modification, as well as their irreversible proteolysis effectivity. One of the degradation systems, present in eukaryotic cells, is ATP-depended ubiquitin-proteasome pathway [1]. Proteasome is multicatalytic protein complex responsible for intracellular protein degradation. The process start when the chain containing several ubiquitin residues is bonded to the protein. The poliubiquitin-protein complex is transported to proteasome interior, where it is proteolytically degraded [2]. Proteasome activity has major role in homeostasis of the whole organism. Disturbance of regular functioning of proteasome is observed in many diseases, for example neurodegenerative illnesses such as Parkinson, Alzheimer or Huntington [3]. Increase of proteasome activity is also observed in case of immunological and pulmonary illnesses like mucoviscidosis [4].

Due to its function nowadays researchers of the world try to find new regulators of proteasome activity. Peptides, which we have synthesised, are based on the sequences of 11S, PA26, PR39 and HIV-1 Tat proteins, which are the natural modulators of proteasome activity [2]. Designed molecules contains the active loop or binding fragment of the mentioned proteins. Biological studies shows that the inhibitory activity of these peptides is not very high. In order to increase biological activity we are searching for compounds with less flexible conformation.

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New galanin(1-15) analogues modified in positions 9, 10 and 11 act as galanin antagonists in GI tract

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Galanin (GAL) is a 29-amino-acid residue neuropeptide widely distributed in the central and peripheral nervous systems of several vertebrate species including mammals. Galanin, originally isolated from porcine intestine, shows many interesting physiological and behavioral actions. GAL has the ability to modulate pituitary hormone release, gastric acid secretion, affects memory, learning, feeding, pain threshold control and sexual behaviour. GAL as well as its N-terminal GAL(1-15) fragment, are known as contractile agents in gastric smooth muscles and as inhibitors of insulin secretion from islets of Langerhans. However due to the lack of specific agonists and antagonists in the gastrointestinal (GI) tract the actual role of GAL in GI remains unknown.

In our studies we have designed and synthesized several new 15-amino-acid residue galanin fragment analogues modified in positions: 9, 10 and 11. Next we characterized the biological properties of these new analogues investigating their action on rat isolated gastric smooth muscles and glucose-induced insulin secretion from isolated rat pancreatic islets of Langerhans. Among analogues derived from GAL(1-15) peptide: [Phe^{9}]GAL(1-15)NH_2 and [Pro^{11}]GAL(1-15)NH_2 were found to be the potent antagonists against the inhibitory effect of GAL on glucose-induced insulin secretion from the isolated rat pancreas. These analogues block the GAL-mediated inhibition of insulin secretion. In gastric smooth muscles two analogues: [Phe^{9}]GAL(1-15)NH_2 and [$D\text{-}Leu^{10}$]GAL(1-15)NH_2 did not show the contractile activities. These two analogues were found to be a partial GAL antagonists and significantly depressed the contractile action of GAL in gastric smooth muscle cells. Our studies have shown that GAL(1-15) analogues modifies in positions: 9, 10 and 11 may be a key compounds for developing a more potent GAL antagonists in GI tract.

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Application of microwaves in peptide cleavage from Merrifield resin

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Recently a dynamic progress in the studies of microwave-assisted solid phase peptide synthesis has been observed. It has been suggested that microwave (MW) heating is able to reduce reaction time, increase yield and facilitate synthesis of difficult peptide sequences.

There are numerous publications concerning the application of microwave irradiation in solid phase peptide synthesis [1]. However, there are only a few reports on application of MW to the product cleavage step from Wang resin, usually the classical product release procedure was used. The microwave assisted cleavage from Merrifield resin was, in our best knowledge, described only as a high temperature procedure [2], which seems not suitable for peptide synthesis. The TFA removal of peptide from Wang resin could be monitored by electrospray ionisation mass spectrometry (ESI-MS), whereas for Merrifield resin this method is not feasible because of incompatibility with strong acids such as liquid HF, TFMSA or HBr/TFA.

The aim of our work was to investigate the possibility of using MW to peptide cleavage from the Merrifield resin with TFA only. We have studied a low power microwave method for peptide cleavage from Merrifield resin using 8 model peptides and compared results obtained in MW assisted TFA cleavage, classical TFMSA procedure and application of TFA at elevated temperature without MW.

We have developed an easy method for peptide cleavage from Merrifield resin that allows rapid analysis of the product using ESI-MS and could be applied to monitor reaction progress.

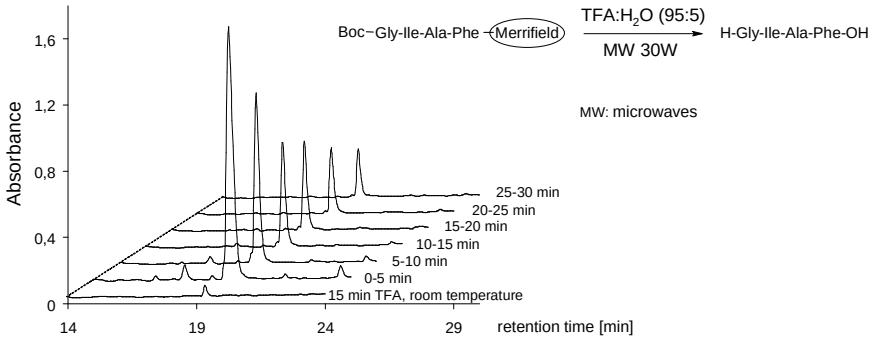

Fig. 1 The HPLC chromatograms obtained after cleavage of the product from Merrifield resin using consecutive 5 min MW irradiation.

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Deuterium kinetic isotope effect in biodegradation of L-Histidine derivatives

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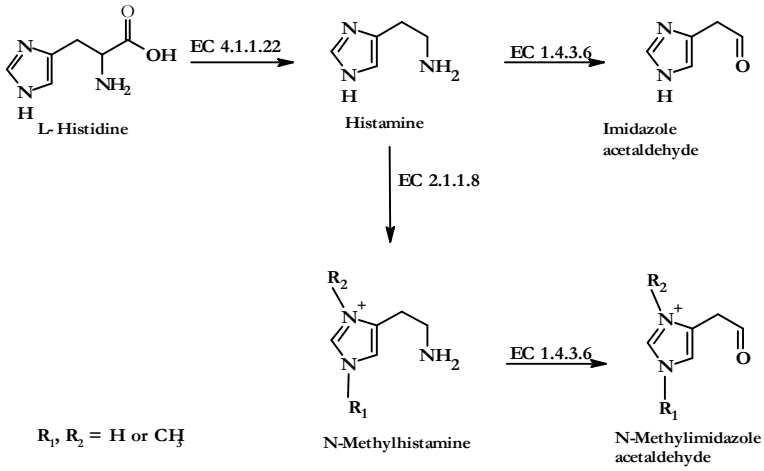
One of the most important amino-acid metabolism pathways is the decarboxylation of L-histidine to histamine catalyzed by Histidine Decarboxylase (EC 4.1.1.22).. An excess of histamine released into a living organism causes several allergic symptoms, such as inflammation, skin rash, nausea, etc. In the brain histamine is converted by enzyme Diamine Oxidase (EC 1.4.3.6.) into biologically inactive aldehydes by three different pathways: 1) histamine to imidazoleacetaldehyde; 2) indirectly by N^{π}-methyl-histamine to N^{π}-methylimidazoleacetaldehyde and similarly, 3) by N^{τ}-methylhistamine to N^{τ}-methylimidazoleacetaldehyde.



Although there have been many studies concerning the enzymatic oxidation of histamine and N-methylhistamines to their aldehydes, the mechanisms of those reactions remain unknown. This work is reporting the results of investigation of the deuterium kinetic isotope effect (KIE) and solvent isotope effect (SIE) in the reaction of oxidation of histamine catalyzed by DAO. For these studies two isomers of methylhistamine labeled with deuterium, i.e., [*(αR)*-^{2}H]-N^{τ}-methylhistamine and [*(αR)*-^{2}H]-N^{π}-methyl-histamine were used. Determination of numerical values of these effects could be useful to understanding the details of the mechanisms of above processes.

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Synthesis of new desmuramylpeptide derivatives as potential antibacterial agents

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The D-lactyl-alanyl-g-D-glutamyl-(L)-meso-2,6-diaminopimelyl-(L)-glycine (FK-156) isolated from *Streptomyces olivaceogriseus* [1,2] and its synthetic analogue of heptanoyl-g-D-glutamyl-(L)-meso-2,6-diaminopimelyl-(L)-D-alanine (FK-565) have been reported to be a potent stimulant of antibody production and free of pyrogenicity. These compounds with close structural resemblance to bacterial cell wall peptidoglycan peptides, exhibit very interesting biological activities. Both FK-156 and FK-565 enhance host defense ability against microbial infections [3], exhibit strong antiviral activity [4] and remarkable antitumor potency [5,6].

Continuing our studies of the synthetic therapeutic agents, we synthesized a new heptanoyl analogues of desmuramylpeptides: heptanoyl-L-Ala-L-Glu-L-Val, heptanoyl-L-Lys-L-Ala-L-Val, heptanoyl-L-Glu-L-Lys-L-Ala, heptanoyl-L-Val-L-Glu-L-Ala, heptanoyl-L-Lys-L-Glu-L-Ala using the solid phase chemistry. The obtained products were purified by solid-phase extraction (SPE) and characterized by MS and analytical RP-HPLC. All compounds were sent to assay their antibacterial activity.

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Tritium kinetic isotope effect on enzymatic decomposition of L-phenylalanine

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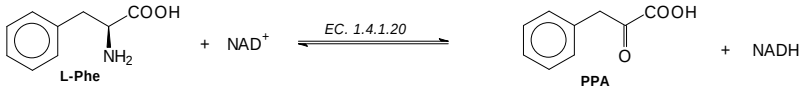
Phenylketonuria (PKU), a human genetic disease, is accompanied by elevated levels of L-phenylalanine (L-Phe) metabolites such as phenylacetate, phenyllactate and phenylpyruvate in body fluids. The knowledge about the mechanism of enzymatic conversionof L-Phe into phenylpyruvic acid (PPA) is essential for proper therapy of PKU patients. One of metabolic path of conversion of L-Phe into PPA is revesible, oxidative deamination catalyzed by enzyme L-Phenylalanine Dehydrogenase (EC 1.4.1.20), PheDH, Scheme 1.



Scheme1. Enzymatic conversion of L-Phe into PPA

The mechanism of above reaction is not clear up to now. Our studies are directed at investigation of the mechanism of this reaction by applying kinetic (KIE) isotope effect method. For this reaction we investigated the tritium kinetic isotope effect using competitive (combined with internal ^{14}C-radioactive standard) method. For purposes of this research the labeled with tritium and ^{14}C isotopomers of L-Phe ([2-^{3}H]- and [1-^{14}C]-L-Phe were obtained.

The isotopomer of [2-^{3}H]-L-Phe, needed for KIE studies, was afforded by reductive amination of PPA catalyzed by enzyme PheDH carried out in tritiated medium.

The isotopomer [1-^{14}C]-L-Phe, used as a radioactive internal standard, was obtained by addition of ammonia to [1-^{14}C]-*(E)*-cinnamic acid, catalyzed by enzyme Phenylalanine Ammonia Lyase (EC 1.4.3.5).

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Synthesis of peptidomimetics containing tetrahydro-β-carbolines skeleton

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Peptide-protein and protein-protein interactions are fundamental events that modulate diverse biological processes such as signal transduction, enzymatic specificity and immunomodulation. Tryptophan is often a key pharmacophore which determines the affinity of peptide ligands for their receptors. Various constrained tryptophan analogues or tryptophan containing motifs have been utilized to generate highly potent and selective ligands to biological target receptors. Cyclic analogues of tryptophan which introduce local constraints reduce the flexibillity of the indol moiety and are very valuable tools to probe the bioactive conformation of the peptide ligands.[1] The Pictet-Spengler reaction [2] has been one of the possibilities to prepare such analogues containing 1,2,3,4-tetrahydro-β-carboline skeleton. The main advantage of this reaction is the formation of a product with freezed the indol moiety in tryptophan in one single step. The heterocyclic skeleton of 1,2,3,4-tetrahydro-β-carbolines possesses multiple sites for functionization, therefore they are an ideal choice for the design of pharmacophore-based libraries in drug discovery.

We report the synthesis of 1,3-disubstituted 1,2,3,4-tetrahydro-β-carbolines by the Pictet-Spengler condensation of L- and D-α-aminoaldehydes as carbonyl compounds with dipeptides with N-terminal α-Trp or β-3-Trp as arylethylamine substrates. The reaction was studied in terms of double stereodifferentiation. The influence of the carboxyl terminus of α-Trp and β-3-Trp on the ratio of *cis/trans* products was also investigated. The conformations of cyclic products were studied by 2DNMR ROESY spectra.

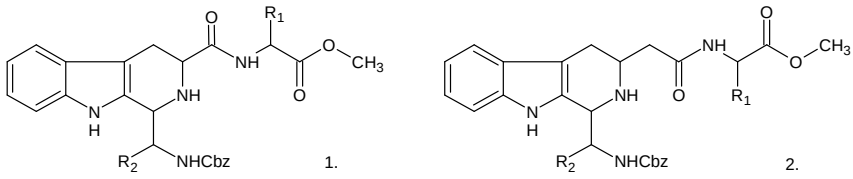

Fig. 1. The peptidomimetics containing tetrahydro-β-carbolines skeleton as a products of Pictet-Spengler condensation of L- and D-α-aminoaldehydes with dipeptides with N-terminal (1) α-Trp or (2) β-3-Trp.

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In search of antimicrobial compounds structurally based upon Cystapep 1

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In late 50ies it was believed that bacterial disease was at last controlled. But decades of overuse, not just by doctors – but by vets, dentists and also by the farming industry – have encouraged the growth of a new breed of antibiotics resistant superbugs. Methicillin resistant *Staphylococcus aureus*, *Streptococcus pyogenes* and newly discovered *Clostridium difficile* are recognized as a major cause of abscess, boil, diarrhoea and colitis. Due to the appearance of superbugs researchers are struggling to create new era agents, which would be active against resistant bacteria.

In 1989 a new group of potential antimicrobial peptide compounds structurally based upon the inhibitory centre of human cystatin C was found to inhibit growth of *Streptococcus pyogenes*. Because of their origin it was thought that they inhibit cysteine protease, but subsequent analysis of a number of similar compounds showed that their antimicrobial effect does not include protease inhibition. Further research found that one of the compounds, called later Cystapep 1, has comparatively low MIC and MBC values for gram-positive bacteria. Cystapep 1 is effective against antibiotic resistant staphylococci and streptococci as well as against susceptible strains of these species. Due to the fact that these resistant strains are presently a concern in hospital wards Cystapep 1 and its analogues may prove useful. [1]

The important issue of a new antimicrobial compounds is their mechanism of action. Most antimicrobial peptides form pores in bacteria membranes. Resistance acquisition on this activity rarely has even been seen. [2] Synthesis and conformational studies of Cystapep 1 derivatives, showed that analogues with antimicrobial activity have a turn stabilized by one hydrogen bond, which is essential for their activity. It may have a connection to the fact that Cystapep 1 probably interact with cell membranes. [3]

During my studies were synthesized two compounds with probable translocated hydrogen bond and two with fluorescent moieties. All four compounds activity against bacteria will be investigated as well as structural studies. Active compound with chemosensors can give the answer about Cystapep 1 mechanism of action.

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[3] not published data

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Conformational studies of peptide corresponding to the *C*-terminal fragment of bovine rodopsin

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The *C*-terminal fragment of bovine rhodopsin included residues 325-348 takes a very important part in signal transduction process. Phosphorylation of all serine and threonine residues from this region, followed by its binding to arrestin protein leads to conformational changes, reduces ability to activate the transduction cascade, and rapidly terminates the receptor activation. All current models of bovine rhodopsin refined from crystallographic data reveal that C-terminal fragment of bovine rhodopsin is highly disordered, flexible and very dynamic, with no stable structure defined [1-4]. Earlier limited conformational studies suggested that synthetic peptide corresponding to 330-348 fragment of bovine rhodopsin is completely disordered in solution as well as its phosphorylated analogs. The phosphorylated peptide possesses well organized three-dimensional structure only in the complex with arrestine [5-7].

The aim of this study is to determined structure and conformationals dynamics of 330-348 fragment of bovine rhodopsin and compare those results with similar results of similar studies performed on phosphorylated peptide. Our studies will allowed to determined how phosphorylation affects structure and conformational dynamics of investigated peptide. The 19 amino-acid residue peptide corresponding to 330-348 fragment of bovine rhodopsin was chemically synthesized. Three dimensional structure was obtained using data from NMR experiments such as COSY, ROE and TOCSY suplemented by time-averaged MD simulations.

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Low molecular weight amphiphilic dendrimeric peptides functionalized with β-lactam moiety

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Multi-drug resistance against conventional antibiotics is one of the most serious problems in contemporary medicine. Number of chemical modifications of the existing lead compounds like penicillins, cephalosporins, carbapenams, etc. have recently reached their limits and therefore, necessity for design of new structurally different compounds is obvious. Currently we have observed intensive worldwide research focusing on the use of natural, linear antimicrobial peptides and their derivatives. On the other side, conventional antibiotics used in contemporary medicine are still preferably active on bacteriall wall.

Non-sequential pharmacophore approach developed in our laboratory allows to mimic membrane active conformations postulated for natural antimicrobial peptides, by assembling dendrimeric peptides from the respective amino acids. Several groups of non-symmetrical low molecular weight amphiphilic dendrimeric peptides designed in our laboratory using the above philosophy expressed high antimicrobial activity, particularly against Gram-positive bacteria.

Here we present synthesis and structure of three groups dendrimeric compounds which contain the above mentioned dendrimeric peptides modified by introduction of fragments of commercial antibiotics – penicillin G and 6-aminopenicillanic acid (6-APA). Two groups are modified by residue of penicillin G – functionalizations occur at the C-end and at the periphery, respectively. The last group is functionalized at the C-end by residue of 6-APA. All these peptide dendrimers have been tested for antimicrobial activity and inhibitory potency against D,D-carboxypeptidase.

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Identification of binding sites for hCC-SAA complex

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Cystatin C (hCC) is the most abundant human extracellular inhibitor of cysteine proteinases. Cystatin C is the single-chain protein which dimerize throught 3D domain swapping mechanizm. HCC is monomeric in its native physiological states while in pathological conditions it is present as dimer. Serum amyloid A (SAA) play major, but relatively uncharacterized roles in the acute phase response and are important complnentes of the innate immune systems of humans. Analysis of primary structur of human SAA suggests that approximately 80% of the molecules may consist of a helical bundle while the remaining C-terminal part is disordered.

Cystatin C can create immunocomplex with SAA. The determination of the interacting sites could also be useful in designing new tools for diagnostics in many neurodegenerative disorders. The identification of the binding site in hCC should be very important for oligomeryzation studies of new oligomerisation inhibitors may be designed baded on SAA binding fragments. In this work we we present a novel affinity method for protein-peptide interaction studies that enabled identification of the interactions between human cystatin C and serum amyloid A. For the identification of the binding sites were applied extraction/excision mass spectrometry method together with digestion by means different proteolytic enzymes. The binding sites of for SAA-hCC complex are located in C-terminal part of both protein, namely residues (96-102) in hCC and (87-105) in SAA.

Acknowledgements:
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Proteolytical stability of conformationally restricted opioid peptides

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Proteolytic stability, one of the important factors in designing of peptide-based drugs, can be enhanced by the side-chain to side-chain cyclization. Previously we described the synthesis and biological activity of deltorphin and enkephalin analogs restricted by cyclization via thy urea bridge. The analogs contained a carbonyl bridge, linked the two side-chain amino groups to form an ureido moiety. Several of these compounds showed very high δ-receptor agonist potency. [1]

In the present study we analyzed proteolytical stability of the cyclic peptides and we identified their degradation products by electrospray mass spectrometry. Our results indicated that cyclization via the urea bridge increased the resistance of the peptides incubated with pepsin and chymotrypsin to proteolytic digestion. Notable enhancement of stability to proteolysis was observed for peptide bonds located not only within the formed ring but also in N-terminal exocyclic linear peptide segments. The observed stability depends not only on the ring size but also on localization of the urea bridge within the ring. One of the interesting observations is the proteolytical stability of a potent opioid, {[H-Tyr-D-Lys(&1)-Phe-Dap(&2)-NH_2] [&1CO&2]} (compound **A** in Fig.1), whereas its isomer with the {[H-Tyr-D-Lys(&1)-Phe-Dap(&2)-NH_2][&1CO&2]} sequence (**B**) hydrolyzed rapidly in presence of chymotrypsin.

We will present a possible explanation of this phenomenon.

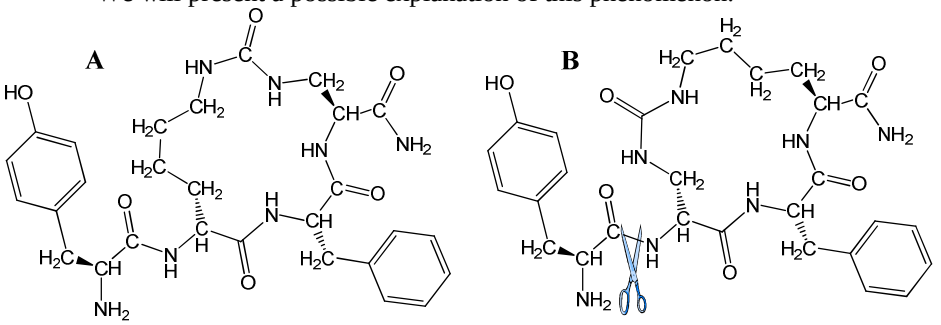

Fig. 1. Structures of two tested isomers. The observed cleavage site of compound **B** is marked.

[1] Filip K., Oleszczuk M., Pawlak D., Wojcik J., Chung N.N., Schiller P.W., Izdebski J. J. Peptide Sci., **9**, 649-657, 2003.

In vitro antifungal activity of antimicrobial peptides against Candida species

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Candida albicans is a common pathogen which causes opportunistic oral and genital infections in humans. Systemic fungal infections (fungemias) have emerged as an important cause of morbidity and mortality in immunocompromised patients. In view of the fact that non-albicans infections are becoming more common and non-albicans species are more resistant to antifungal drugs, we have made an attempt to find new active substances active against those pathogens.

In this report we tested the following synthetic antimicrobial peptides: protegrin 1, tachyplesin 3, temporin A and citropin 1.1 and a short-chain lipopeptide, Palm-KK-NH_2. As a control we used antifungal drugs such as nystatin, amphotericin B and clotrimazole.

All peptides were tested using the methods recommended by NCCLS. MIC (Minimal Inhibitory Concentration) and MBC (Minimal Bactericidal Concentration) were determined on Sabouraud (2% glucose) medium. The microorganisms were isolated from the skin, oral and vaginal mucosa.

Our results have shown that Palm-KK-NH_2, citropin 1.1 and tachyplesin 3 were very effective against C.albicans and non-albicans species. Further details will be presented on a poster.

Identification of the epitope for anty cystatin C antibodies (Cyst-13)

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The immune system has a major role in the defense of organisms against infections. Nowadays scientists are working on the use of antibodies in a search of inhibitors for many kinds of amyloidogenic diseases. The great potential of this concept is hidden in monoclonal antibodies (mAb) , which recognize only one epitope of the antigen and are highly specific to the particular antigen. Grubb and coworkers described the influence of monoklonal antibodies on dimerization process of human cystatin C (hCC), even catalytical amount of the monoclonal antibodies visibly diminished the process. This clearly showed that the mAb can be considered as potential therapeutic agent in amyloidosis caused by aggregation of hCC and its L68Q mutant. The physiological role of cystatin C is to regulate extracellular cysteine protease activity during microbial invasion or release of lyzosomal proteinases from dying or diseased cells. Cystatin C is monomeric in its native physioloigical states while in pathological conditions it is present as dimer.

In this work we present the preliminary results of studies on the influence of monoclonal antibodies Cyst-13 on dimerization process of human cystatin C and identification of the epitope for those antibodies with the use of epitope extraction-, excision- mass spectrometry method.

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Bradykinin analogues with unchanged main chain of the molecule

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The present study is a continuation of previous investigations aimed at finding structural requirements in effective bradykinin antagonists for B_2 receptors.

Previously, it was demonstrated that the presence of a bulky acyl substituent (1-adamantaneacetic acid, 4-tert-butylbenzoic acid, 1-adamantanecarboxylic acid, etc.) at the N-terminus of B_2 antagonists consistently improved their antagonistic potency in the rat blood pressure assay [1]. Reported results also suggested that the effects of acylations might vary substantially with the chemical character of the acyl group [2]. It seemed that either the positive or the negative charge on the N-terminal acyl group influenced the activity of the analogues, as was highlighted by a suppressed antagonistic potency due to these modifications. Recently we decided to learn how N-terminal acylation would affect pharmacological activity of the bradykinin molecule. Of the many acylating agents tested previously on B_2 antagonists, acridin-9-ylacetic acid and anthracen-9-ylacetic acid were used. These two analogues, which do not contain any changes in the main chain, were able to antagonize the bradykinin activity in the rat blood pressure test [3].

In the current work we present some pharmacological properties of ten new analogues of bradykinin modified in the N-terminal part of the BK molecule with a variety of acyl groups (1-adamantaneacetic acid, 1-adamantanecarboxylic acid, 4-tert-butylbenzoic acid, 4-aminobenzoic acid, 12-aminododecanoic acid, succinic acid, 4-hydroxybenzoic acid, 4-hydroxy-3-methoxybenzoic acid, 3-(4-hydroxyphenyl)propionic acid and 6-hydroxy-2-naphthoic acid). Our results provide new information on the structure–activity relationship of BK analogues and may have an impact on designing selectively acting BK antagonists.

[1] Lammek B., Pol. J. Chem., **68**, 913-920, 1994.
[2] Trzeciak H.I., Kozik W., Melhem S., Kania A., Dobrowolski D., Prahl A., Derdowska I., Lammek B., Peptides, **21**, 829–834, 2000.
[3] Prahl A., J. Pept. Sci., **13**, 206-210, 2007.

The effects of *C*-terminal modifications of bradykinin analogues with 1-aminocyclohexane-1-carboxylic acid and 1-aminocyclohexane acetic acid on pharmacological properties of the derivatives

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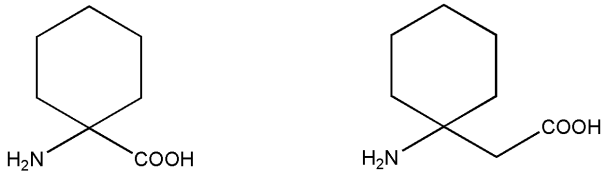
In the present work, sterically constrained non-coded amino acids, 1-aminocyclohexane-1-carboxylic acid (Acc) and 1-aminocyclohexane acetic acid (Aca), were substituted in the C-terminal part of the model B_2 receptor antagonist [D-Arg^0,Hyp^3,Thi^{5,8},D-Phe^7]BK, previously synthesized by Stewart's group. The analogues were N-acylated with 1-adamantaneacetic acid (Aaa).

Recently we reported that the Acc residue could be substituted in positions 7 and 8 of the Stewart's peptide, since the position in which it is introduced is very important for analogue's antagonism [1]. Two compounds: [D-Arg^0,Hyp^3,Thi^5,D-Phe^7,Acc^8]BK and its acylated counterpart are potent B_2 antagonists in the rat blood pressure test as well as in the rat uterus assay. We found it interesting to learn how the Acc^5 and Acc^{5,8} substitution would influence pharmacological properties of the resulting analogues. We also decided to replace the amino acid residue at position 7 of the model peptide with the Aca residue whose structure differs only slightly from that of the Acc modification. Our results may be useful for designing new B_2 agonists and antagonists.



Structures of 1-aminocyclohexane-1-carboxylic acid (Acc) and 1-aminocyclohexane acetic acid (Aca)

[1] Labudda-Dawidowska O., Wierzba T.H., Prahl A., Kowalczyk W., Gawiński Ł., Plackova M., Slaninová J., Lammek B., J. Med. Chem., **48**, 8055-8059, 2005.

Synthesis of tritiated ligand for binding assays to the tachykinin receptors

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Tachykinins belong to the family of neuropeptides and are characterized by a common *C*-terminal sequence, Phe-X-Gly-Leu-Met-NH_2, where X is lipophilic amino acid either an aromatic (Phe, Tyr) or an aliphatic (Ile, Val). Tachykinins interact with specific membrane proteins, belonging to the family of G protein-coupled cell membrane receptors. Tachykinins are involved in broad spectrum of normal neuromodulatory functions as well as pathological cancer self-activation. To be able to measure the affinity of new synthesized compounds to the tachykinin receptors tritiated compounds are needed.

Our aim was to synthesize the tritiated standard compound for our further studies to measure binding affinities to tachykinin receptors. First, the cold precursor peptide with the sequence: Lys-Phe(I)-Phe(I)-Gly-Leu-Met-NH_2 have been synthesized. This compound was later tritiated in the manual apparatus (BRC, Szeged, Hungary), and hot compound was isolated by HPLC. We will present the details of the synthesis and obtained radioactivity of the final product.

Acknowledgement:
Project supported by EU grant Normolife (LSHC-CT-2006-037733).

β^2-homo-amino acid scan of Endomorphin-2 and its D-Ala^2-analogue (TAPP)

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A major challenge in opioid peptide chemistry is the synthesis of novel compounds mimicking the endogenous peptide ligands. These new peptidomimetics should be biologically active and more stable against enzymatic degradation than their parent ligands. One of the possibilities is the introduction of β-amino acids into the peptides sequence. Monosubstituted β-amino acids (β^2- or homo β^3-) due to their similarity in structure to α-amino acids, moreover their tendency to give folded structures even in short peptides and to the stability towards mammalian peptidases may be very useful in the creation of the new potentially active compounds.

We have developed simple and efficient two step conversion of the cyanoacetate into fully protected, both aromatic and aliphatic, β^2-amino acids. We focused our attention on applying this method to the synthesis of different β^2-amino acids (as homologues of α-amino acids) as elements for the synthesis and structure – activity relationship study of endomorphin analogues. Small library of analogues has been created in which α-amino acids (fig.1a) in every position with exception of Pro were substituted by their respective (*R*)- or (*S*)-β^2-analogues (fig.1b). As templates, endomorphin-2 (Tyr-Pro-Phe-Phe-NH_2) and its D-Ala^2-analogue (TAPP) have been used. In this communication, the conformational and metabolic consequences of such modification will be discussed.

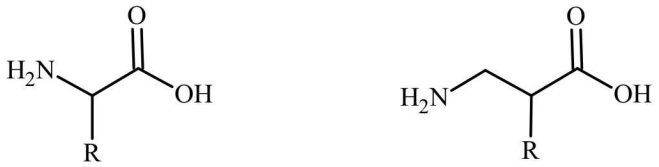

Fig. 1. Structure of α-amino acids (a) and β^2-homo-amino acids (b)

Acknowledgement:
Project supported by EU grant Normolife (LSHC-CT-2006-037733).

Development of new phosphonic-type human neutrophil elastase inhibitors

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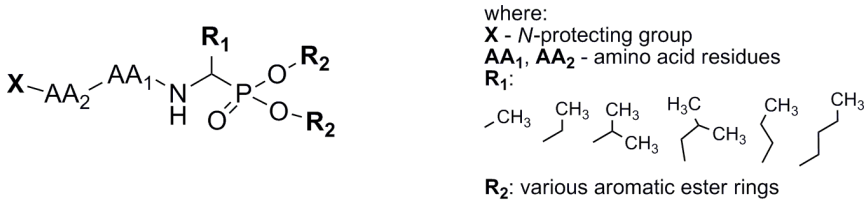
Neutrophil elastase (NE) is a 30kD glycoprotein which belongs to serine protease family. NE is the only neutral protease able to degrade insoluble elastin. It can also hydrolyze other extracellular matrix proteins including fibronectin, proteoglycans, and type IV collagen. Its major endogenous inhibitor is alpha-1-proteinase inhibitor (α-1 PI). It has been well established that deficiency of α-1 PI may lead to lung cancer development [1]. The imbalance between neutrophil elastase and α-1 PI can't be suppressed by reversible inhibitors. Only irreversible inhibitors with a half life of more than 48h such as 1-aminoalkylphosphonate diaryl esters have the ability overcome the elastase/serpin imbalance. 1-aminoalkylphosphonates and their peptidyl derivatives belong to the family of irreversible, potent and selective inhibitors of serine proteases [2].



Scheme 1. Schematic representation of synthesized HNE inhibitors.

Here we present the synthesis and biological evaluation of α-aminophosphonic human neutrophil elastase inhibitors library – phosphonic analogues of Val, nVal, Leu, nLeu, Ala, Abu having structurally diverse aromatic ester groups and their peptidyl derivatives (Scheme 1). The inhibition data against HNE as well as toward related proteases of this class will be presented.

[1] Zhifu S., Ping Y., Lancet Oncol., **5,** 182–190, 2004.
[2] Oleksyszyn J, Powers J.C., Biochemistry, **30**, 485-493, 1991.

The kinetic and solvent deuterium isotope effects in 5-position of indole ring on the enzymatic decomposition of L-tryptophan

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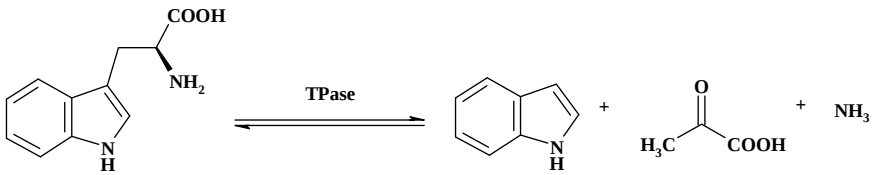
L-Tryptophan (L-Trp) and its derivative 5'-hydroxy-L-tryptophan (5'-OH-L-Trp), play the role of primary intermediates in the biosynthesis of hormones, i.e., tryptamine and serotonine. Some of their derivatives also exhibit pharmaceutical activity. In the living cells the enzyme tryptophanase (*L-Tryptophan Indole Lyase* EC, 4.1.99.1), TPase, catalyses the decomposition of L-Trp to the corresponding indole, pyruvic acid, and ammonia (Scheme 1).



Scheme 1. Decomposition of L-Trp by enzyme Tpase.

Under some conditions TPase also catalyzes the reverse reaction leading to the formation of L-Trp. The mechanism of above reaction involving multiple proton transfer is not clear up to now. Our studies are directed at investigation of the mechanism of this reaction by applying kinetic (KIE) and solvent (SIE) isotope effect methods.

The isotopomer of [5'-^{2}H]-L-Trp, needed for KIE studies, was synthesized by coupling of [5-^{2}H]-indole with *S*-methyl-L-cysteine using the of enzyme TPase. The deuteriated [5-^{2}H]-indole was prepared by reduction of 5-bromoindole with sodium borodeuteride dissolved in methanol (deuteriated in hydroxyl group). The reaction was catalyzed by $PdCl_2$.

This work is reporting the kinetic studies needed to calculate the deuterium KIE and SIE in enzymatic decomposition of L-Trp, Scheme 1. For determining KIE and SIE noncompetitive method was chosen. The parameters in Michaelis equation, i. e., K_m and V_{max} were determined separately for normal and deuterium incubation medium.

This work was supported by the grant BST-132623.

Analogues of Cyclolinopeptide A modified by tetrozole ring and by homophenylalanine

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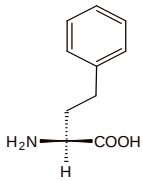
The search of new immunosupressants, exhibiting the same mechanism of action as for Cyclosporine A (CsA) and FK-506 is an important challenge for medicinal chemistry. Cyclolinopeptide A (CLA) a natural cyclic nonapeptide [cyclo(Leu[1]-Ile[2]-Ile[3]-Leu[4]-Val[5]-Pro[6]-Pro[7]-Phe[8]-Phe[9])] [1] possesses a strong immunosuppressive activity comparable with that of CsA in low doses. The possibility of practical application of CLA as a therapeutic agent is limited due to its high hydrophobicity. It has been suggested that the tetrapeptide sequence Pro[6]-Pro[7]-Phe[8]-Phe[9] is responsible for the interaction of the CLA molecule with the proper cellular receptor. In order to evaluate the role of this tetrapeptide unit for biological activity, we decided to modify this fragment.

In this communication we present linear and cyclic CLA analogues in which penylalanine residues in position 8 have been replaced by homophenylalanine (HoPhe) **1** [2] and in position 5-7 the 1,5-disubstituted tetrazole ring have been incorporated **2** [3] as an effective *cis*-amide bond mimic.



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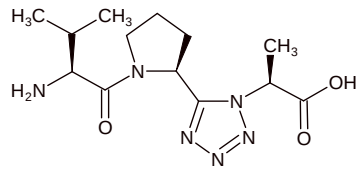

2

The Synthetic strategy and biological activity of all analogues will be discussed.

[1] Kaufman H. P., Tobschirbel A., Chem.Ber., **92**, 2805, 1959.
[2] Li X., Yeung Ch., Chan A. S. C., Lee D., Yang T., Tetrahedron: Asymmetry, **10**, 3863, 1999.
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Synthesis and biological evaluation of cyclic endomorphin-2 analogs

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Incorporation of a conformational constraint is a well-known approach used to restrict the flexibility of peptides and increase their selectivity towards one specific receptor type.

This approach has been successfully used in the field of opioid peptide analogs, either by introducing sterically hindered amino acids or by cyclization of linear sequences. Cyclization reduces the molecular conformational freedom, which is responsible for the contemporary activation of different receptors, increases metabolic stability and generally increases lipophilicity, which often improves the blood-brain barrier permeability of peptides.

Here we have described the synthesis and antiociceptive activity of a series of endomorphin-2 (EM-2, Tyr-Pro-Phe-Phe-NH_2) analogs obtained by cyclization through an amid bond between the side-chain amino and carboxy groups of the diamino and dicarboxy amino acids introduced into the peptide sequence in positions 2 and 5.
New analogs are cyclic pentapeptides of a general structure:

Xaa-c(Yaa-Phe-Phe-Zaa)-NH_2 Xaa = Tyr or NMeTyr
Yaa = D-Lys or D-Asp
Zaa = Asp or Lys

All new analogs had μ-opioid receptor affinity in a nanomolar range and were much more resistant to enzymatic degradation than EM-2. Their analgesic effect was assessed in the hot-plate test in mice (supraspinally mediated analgesia), after i.c.v. administration. All cyclic analogs showed an extremely strong analgesic effect, which was dose-dependent. In case of the best analog, Tyr-c(D-Lys-Phe-Phe-Asp), the antinociceptive effect was observed at a dose as low as 3 ng, whereas for EM-2 much higher doses were required (about 100 ng/animal).

Acknowledgement:
This work was supported by grants from Polish Ministry of Science No 125/N-POLONIUM/2008/0 and No NN 401 0064 35, from the Medical University of Lodz No 503-1099-1 and Polpharma Foundation For Development of Polish Pharmacy and Medicine.

The characterization of staphopains enzyme family from *Staphylococcus aureus* using combinatorial chemistry methods

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Staphylococcus aureus produces a large number of extracellular proteins, many of which are important virulence factors toxic to humans and animals . Among the proteolytic enzymes secreted by this bacteria, there are three cysteine proteases, referred to as staphopain A (StpA) staphopain B (StpB) and staphopain C (StpC) [1]. They are involved in several function of this pathogen among them neutrophil degradation, skin necrosis ect.
In this work we described the substrate specificity of the staphopains (A, B and C) using combinatorial chemistry methods. In order to established substrate specificity of individual enzyme the library of FRET peptides was applied. The library was synthesized on the solid phase using portioning – mixing approach methods. General formula of such library is given below:

ABZ-X_4-X_3-X_2-X_1-ANB-NH_2
where:
ANB-NH_2 – amid of 5-amino-2-nitrobenzoic acid (acceptor of fluorescence);
ABZ – 2-aminobenzic acid (donor of fluorescence);
X_1; X_2, X_3, X_4 were 19 proteinogenic amino acid residues excluding Cys.

Partial results of the substrate mapping of those three enzymes indicate that StpA and StpB display very similar substrate specificity. StpC reveal quite different substrate preferences.
The obtained substrate sequence(s) of all three proteases will be characterized biochemically.

[1] Dubin G., Acta Biochim Pol. 2003, 715-24.

Acknowledgment:
This work was supported by Ministry of Science and Higher Education under grant 1600/B/H03/2009/36/.

Synthesis and antimicrobial properties of amphiphilic peptide with "flexible" structure

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[a]Mossakowski Medical Research Centre Polish Academy of Sciences
[b]National Institute of Hygiene
[c]Institute of Organic Chemistry Polish Academy of Sciences

Amphiphilicity of antimicrobial peptide is determined by hydrophilicity/lipophylicity of its amino acid side chains. Their assumed biological target is cytoplasmic membrane that in case of bacteria is slightly negatively charged. In addition, ability of the peptide to adopt amphipatic conformation in which positively charged and hydrophobic centers are in well defined areas usually associated with defined secondary structure like α-helix or β-sheet. It is confirmed that these peptides are capable to interact with the membranes of living cells and to modify its life-process . Significant number of amphiphilic peptides occur in nature, other have been produced by synthetic methods. Our research team developed and antimicrobially tested new type of low molecular amphiphilic dendrimeric peptides that expressed antimicrobial properties. Until now peptides studied by us had very compact structure obtained by combinations of phenylalanine and lysine residues. This communication presents the synthesis and properties of more flexible structure in which functional amino acid residues are separated by flexible glycine residue. Final dendrimeric peptide has higher flexibility and in consequence higher ability of adaptation to the target surface.

Acknowledgement:
The project is financially supported with Polish grant MNiSzW NN204239436.

Structure-activity relationship of peptidomimetics inhibiting osteoclastogenesis process

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Osteoclastogenesis is the complex process generating osteoclasts which are the only cell capable of resorbing bone. The activity of osteoclasts plays essential role in normal skeletal development (growth and modeling), in the maintenance of its integrity throughout life, and in calcium metabolism (remodeling). Excessive bone resorption is the cause of bone lose in pathological conditions such as osteoporosis, rheumatoid arthritis and periodontal disease.

It has been known that cystatin C and other cysteine proteinase inhibitors such as E-64 effect on osteoclast formation and differentation, but the complete mechanism of this process is still unresolved [1,2]. It has been suggested that cysteine proteinase inhibitors decrease formation of osteoclasts by interfering at a late stage of pre-osteoclast differentiation. In this work we focused on the investigation of peptidomimetics which structures are based on the N-terminal fragment of human cystatin C. A series of peptidomimetics were designed, synthesized and evaluated for their ability to inhibit the of formation of multinucleated osteoclasts. The preliminary evaluation of the activity of obtained peptidomimetics has shown that most of the them inhibit osteoclastogenesis. Additionally, for chosen osteoclastogenesis inhibitors, we have carried out conformational investigation which indicated that the level of their structure similarity is very high.

[1] Brage, M., Lie, A., Ransjö, M., Kasprzykowski, F., Kasprzykowska, R., Abrahamson, M., Grubb, A., Lerner, U.H. Bone, **34**, 412-424, 2004.
[2] Brage, M., Abrahamson, M., Lindström, V., Grubb, A., Lerner, U.H., Calcif Tissue Int., **76**, 439-447, 2005.

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Oferowane rozwiązania dedykowane są badaniom w **biologii, biochemii, biofizyce, farmacji, naukach medycznych** – **mikrobiologii i diagnostyce klinicznej** oraz **analizie strukturalnej** związków chemicznych.

Cześć oferty skierowana jest do specjalnych zastosowań w **przemyśle farmaceutycznym, chemicznym,** spożywczym, jako systemy: kontroli jakości, badania tożsamości oraz badań mikrobiologicznych i kontroli procesów produkcyjnych.

Nowoczesne i przyjazne użytkownikowi oprogramowanie kontrolno-pomiarowe, z rozbudowaną bazą dedykowanych programów użytkowych, symulacyjnych i bibliotek, stanowi doskonałą podporę wspomagającą procesy pomiarowe.

Bruker Polska ze swoją ponad dwudziestoletnią tradycją zapewnia najwyższą jakość usług serwisowych i aplikacyjnych, a bliskość macierzystych zakładów z Niemiec pozwala na szybkie i kompetentne realizowanie zadań związanych z instalacją i eksploatacją naszych przyrządów oraz pomoc w ich przystosowaniu do Państwa potrzeb. Naszym klientom i użytkownikom oferujemy szkolenia z zakresu obsługi spektrometrów, użytkowania oprogramowania i kursy aplikacyjne w siedzibie firmy w Poznaniu oraz w głównych oddziałach w Niemczech.

Oferta obejmuje:

- spektrometry NMR (200 - 1000 MHz)
- spektrometry EPR (od pasma L - 2,4GHz do W - 94GHz)
- spektrometry masowe MS (pułapki jonowe, ESI-(Q)-TOF, MALDI-TOF(/TOF), FTMS)
- spektrometry rentgenowskie XRF
- dyfraktometry rentgenowskie XRD i monokrystaliczne SCD
- spektrometry OES
- analizatory gazowe CS/ONH

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In the area of peptides, proteins and other short and long chain polymer analysis we offer equipment like UV-Vis spectrophotometers, DNA and protein analyzers, FTIR, RF, GC chromatographs, LC and electrovalent mass spectrometers of the GC-MS, LCMS, LCMS-IT-TOF type, as well as MALDI-TOF; TOC analysers – of organic coal and nitrogen in solution and solid state and AAS.

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